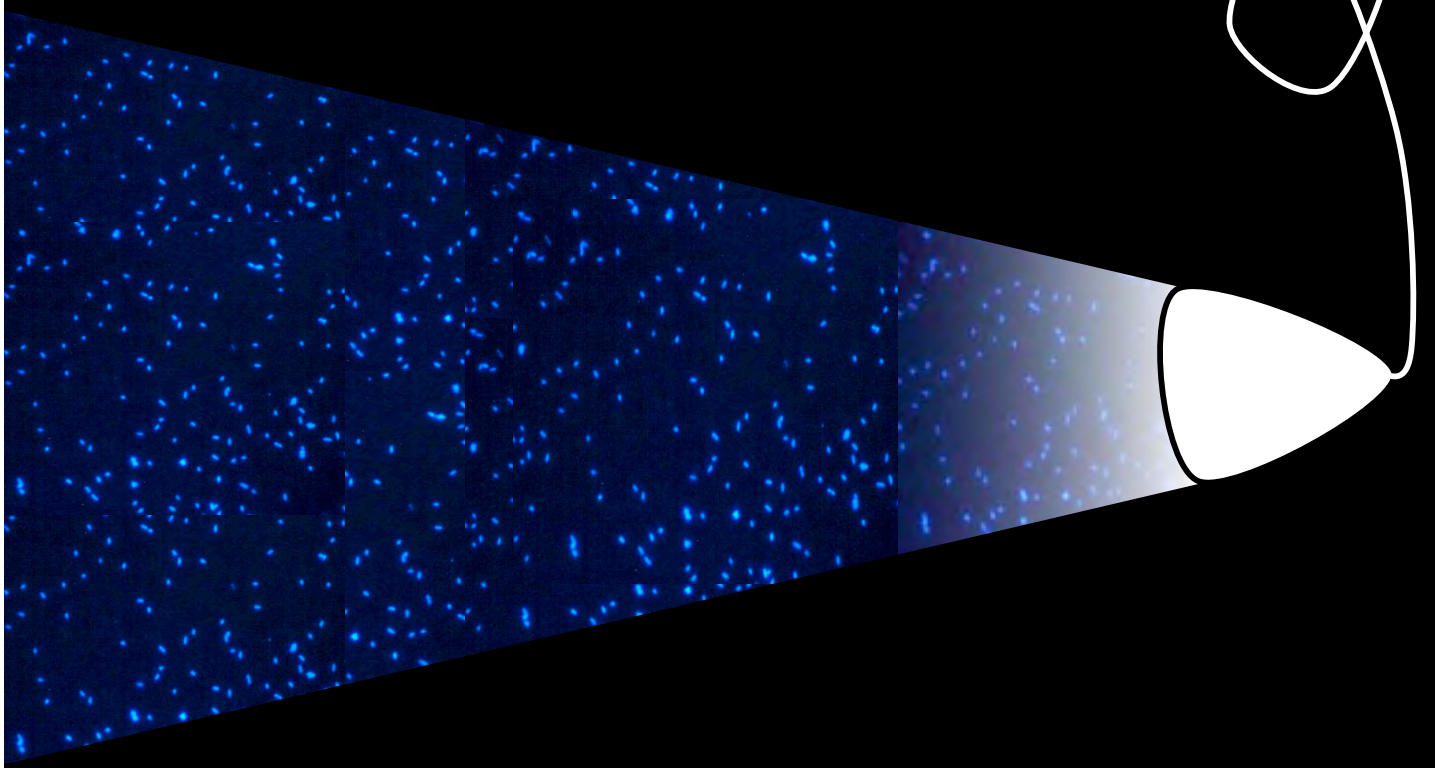
TESIS DOCTORAL

Shedding light on the role of the prokaryotic assemblage in the biogeochemical cycles of the dark ocean

Federico Baltar González



UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

Departamento de Biología

Las Palmas de Gran Canaria

Marzo 2010

D/Dª Juan Luis Gómez Pinchetti SECRETARIO/A DEL DEPARTAMENTO DE Biología DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA,

CERTIFICA,

Que el Consejo de Doctores del Departamento en sesión extraordinaria tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada "*Shedding light on the role of the prokaryotic assemblage in the biogeochemical cycles of the dark ocean*" presentada por el/la doctorando/a D/Dª. Federico Baltar González y dirigida por el Dr. Javier Arístegui Ruiz, el Dr. Gerhard J. Herndl y el Dr. Josep M. Gasol Piqué.

Y para que así conste, y a efectos de lo previsto en el Artº 73.2 del Reglamento de Estudios de Doctorado de esta Universidad, firmo la presente en Las Palmas de Gran Canaria, a 25 de Enero de 2010

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
Departamento de Biología

Programa de doctorado de Oceanografía.

Bienio 2005-2007. Con Mención de Calidad de la ANECA.

Título de la Tesis:

Shedding light on the role of the prokaryotic assemblage in the biogeochemical cycles of the dark ocean

(Arrojando luz sobre el papel de los procariotas en los ciclos biogeoquímicos del océano oscuro)

Tesis doctoral presentada por D. Federico Baltar González para obtener el grado de Doctor por la Universidad de Las Palmas de Gran Canaria.

Dirigida por Dr. D. Javier Arístegui Ruiz
Dr. D. Gerhard Herndl
Dr. D. Josep María Gasol Piqué

El/la Director/a **El/la Co-Director/a** **El/la Co-Director/a** **El/la Doctorando**

A mis padres, hermanos y Ángela

Cover and back-cover explanation:

The proportional size of the surface epipelagic layer (< 200 m depth, bright blue) and the dark ocean (> 200 m depth, dark blue and black) is depicted in the cover to illustrate the greater importance of the dark ocean in terms of size despite the disproportional low number of studies done in the dark ocean as compared to the shallower waters. A DAPI image is also shown where no information is available concerning the prokaryotic function, community structure,… In the back-cover (after "shedding some light" with this Thesis) the DAPI image turns into a more specific picture where different kind of prokaryotes are painted interacting with each other, with particles,…

Explicación de la portada y contraportada:

En la portada se ha dibujado proporcionalmente el tamaño que ocupa la capa superficial epipelágica (< 200 m de profundidad, en azul claro) y el océano oscuro (> 200 m de profundidad, de azul oscuro a negro). Esta distinción se ha hecho para ilustrar la mayor importancia del océano oscuro en lo que al tamaño se refiere al compararlo con las aguas someras. También se muestra una imagen de DAPI de la que no es posible obtener información sobre la función de los procariotas, sobre la estructura de la comunidad,… En la contraportada (después de "arrojar algo de luz" con esta Tesis) la imagen de DAPI se transforma en una más específica donde se pueden distinguir distintos tipos de procariotas interactuando entre ellos, con partículas,…

AGRADECIMIENTOS / ACKNOWLEDGEMENTS

Esta tesis se ha llevado a cabo gracias a la financiación del Ministerio de Educación de una beca de Formación del Personal Universitario (AP2005-3932) en el marco del proyecto de investigación RODA (CTM 2004-06842-C03/MAR), concedido al Dr. Javier Arístegui. El trabajo de tesis se ha visto también beneficiado por campañas llevadas a cabo en el marco de los proyectos CONAFRICA (CTM2004-02319/MAR) y ARCHIMEDES (ALW-NWO; 835.20.023), este último otorgado por la División de Ciencias de la Tierra y de la Salud de la Fundación Holandesa de la Ciencia.

¡Qué alegría!....por fin aquí puedo escribir lo que me apetezca, sin necesidad de tener en cuenta la estructura general, la expresión o incluso que la cita elegida sea la correcta… Pido de antemano disculpas porque tengo tanta gente a la que agradecer que probablemente se me olvide alguien. Intentaré condensar y ser breve, dificultando aún más esta complicada tarea.

Antes que nada me gustaría agradecer a mis directores de tesis, a quienes yo considero "Los tres mosqueteros" (Fig. 1). ¡Qué suerte tuve al tener a estas tres personas como directores y como amigos!. Me gustaría empezar por Javier (o Athos). ¿Qué puedo decir?. Probablemente todo lo que diga sea poco. Ha sido él quien, desde que en el verano de tercero a cuarto de carrera, cuando me atreviera a ofrecerle colaborar, siempre me ha guiado por el "buen camino". Durante todo este tiempo me ha enseñado muchísimas cosas, no limitándose a la ciencia, sino también relacionado con la vida. Ha sido todo un ejemplo a seguir. Siempre ha confiado en mí, desde un principio, dotándome de unas herramientas valiosísimas para afrontar los desafíos que están aun por llegar. Además de todo esto (y de lo que me olvido…) hemos desarrollado una muy buena amistad, de la cual me siento muy orgulloso. Muchas gracias a mi "padre científico".

Me gustaría también agradecer mucho a Pep (o Porthos). ¡Qué grande eres Pep!...en todos los sentidos. Desde un principio siempre estuviste cuando se te necesitaba. He aprendido muchísimas cosas de ti, y muchas me gustaría también ser capaz de desarrollarlas más aún en los próximos años. Por ejemplo, tu manera, tan amena, de hacer las presentaciones…siempre soltando una coña en el momento más inesperado…Eso es precisamente algo que intentaré seguir haciendo, reduciendo esa "seriedad excesiva" (desde mi punto de vista) que parece ser inherente a este mundo de la ciencia… Intentaré seguir riéndome y disfrutando todo lo que pueda, poniendo cada cosa en su debido lugar.

Fig. 1. "Los tres mosqueteros y D'Artagnan". Imagen usada como diapositiva final en mis primeras comunicaciones orales en congresos

Y como no agradecer a Gerhard (permítanme escribir las siguientes líneas en inglés porque van dedicadas a su persona). Thank you very much Gerhard (Aramis). I could not describe precisely enough with words all the good moments we have shared together and all the great things I have learnt from you. You have been always there, at any time and for every issue. Thank you very much not only for allowing me to visit your group in Texel and in Vienna all this 4 years, and for inviting me to join the 3 ARCHIMIDES cruises, and for allowing me to give a talk in the Euroceans

Workshop, and so on… so many things to thank you that I would need the space of another full PhD to be able to finish….Thank you so much!!!. But beside all this "scientific education" you have shown me, I really appreciate (actually even more) the great friendship we have developed during all this time. I have really enjoyed a lot the thousands of tennis matches we have played (although I never won any single match), as well as the after-match's beers or smoothies. We still have a lot of places to conquer with our racquets …

Agradecer también a toda la gente de la Facultad de Ciencias del Mar, los que están y los que se han ido, por toda su ayuda y consejos. Empezando por el laboratorio B-201: desde los 2 Carlitos, Juan Carlos, Sebastien, Pierick, Pascal, Iraida, Minerva, Nandi, Iván (y Vero), Martita, Ted, May, Claire, Paula, Mar, Valeria, Ico, Inma, Gara, Federico, Igor, Natalia, Alicia, Manuela… Por supuesto agradecer también a una persona que me conoce desde que era un renacuajo: al señor Chago… del que aprendiera una frase célebre que no se me olvidará: "No somos nadie, y menos…", ¡qué bien lo pasamos en Miami!.

Me gustaría también darle las gracias a los profesores Antonio M., Pepe M., Alonso H. y a Nacho. Los cuales siempre me alegraban el día cuando me los encontraba por el pasillo y nos poníamos al día. Por cierto Pepe, muchas gracias también por los partiditos de tenis en tu casa y por las fantásticas limonadas al acabar cada partidillo.

Gracias a todos los alumnos de la Facultad de Ciencias del Mar que tuvieron que sufrirme en las prácticas de la carrera durante estos años, gracias por su paciencia y por permitírme pasármelo "pipa".

No me puedo olvidar de la gente que me acogió tan amablemente, cuando empezaba a hacer mis primeros pinitos: la gente de "Algología" (ahora conocido como Centro de Biotecnología Marina). Muchísimas gracias a Juan Luis, Antera, Emilio, Águeda, Elena, Leire, Guillermo, Sonia… por echarle una mano al pesado de Fede cada vez que le hizo falta (que era muchas veces).

Aún en la Universidad, me gustaría agradecer a Francis por estar siempre ahí, desde hace más de 6 añitos, aconsejándome y, de cuando en cuando, aguantando mis chistes malos…!viva el Bienmasabe!...Por cierto, mamá, Francis es "el de la corriente de Canarias"… Sí, Francis, así es como te conoce mi madre cuando hablamos de ti… Otro que ha tenido que sufrir demasiadas veces mis chistes ha sido Pedrito…Muchas gracias Pedrito por esos descansitos que nos tomamos para coger aire fresco cuando estamos trabajando en nuestra "oficina" (vamos, la biblioteca de la Facultad)…

No puedo olvidarme de la gente de Investigación y Tercer Ciclo de la ULPGC. Es especial Alejandro y Cristina. Muchísimas gracias por ser tan pacientes y serviciales.

También me gustaría agradecer a Laura Alonso y a María Vila, antiguos miembros del CMIMA (Barcelona) por ayudarme siempre que me hizo falta. Gracias también a Txetxu (IMEDEA) por toda la ayuda y los truquillos que me enseño cuando yo empezaba.

Muchísimas gracias también a Pere (en el IMEDEA actualmente). El señor Pere y yo coincidimos al comienzo y al final de esta andadura. Tuve el privilegio de hacer la tesina de último año de carrera con él. Y también tuve la fortuna de hacer mi última campaña con él a bordo. ¡Qué bien nos lo pasamos haciendo "ciencia" juntos!

Gracias también a los UTM (Kim, Xavi, Jose, Alberto, Dulce,...), que tanto nos ayudaron en las campañas. Muchas gracias Kim por motivarme siempre y por todas las palabras bonitas que me dices cuando nos vemos. Por cierto Xavi, que rico estaba el guacamole y la tortilla "a fuego lento" que nos preparaste en tu casa…

Agradecer a toda la gente con la que tuve el placer de compartir todas las campañas en el Atlántico: Iraida, Celso, Pingu, Jordi D., Julia, Itziar, Craig, Adam Snow, Georgina, y muchos más…Como no agradecer al grupo entero de oceanografía microbiana de Gerhard Herndl. (ahora también escribo en inglés para que me puedan entender). Thank you very much to all the people of Gerhard's lab during all these years: Taichi, Catherine, Daniele, Eva, Thomas, Dominique, Alejandra, Kristin, Simone, Adam, Christian, Michaele, Txetxu, Craig, Georgina… Thank you so much for hosting me so well every year I visited you! And also for the great times we shared together, not only in the lab but also on board the 3 cruises we shared (Fig. 2). I would also like to thank Alex Bochdansky (Old Dominion University, Virgina, USA) for being such a great guy, always laughing and with an enormous scientific vocation. By the way Alex, thank you for the original image of Fig. 2, great shot!

I would also like to thank Doug Bartlett and Emiley Eloe (from Scripps Institution of Oceanography) for all the help kindly provided to me while preparing my postdoctoral proposal.

Fig. 2. "Nos vemos en el océano oscuro", con Taichi, Daniele y Thomas. Imagen usada como diapositiva final en la últimas comunicaciones orales en congresos

Me acabo de acordar que una de esas noche mientras trabajaba en el laboratorio del Sarmiento de Gamboa pensé que debía agradecer también a Barry White, Miles Davis, Joaquín Sabina,… (entre otros) por todas esas madrugadas en las que me amenizaron la tarea durante todas las campañas.

También quisiera agradecer , fuera del ambiente laboral, a mis amigos (Kiko, Laura, Alberto, Jordi, Elena, JuanCri, David, Yeray, Elena…) y mis tíos, por todo su apoyo y por las risas compartidas.

Por último me gustaría agradecer especialmente,

A Ángela por todo lo que hemos vivido juntos durante casi una década, y que tanto ha significado para mí. Gracias por tu apoyo incondicional y tu confianza ciega en mí. Has sido la pieza clave de este engranaje, hubiese sido mucho más difícil sin ti.

A José y Julia por esta amistad tan grata y sincera que sólo crece con los años. Pocas veces he visto a alguien disfrutar tanto con un Filipino de chocolate como a D. José aquella tarde…

A Juanma por una hermosa amistad, eres parte de mi vida, no en vano has estado ahí desde que tengo uso de razón, muchas gracias por aguantarme desde la guardería,… ¡y lo que te quedan!

A mis hermanos Pepe, Roberto y Lucía por recordarme cada año la suerte que tengo de tener unos hermanos como los que tengo.

A mis padres por cuidar de mí desde siempre, por darme la oportunidad de acceder a una buena educación, por prepararme para vivir en este mundo y por echarme de menos cuando me voy.

"If I have seen further it is only by standing on the shoulders of giants."

Sir Isaac Newton

RESUMEN

Los procariotas son la piedra angular que sustenta los ciclos biogeoquímicos oceánicos. Debido a las extremas condiciones abióticas (alta presión, baja temperatura y poca disponibilidad de materia orgánica) características del océano oscuro (> 200 m de profundidad), se asumía que era un lugar donde imperaba una insignificante actividad biológica, distribuida homogéneamente. A ello se debe que la mayor parte de la investigación realizada concerniente a la ecología de los procariotas se ha limitado a las aguas superficiales, olvidando el estudio del mayor hábitat de la biosfera: el océano oscuro. En el presente estudio hemos contribuido a desgranar el papel que juegan los procariotas del océano profundo en los ciclos biogeoquímicos marinos. La distribución del pool de materia orgánica (disuelta y particulada), la estructura de la comunidad procariota, la abundancia y el metabolismo (producción heterotrófica, respiración, actividad enzimática extracelular) fueron analizados a lo largo de la columna de agua del Atlántico Norte en seis campañas. Encontramos que el océano oscuro juega un papel clave en los procesos relacionados con la remineralización del carbono (presentando a veces la misma actividad por célula que la zona epipelágica), encontrándose lejos de ser un ecosistema no activo, homogéneamente distribuido. Esa heterogeneidad encontrada en el océano oscuro parece estar controlada por un efecto “bottom-up”, donde la distribución de la materia orgánica particulada suspendida controla la actividad procariota. Esta fuerte asociación entre partículas suspendidas y procariotas del océano profundo facilitaría interacciones sinérgicas en el ciclo de la materia del océano oscuro. Por último, también encontramos que el enigmático desequilibrio existente entre el aporte de carbono orgánico al océano oscuro y la demanda de carbono de los procariotas mesopelágicos puede ser reducida en gran medida cuando se tiene en cuenta la fijación de carbono inorgánico disuelto (CID). Nuestros números indican que el hundimiento de carbono orgánico particulado sólo cubre un 4-12% de la cantidad de carbono orgánico requerido diariamente, mientras que la fijación oscura mesopelágica de CID puede aportar un 12-72% de este requerimiento procariota. Resumiendo, esta tesis contribuye a arrojar luz sobre el crucial papel que juegan los procariotas del océano oscuro en los ciclos biogeoquímicos globales, sugiriendo que los microbios del océano profundo son protagonistas esenciales en el ciclo de materia de la biosfera.

ABSTRACT

Prokaryotes are the cornerstone mediating the oceanic biogeochemical cycles. Due to the typical extreme abiotic conditions (e.g. high pressure, low temperature, low availability of organic matter) found in the dark ocean (> 200 m depth), it was generally assumed to be a site holding negligible, homogeneously-distributed biological activity. That is why most of the research done concerning the ecology of prokaryotes have been carried out in surface waters, further leaving behind the study of the largest habitat in the biosphere: the dark ocean. In this study we assessed the actual role of the deep-sea prokaryotes in the marine biogeochemical cycles. The distribution of the organic matter pool (dissolved and particulate), the prokaryotic assemblage structure, abundance and metabolism (heterotrophic production, respiration, extracellular enzymatic activity) were analyzed along the water column of the North Atlantic, in six different research cruises. We found that the dark ocean plays a key role in the carbon mineralization processes (sometimes being, on a per-cell level, as active as the epipelagic waters), being far from a homogenously-distributed non-active ecosystem. The heterogeneity found in the dark ocean seemed to be controlled by a "bottom-up" effect, where the suspended particulate organic matter distribution modulates the prokaryotic activity. This stronger association between suspended particles and deep-sea prokaryotes than assumed hitherto, would facilitate synergistic interactions in the cycling of matter in the dark ocean. Finally, we also found that the enigmatic imbalance between the organic carbon supply to the dark ocean and the mesopelagic prokaryotic carbon demand could be greatly reduced when taking into account the dissolved inorganic carbon (DIC) fixation. Our numbers indicates that the sinking particulate organic carbon could only account for 4-12% of the daily-required organic carbon, while the mesopelagic dark fixation of DIC can supply 12% to 72% of this prokaryotic carbon demand. Overall, this thesis contributes to shed light on the actual paramount role that dark ocean's prokaryotes play in the global biogeochemical cycles, suggesting that the deep-sea microbes are principal characters in the material cycling of the biosphere.

PRESENTACIÓN DE LA TESIS

La presente tesis titulada *Arrojando luz sobre el papel de los procariotas en los ciclos biogeoquímicos del oceáno oscuro,* resulta de la recopilación de una serie de trabajos encuadrados dentro de los proyectos de investigación RODA (CTM 2004-06842-C03/MAR), ARCHIMEDES (ALW-NWO; 835.20.023) y CONAFRICA (CTM2004-02319/MAR), dirigidos los doctores Javier Arístegui, Gerhard Herndl y Santiago Hernández León, respectivamente. El Dr. Arístegui (Universidad de Las Palmas de Gran Canaria) ha dirigido esta tesis en co-dirección con el Dr. Gerhard Herndl (Universidad de Viena) y el Dr. Josep Gasol Piqué (Instituto de Ciencias del Mar-CSIC de Barcelona).

Esta tesis consta de una primera parte realizada íntegramente en inglés, estructurada en Introducción, Objetivos, Contribuciones originales, Síntesis de resultados y Discusión General, Conclusiones y Líneas futuras de investigación. De este modo, el resumen y las conclusiones de la tesis se encuentran en una lengua distinta a la lengua Española (Inglés), de acuerdo a la normativa para la obtención de la Mención europea del Título de Doctor (BOULPGC. Art.1 Cap. 4, 5 de noviembre 2008).

La segunda parte de la tesis está escrita en castellano y, por lo tanto, consta de las 50 páginas en castellano requeridas por el Reglamento de Elaboración, Tribunal, Defensa y Evaluación de Tesis Doctorales de la Universidad de Las Palmas de Gran Canaria (BOULPGC. Art.2 Cap.1, 5 de noviembre 2008). Además, sigue la estructura exigida por este Reglamento: Introducción, Objetivos, Planteamiento y Metodología, Resultados, Discusión General, Conclusiones y Futuras Líneas de Investigación.

THESIS PREVIEW

This thesis entitled *Shedding light on the role of the prokaryotic assemblage in the biogeochemical cycles of the dark ocean* compiles different studies carried out in the frame of the research projects RODA (CTM 2004-06842-C03/MAR), ARCHIMEDES (ALW-NWO; 835.20.023) and CONAFRICA (CTM2004-02319/MAR). These projects were granted to Dr. Javier Arístegui, Dr. Gerhard Herndl and Dr. Santiago Hernández León, respectively. Dr. Arístegui (Universidad de Las Palmas de Gran Canaria) has co-supervised this thesis together with Dr. Gerhard Herndl (University of Viena) and Dr. Josep Gasol Piqué (Instituto de Ciencias del Mar-CSIC, Barcelona).

This thesis is structured into a general introduction that explains the role of prokaryotes in the oceanic ecosystems, their main carbon sources, and introduces the main questions that are the focus of the present thesis against the actual background of each topic. Then, the original contributions are compiled in manuscript format and are at different stages in the peer-review publication process. Afterwards, there is a synthesis of results and general discussion section, which precedes conclusions. Finally, further research studies on the microbial ecology of the dark ocean are suggested.

At the end, a summary in Spanish is included, containing more than 50 pages. This is a requirement from the PhD Thesis Regulations from the Universidad de Las Palmas de Gran Canaria (BOULPGC. Art.2 Chap.1, November 5th 2008). Besides, in order to obtain the Doctor Europeus Mention (BOULPGC. Art.1 Chap.4, November 5th 2008), the Summary and Conclusions have been translated into English.

"We have widely explored the shallow layer of the ocean, but only some robots and a handful of men, inside especial immersion vehicles, have been in the deep waters. If we compare these occasional incursions with needles, then we would have to imagine a haystack of the size of our planet. It is like if some extraterrestrial organisms would bring down to the deep ocean some cameras that could just cover a few meters. One of these cameras films a piece of the Mongolia's desert, and the other ones from the Kalahari and the Antarctica. Another one reaches to a big city, for instance the New York's Central Park, where it films a couple of square meters of green grass and a dog peeing beside a tree. What would then be the conclusion that the extraterrestrial organisms would obtain? That this is an inhabited planet where it is possible to find sporadically primitive living forms."

Frank Schätzing, *The Swarm* (2004)

INDICE / CONTENTS

INTRODUCTION

GENERAL INTRODUCTION

"Bacteria represent the world's greatest success story. They are today and have always been the modal organisms on earth; they cannot be nuked to oblivion and will outlive us all. This time is their time, not the "age of mammals" as our textbooks chauvinistically proclaim. But their price for such success is permanent relegation to a microworld, and they cannot know the joy and pain of consciousness. We live in a universe of trade-offs; complexity and persistence do not work well as partners."

Stephen Jay Gould, "An Earful of Jaw", *Eight Little Piggies: Reflections in Natural History* (1993)

Role of prokaryotes in the oceanic ecosystem

Prokaryotes play a key ecological role in the oceans, mediating most of the biogeochemical cycles and acting as a trophic link through the microbial loop (Pomeroy 1974, Williams 1981, Azam et al. 1983). They are the main responsible group of organisms for converting dissolved organic matter (DOM) into living particulate organic matter (POM); therefore supplying organic matter to higher trophic levels of the food web, which would otherwise not be accessible. This 'microbial loop' concept has been refined during the last decades (Azam 1998, Fuhrman 1999), incorporating among others, the ideas that prokaryotes might compete with eukaryotic phytoplankton for inorganic nutrients (Obernosterer & Herndl 1995, Thingstad 2000), consume half or more of primary production (Ducklow 2000, Robinson 2008) and are in turn lysed by virus (Fuhrman 2000, Breitbart et al. 2008) and consumed by protists (Jurgens & Massana 2008). Therefore, nowadays it is accepted that the microbial food web exerts a major influence on flux patterns of carbon and nutrients in the ocean (Nagata 2008). This notion was based on a series of technical advances.

Initially, the number of prokaryotes in marine systems were severely underestimated. First estimates of prokaryotic abundance were based on the number of

cells able to grow on agar plates (e.g. Zobell 1946); a technique that was later shown to underestimate the in situ abundance of prokaryotes by several orders of magnitude (Jannasch & Jones 1959). In the 1970s, after the development of epifluorescence microscope techniques, it was found that the abundance of prokaryotes in surface waters is in the range of 10^5 - 10^6 ml^{-1} (Francisco et al. 1973, Hobbie et al. 1977, Porter & Feig 1980). This discrepancy between plate and microscopic counts was later coined the "Great plate count anomaly" (Staley & Konopka 1985). Moreover, most of the findings achieved concerning the microbial loop were made using a black-box approach, disregarding the phylogenetic and functional groups within the individual components of the microbial community. Again, the development of techniques (this time molecular ones) in the early 1990s allowed us to begin to answer questions like who is doing what in the ocean.

Due to this key ecological role played by prokaryotes in the ocean, responsible for up to >90% of the community respiration (Robinson & Williams 2005), the estimation of their metabolic activities is imperative if we want to constrain carbon fluxes. In fact, the amount of organic carbon finally flowing through prokaryotes to higher trophic levels and/or the organic carbon concentration respired in the ocean greatly depend on the prokaryotic growth efficiency (PGE —the proportion of the organic carbon incorporated into biomass (production) compared to the total organic carbon uptake (production plus respiration) (Carlson et al. 2007). However, obtaining the metabolic rates involved in the PGE assessment is not simple. Due to the difficulties related to the prokaryotic respiration (PR) measurement, PGE is frequently calculated by measuring prokaryotic heterotrophic production (PHP) via leucine or thymidine incorporation applying an empirically obtained conversion factor to convert the leucine or thymidine incorporation rates into C production (del Giorgio & Cole 1998). However, this leucine to carbon conversion factor (LeuCF) is not constant and may vary by one order of magnitude within the same system (e.g. Sherry et al. 2002, Alonso-Sáez et al. 2007). Consequently, it is generally recommended to estimate empirical LeuCFs to better constrain carbon fluxes (Bell 1990, Pulido-Villena & Reche 2003, Buesing & Marxsen 2005, Calvo-Díaz & Morán 2009). Overall, the efficiency of channeling DOM into either biomass production or remineralization will depend on the quality of the organic matter and the stoichiometric balance between available carbon, nitrogen and

phosphorus (Goldman et al, 1987, Obernosterer & Herndl 1995, del Giorgio & Cole 1998, 2000). Therefore, to better understand and predict the effect of the prokaryotic metabolic variability on the ecosystem, it is necessary to relate this metabolic variability to variations in environmental (e.g. organic matter pool) and assemblage composition, trying to arrive at a mechanistic understanding of the microbial loop in the ocean.

Main carbon sources for prokaryotes in the ocean

The organic matter pool in the ocean is divided into two functionally separated groups (dissolved and particulate), although they represent interconnected pools (Simon et al. 2002, Engel et al. 2004, Verdugo et al. 2004). It is considered particulate organic matter (POM) when it is retained on filters with a pore size of 0.2-0.7 μm. Within the POM pool, there are two kinds of particles distinguished by their sinking properties: sinking and buoyant particles. The bulk of POM is commonly formed by small, neutrally buoyant, suspended particles (Kepkay 2000, Verdugo et al. 2004). These suspended particles are generally more labile than sinking particles collected at comparable depths (Repeta & Gagosian 1984, Wakeham & Canuel 1988, Druffel et al. 1998). Moreover, large gels (i.e., several hundreds of μm or larger) may form from the assemblage of macromolecules or colloidal material, transforming organic molecules up the particle size spectrum to sinking particles (Verdugo et al. 2004, Engel et al. 2004). Transparent exopolymer particles (TEP; Alldredge et al. 1993, Passow 2002) and other macro-gels are mainly responsible for the formation of large sinking aggregates > 500 μm ("marine snow") (Suzuki & Kato 1953, Alldredge & Silver 1988, Alldredge & Gotschalk 1988, Thornton 2002). These large particles have been shown to harbor complex microenvironments supporting a particularly active microbial community (Alldredge et al. 1986, Kaltenböck & Herndl 1992, Müller-Niklas et al. 1994).

DOM is mainly generated by phytoplankton extracellular release and grazing processes (and viral infections) either directly on phytoplankton ('sloppy feeding') or within the microbial food web (Nagata 2000). Also, heterotrophic prokaryotes release copious amounts of DOM via their polysaccharidic envelope surrounding the cells (Heissenberger & Herndl, 1994; Heissenberger et al., 1996; Stoderegger & Herndl,

1998). However, not all the DOM is accessible to prokaryotes since it is generally composed of a mixture of very old refractory DOM and a smaller fraction of young bioavailable DOM (mainly mono- and polysaccharides, proteins, peptides and free aminoacids) (Benner 2002). Most of the grazing- and virus-related DOM release is in the form of high molecular weight DOM (HMW-DOM, >1000 Da) and also senescent phytoplankton release mainly HMW-DOM (Lignell 1990). The concentration of the free sugars and amino acids is generally low (<10% of total organic carbon), while combined forms (i.e., as oligomers and polymers) are more common. Moreover, prokaryotes preferentially utilize HMW-DOM, due to its higher bioreactivity compared to the bulk of refractory low-molecular-weight DOM (Amon & Benner 1994, Benner 2002). However, prokaryotes are only able to directly assimilate low-molecular-weight compounds (i.e. mono- and oligomers), to use HMW-substrates, they require substrate cleavage prior to uptake, since only molecules <600 Da can be transported through the prokaryotic cell wall (Weiss et al., 1991). Hoppe et al. (2002) showed that the ectoenzymatic hydrolysis of DOM is tightly linked to the uptake of the resulting oligo- or monomers. Consequently, the measurement of the kind and quantity of prokaryotic extracellular enzymatic activities (EEA) in the ocean is a valuable proxy for substrate utilization and to estimate the regulation of the carbon flux through prokaryotes.

Prokaryotes in the dark ocean: the largest but least studied habitat of the biosphere

Despite being the least understood aquatic habitat on the planet, the dark ocean (>200 m depth) is a "record holder" within the habitats on Earth. It is the largest habitat (~ 1.3×10^{18} m^3) and the major reservoir of active organic carbon (mainly in the form of dissolved organic carbon) in the biosphere (Libes 1992, Hansell & Carlson 1998, Benner 2002), containing also more than 98% of the global dissolved inorganic carbon (DIC) pool (Gruber et al., 2004). The deep ocean also contains the largest pool of microbes in aquatic systems (Whitman et al. 1998), harboring nearly 75% and 50% of the prokaryotic biomass and production, respectively, of the global ocean (see Arístegui et al. 2009 for a review).

However, generally prokaryotic abundance (PA) and biomass production decline exponentially with depth by about 2 and 3 orders of magnitude (Reinthaler et al. 2006, Arístegui et al. 2009). Despite the mentioned decrease in PA with depth, prokaryotic richness decreases only about 30% from the epipelagic to the bathypelagic layers (Moeseneder et al. 2001a, Hewson et al. 2006). Another common feature of deep-ocean prokaryotes is the distinct stratification of populations found in different water masses (DeLong et al. 2006); attributed to changes in the organic matter field with depth (Moeseneder et al. 2001a, DeLong et al. 2006), and to adaptations to pressure and temperature (Vezzi et al. 2005, Lauro et al. 2006). The relative contribution of Bacteria to total PA decreases, concomitantly those of the Crenarchaea increases with depth (Karner et al. 2001, Moeseneder 2001b, Teira et al. 2006). There is growing evidence that a substantial fraction of the Crenarchaea uses CO_2 as a carbon source and ammonia as an energy donor through ammonia oxidation (Herndl et al. 2005, Könneke et al. 2005, Ingalls et al. 2006, Wutcher et al. 2006). However, the bathypelagic crenarchaeal community seems to be basically heterotrophic (Teira et al. 2006, Kirchman et al. 2007). Nevertheless, the CO_2 fixation carried out by the deep-ocean Crenarchaea has been estimated to be about 1 mmol C m^{-2} d^{-1} in the North Atlantic (Herndl et al. 2005). This is a substantial supplement to the carbon flux of organic matter originating from the surface waters, providing freshly produced organic matter of higher nutritional value than the refractory deep-water organic matter. Consequently, this dark CO_2 fixation has been considered as the "primary production of the dark ocean" (Herndl et al. 2008). But, *what is the role of this new source of deep-ocean organic matter in the dark ocean food web?*

It is generally assumed that the DOM pool is not driving the metabolism of the dark-ocean prokaryotes. The dissolved fraction of the organic matter becomes increasingly refractory with depth, leading to a successively increasing DOC:DON:DOP ratio (Benner 2002). Therefore, the bulk of the DOM pool of the dark ocean is supposed to be carbon-rich low-molecular-weight degradation products from the remineralization of organic matter (Benner 2002). In fact, although the majority of the deep-ocean DOM is still not characterized at a molecular level, about 25% of the detrital

carbon pool (dissolved and particulate), and about 50% of the detrital nitrogen pool of the deep-water DOM, have been shown to be of prokaryotic origin (Kaiser & Benner 2008). Hence, *how does the change in the quality and quantity of the organic matter (OM) with depth affect the metabolism (respiration, production, EEA, PGE) of deep-ocean prokaryotes? Do these differences in the OM composition generate variations in the carbon to leucine (LeuCF) conversion factor used to estimate heterotrophic prokaryotic biomass production?*

Despite the refractory nature of the deep-ocean DOM, more than 95% of the prokaryotes are reported to thrive as free-living organisms in the dark ocean while the remaining fraction is thought to be associated with suspended and sinking particles (Cho & Azam 1988, Turley & Mackie 1994). However, recent environmental genomic data challenge this "free-living" hypothesis and suggest a preferentially particle-attached way of life for dark-ocean prokaryotes (López-López et al. 2005, DeLong et al. 2006, Martín-Cuadrado et al. 2007). Nevertheless, the quantification of this particulate material is far from trivial, probably because we disrupt part of this colloidal material during sampling with the routinely used techniques. Direct evidence is needed to substantiate these indications of a predominately particle-associated prokaryotic life style in the dark ocean. *Is there a direct relation between suspended particles and the prokaryotic metabolism in the bathypelagic ocean?*

It is accepted now that the source of labile dissolved organic matter required to support dark-ocean prokaryotic metabolism is not derived from exported surface water DOM, but originates from transformation and solubilization of particles. Arístegui et al. (2002) estimated that resident DOC could account for only 10-20% of the global apparent oxygen utilization in the dark ocean. Karl et al. (1988) postulated the "particle-decomposition" paradox based on the notion that particles-associated prokaryotes solubilize a larger amount of POC than actually required to support the observed growth and hence, releasing DOM into the ambient water. In agreement to this, Cho & Azam (1988) and Smith et al. (1992) reported that marine-snow prokaryotes express high EEA, transforming POM into DOM at a higher rate than that of substrate uptake. This uncoupling of solubilization and uptake has been postulated

to be a key biochemical mechanism for a large-scale transfer of sinking POM to the dissolved phase (Smith et al. 1992). Therefore, the EEA is again a key player, as the step in between, "connecting" the organic matter pool with prokaryotic metabolism in the dark ocean. Taking into account the tendency of particle-associated prokaryotes to release extracellular enzymes into the surrounding (Smith et al. 1992), one might suggest that finding a high proportion of dissolved EEA would be indicative of a possible particle-attached way of life. Therefore the question can be raised: *Is there a high proportion of free EEA in the dark ocean? Is this dissolved EEA related to the suspended POM pool?*

Little is known about how deep-ocean prokaryotes respond to the shifts in the composition, distribution and supply of organic carbon (Azam 1998). It is accepted that most of organic carbon is generated in the euphotic zone and transported downward, although most (>90% of the export flux) is respired in the mesopelagic (Arístegui et al. 2005a). In fact, one third of the oceanic biological respiration of OM takes place in the dark ocean (del Giorgio & Duarte 2002, Arístegui et al 2005a). However, compiled global budgets and intensive local field studies suggest that estimates of metabolic activity (prokaryotic carbon demand) in the dark ocean exceed the influx of organic substrate (generally calculated as the sinking POC flux) (Carlson et al. 1994, Reinthaler et al. 2006, Steinberg et al. 2008). This imbalance indicates either an overestimation of the metabolic activity of the deep-ocean prokaryotes or the existence of unaccounted sources of organic carbon (Arístegui et al. 2005a, Burd et al. in press). The high abundance of suspended POC in the ocean (Kepkay 2000, Verdugo 2004) has generally been neglected in the carbon budget calculations (Arístegui et al. 2005a). *Can then the suspended POC and/or the dark CO_2 fixation close the gap between the prokaryotic carbon demand and the organic matter supply in the dark ocean?*

Despite the growing evidence that the dark ocean plays a central role in ocean's biogeochemistry and holds a unique reservoir of high genetic and metabolic microbial diversity (see review of Arístegui et al. 2009), the deep-ocean has been much less studied than the surface waters. This is basically due to the difficulty related to the deep-ocean sampling (time and expenses involved) and to the fact that, until recently,

the dark ocean was supposed to be a place with only insignificant biological activity. Only over the last two decades the notion emerged of the deep ocean as a site harboring a diverse and active community of prokaryotes (Bacteria and Archaea) (Karner et al. 2001, López-García 2001, Kirchman et al. 2007). For a long time the general perception of the dark ocean as a homogenous system with a rather stable prokaryotic assemblage and low activity was accepted as common knowledge. But, *is the dark ocean prokaryotic activity and assemblage composition really homogeneously distributed?*

AIM & OUTLINE OF THESIS OUTLINE

The aim of this thesis work was to respond to several of the questions and uncertainties raised in the General Introduction. Basically they can be summarized by the following questions:

1. What is the variability in depth-gradients of prokaryotic activity and assemblage composition in the dark ocean? **Chapter I**.

2. Are these gradients related to variations in the organic matter pool of the dark ocean? If so, would this deep-ocean "bottom-up" control have an influence on the global carbon balance? **Chapter II**.

3. Is there significant hydrolysis of organic matter in the dark ocean? How does the variability of the quality and quantity of the organic matter affect the prokaryotic metabolism (PHP, PR, EEA)? **Chapter III**.

4. Are those prokaryotic enzymes preferentially attached to the cell wall or are they actively released, and what would be the consequences of this? **Chapter IV**.

5. Do mesoscale processes (e.g. island-induced eddies) affect the variability in the diversity and function of prokaryotes? If so, would this influence translate to the dark ocean's assemblages? **Chapter V**.

6. Are deep-ocean carbon-to-leucine conversion factors different to those found in surface-waters? If so, is the flux of carbon channeled through deep-ocean prokaryotes comparable to what is found in the epipelagic layer? **Chapter VI**.

To answer the first question, a study of the distribution of prokaryotic abundance (PA), activity and assemblage composition of the major prokaryotic groups (marine *Crenarchaeota* Group I, marine *Euryarchaeota* Group II, SAR 11, *Roseobacter*, *Gammaproteobacteria* and *Bacteroidetes*) was conducted from surface

layers to 2000 m depth. This research was carried out following a transect along a coastal transition zone (from the NW African upwelling to the offshore waters of the Canary Islands region) where a trophic gradient was expected, at least in surface waters (**Chapter I**).

To address the second question, more than 9000 km were covered in the (sub)tropical North Atlantic analyzing regional gradients and depth distribution of the organic matter pools (dissolved and particulate) and the microbial metabolism (prokaryotic heterotrophic production [PHP] and respiration [PR]) in the core of the main water mass (from the surface mixed layer to 4500 m depth) (**Chapter II**). Also we performed a comparison between the prokaryotic carbon demand and the calculated supply of sinking particulate organic carbon.

To study the relation between the prokaryotic metabolism and the extracellular enzymatic activity (EEA) in the dark ocean, the PA, PHP, PR and the EEA of two glycolytic enzymes (α-glucosidase and β-glucosidase), one proteolytic enzyme (leucine aminopeptidase) and alkaline phosphatase were measured in the meso- and bathypelagic realm of the (sub)tropical Atlantic (**Chapter III**).

In order to account for the proportion of free EEA in the water column, the dynamics of both total and dissolved EEA across the Central Atlantic were investigated from the lower euphotic zone to the bathy- and abyssopelagic layers (to 7000 m depth). To further relate the dissolved and total EEA to the particulate organic matter pool, the PA, PHP, particulate organic carbon and nitrogen were also measured (**Chapter IV**).

The effect of four island-induced eddies (2 cyclonic and 2 anticyclonic) in the diversity and function of prokaryotes were analyzed in the eddy field downstream the Canary Islands. Abundance, relative nucleic acid content, viability, bulk and single-cell activities and prokaryotic community structure were measured to investigate whether the possible effects on diversity and function generated in the surface waters translate down to the meso- and bathypelagic prokaryotic assemblages underneath the eddies (**Chapter V**).

Thirteen experiments were conducted in the mesopelagic zone of the subtropical Northeast Atlantic to study how the different abiotic and biotic deep-water conditions affect prokaryotic growth efficiencies (PGE) and the leucine-to-carbon conversion factor (LeuCF), as well as to check how the LeuCF influences the estimates of the actual deep-water PGE (**Chapter VI**).

METHODOLOGY

A wide range of methodologies has been applied to reach the objectives of this Thesis; covering from molecular biology and ecology to marine biogeochemistry techniques. The particular methodology used in each study within this thesis is described in rigourous detail on the respective chapter. The main sampling and experimental methodologies utilized are briefly explained in this section.

Samples were obtained on board oceanographic research vessels, using a rosette sampler equipped with twenty-four 10-12 l Niskin or NOEX bottles. Mounted on this rosette a SeaBird 911 plus CTD system allowed us to estimate the temperature, salinity and fluorescence throughout the water column. After obtaining the samples different procedures were followed depending on the purpose of each study.

Prokaryotic abundance (PA) was determined by flow cytometry using a FACSCalibur (Becton Dickinson) with a laser emitting at 488 nm wavelength. Prokaryotes were identified by their signatures in a plot of side scatter (SSC) versus green fluorescence (FL1). Beside, **high and low nucleic acid cells (H-NA** and **L-NA** respectively**)** were then separated in the scatter plot of SSC-FL1 attending to Gasol et al. (1999).

Viable and damaged prokaryotic cells were estimated in non-fixed samples following the **nucleic acid double-staining (NADS)** protocol (Gregori et al. 2001, Falcioni et al. 2008).

Prokaryotic heterotrophic activity (PHA) was estimated from the rates of protein synthesis determined by the incorporation of tritiated leucine (Kirchman et al. 1985). **Prokaryotic heterotrophic production (PHP)** was computed from PHA rates, using the conservative theoretical conversion factor of 1.55 kg C mol^{-1} Leu incorporated, assuming no internal isotope dilution (Kirchman & Ducklow 1993).

Prokaryotic respiration (PR) was measured by following the changes in dissolved oxygen concentrations during dark incubations in biological oxygen demand (BOD) bottles. This was done by automated Winkler titrations on the basis of colorimetric end-point detection as described in Arístegui et al. (2005b). Respiration

was also measured via the respiratory activity **of the electron transport system (ETS)** following the modifications of the tetrazolium reduction technique as described in Arístegui & Montero (1995).

Particulate organic carbon (POC) and organic nitrogen (PON) analyses were performed on a Perkin-Elmer 2400 CHN (carbon, hydrogen, nitrogen) elemental analyzer according to the JGOFS (Joint Global Ocean Flux Study) protocol (UNESCO 1994).

Total organic carbon (TOC) analysis was performed using the high temperature combustion method on a modified Shimadzu TOC-5000A. The absorbance of the CO_2 evolving from the combusted TOC was detected with an external infrared cell (LiCor Model LI-6252) and quantified as peak area by the Shimadzu integrator (Benner & Strom 1993).

Dissolved organic nitrogen (DON) analyses were performed according to the method of Valderrama (1981) using an autoanalyzer (TRAACS).

Prokaryotic **extracellular enzymatic activity (EEA)** α-, β-glucosidase, aminopeptidase and alkaline phosphatase potential rates were estimated analyzing the hydrolysis of the fluorogenic substrate analogs 4-methylcoumarinyl-7-amide (MCA)-L-leucine-7-amido-4-methylcoumarin, 4-methylumbelliferyl (MUF)-α-D-glucoside, 4-MUF-β-D-glucoside and MUF-phosphate (Hoppe 1983). The fluorescence resulting from the cleavage of MUF or MCA and was detected spectrofluorometrically using a Fluorolog-3 fluorometer with a MicroMax 384 microwell plate reader (Horiba) at an excitation and emission wavelength of 365 nm and 445 nm, respectively.

Fluorescence in situ hybridization and catalyzed reporter deposition (CARD-FISH), and **MICRO-CARD-FISH (CARD-FISH** combined with **micro-autoradiography)** for the identification of marine *Bacteria* and *Archaea* was done hybridizing with horseradish peroxidase (HRP)-labeled oligonucleotide probes, and tyramide-Alexa488 for signal amplification, following the protocol described in Teira et al. (2004)

For **DNA fingerprinting** of prokaryotic communities (both *Bacteria* and *Archaea*), DNA extraction was performed using the UltraClean Soil DNA Isolation Kit MoBio kit (MoBIO laboratories, Carlsbad, CA, USA) and the protocol of the

manufacturer. **Terminal-restriction fragment length polymorphism (T-RFLP)** was done as described in Moeseneder et al. (2001b). **Automated ribosomal intergenic spacer analysis (ARISA)** was done according to Fisher & Triplett (1999) and Hewson & Fuhrman (2004). Both fingerprinting techniques (T-RFLP and ARISA) were deployed using an ABI Prism 310 capillary sequencer (Applied Biosystem) and the resulting electropherograms were analyzed using the ABI Genescan software. The output from the ABI Genescan software was transferred to the Fingerprinting II (Bio-Rad) software to determine peak area and for standardization using size markers. The obtained matrix was further analyzed with the Primer software (Primer-E) to determine similarities of the fingerprints between samples.

Chapter I

Strong coast–ocean and surface–depth gradients in prokaryotic assemblage structure and activity in a coastal transition zone region

F. Baltar, J. Arístegui, J. M. Gasol, S. Hernández-León, G. J. Herndl

Aquatic Microbial Ecology 50, 63-74 (2007)

Abstract

The distribution of marine *Crenarchaeota* Group I, marine *Euryarchaeota* Group II and some major groups of *Bacteria* (SAR 11, *Roseobacter*, *Gammaproteobacteria* and *Bacteroidetes*) was investigated in the North Atlantic water column (surface to 2000 m depth) along a transect from the coastal waters of the NW African upwelling to the offshore waters of the Canary Coastal Transition Zone (CTZ). Catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH) was used to describe the prokaryotic assemblages. Bulk picoplankton abundance and leucine incorporation were determined. Pronounced changes in prokaryotic assemblage composition were observed from the coast to the open ocean and at the deep chlorophyll maximum (DCM) with decreasing bulk heterotrophic activity. All bacterial groups decreased in absolute abundances from the coast to the open ocean; both archaeal groups increased towards the open ocean. Prokaryotic abundance and activity decreased 2 and 3 orders of magnitude, respectively, from the surface to 2000 m. Prokaryotic growth rates were high in the mesopelagic zone (~0.13 d^{-1}). SAR11 in total picoplankton abundance decreased from 42% in the DCM to 4% at 2000 m, while marine *Crenarchaeota* Group I increased from 1% in the DCM to 39% in the oxygen minimum layer. A clear influence of the different intermediate water masses was observed on the bulk heterotrophic picoplankton activity, with lower leucine incorporation rates corresponding to layers where patches of Antarctic Intermediate Water were detected. Coast–ocean and surface–depth gradients in bulk prokaryotic abundance and production and assemblage composition were comparable to changes observed in basin-scale studies, pinpointing the CTZs as regions of strong variability in microbial diversity and metabolism.

Introduction

Over the past few decades it has become clear that prokaryotes are an important component of marine plankton communities, playing a key role in mediating a range of biogeochemical cycles (Azam et al. 1983). Prokaryotic plankton has been enumerated in a 'black-box' approach, firmly establishing that they represent the largest pool of living biomass in the sea (Gasol et al. 1997). With the advent of molecular biology techniques,

marine microbiologists have been able to analyze the prokaryotic communities from a 'Gleasonian' point of view (Gleason 1926), focusing on distinct prokaryotic groups that can be reliably identified and quantified in marine assemblages.

Small-subunit ribosomal RNA (rRNA) genes, analyzed by molecular techniques, have become the universal phylogenetic markers and the main criteria by which microbial plankton groups are identified (Giovannoni & Rappé 2000). Most of these groups were first identified by sequencing 16S rRNA genes cloned from seawater (e.g. Giovannoni et al. 1990). It soon became apparent that <20 microbial clades accounted for most of the genes recovered (Mullins et al. 1995). Nevertheless, 16S rRNA gene-clone libraries do not accurately reflect the abundance of microbes from particular phylogenetic clades in the environment. Such analyses need to be complemented by other strategies aimed at studying single populations *in situ*. Hybridization techniques such as catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH; Pernthaler et al. 2002) became an indispensable tool in determining the abundance of specific prokaryotic groups in the natural environment. Indeed, some groups of marine bacteria had been known for years from their 16S rRNA gene sequences before their abundances could be determined. Paradigmatic examples are bacteria related to the marine SAR11 (Morris et al. 2002), SAR86 (Pernthaler et al. 2002), SAR116 (Fuchs et al. 2005), SAR202 (Morris et al. 2004), or the SAR406 (Fuchs et al. 2005) clades.

The presence of planktonic prokaryotes in a given oceanic habitat is thought to be determined by local environmental conditions and not by restricted dispersal (Pedrós-Alió 2006). Members of the SAR11 clade are among the most common prokaryotes in the marine plankton. They can contribute >50% to the total picoplankton abundance in the surface waters of the northwestern Sargasso Sea, and 25% of mesopelagic picoplankton assemblages (Morris et al. 2002). *Bacteria* related to *Roseobacter*, also referred to as the SAR83 cluster (Rappe et al. 2000), are another common component of coastal and offshore picoplankton assemblages constituting up to 25% of marine picoplankton (González & Moran 1997, Eilers et al. 2001). The seasonal dynamics in the abundance of *Roseobacter* closely follows the development of phytoplankton biomass in the North Sea (Eilers et al. 2001). Furthermore, it has been shown that members of these 2 clades (SAR11 and *Roseobacter*) efficiently utilize dimethylsulfoniopropionate (Malmstrom et al. 2004a,b). The *Bacteroidetes* cluster has been associated with upwelling events (Fandino et al. 2001, Alonso-Sáez et al. 2007a), and members show a high ability to degrade high-molecular-weight compounds

(Cottrell & Kirchman 2000). Members of the SAR86 lineage, related to the *Gammaproteobacteria*, are characterized by high growth rates, probably efficiently exploiting nutrient pulses (Eilers et al. 2000).

Archaea are widespread among marine prokaryotic plankton (DeLong et al. 1994, Massana et al. 1998). Quantitative studies using FISH have shown the dominance of *Crenarchaeota* in meso- and bathypelagic waters, whereas *Euryarchaeota* were thought to comprise less than 5 to 8% of total picoplankton abundance in deep waters of the Pacific and the Antarctic Oceans (Karner et al. 2001, Church et al. 2003). Using CARD-FISH, in combination with the Proteinase-K permeabilization of the archaeal cell wall, Teira et al. (2004, 2006a,b) and Herndl et al. (2005) found higher (10 to 20%) abundances of Euryarchaeota in deep waters of the North Atlantic Ocean than reported by Karner et al. (2001) for the subtropical North Pacific Gyres. All these studies support the view that diverse prokaryotic groups are linked to different environmental conditions and, hence, changes in assemblage structure are likely to be more pronounced in regions with strong oceanographic gradients.

Most of the studies describing variability in the composition of picoplankton have been carried out in surface oceanic waters (e.g. Alonso-Sáez et al. 2007a). Only a few have quantified the different groups of prokaryotes in the dark ocean. Among these, some studies used PCR-based fingerprinting techniques (Hewson et al. 2006) to identify components of the prokaryotic community, while others applied PCR-independent group-level FISH. The latter, however, have used 2 or 3 prokaryotic probes targeting *Bacteria*, *Crenarchaeota* and *Euryarchaeota* (Karner et al. 2001, Teira et al. 2004, 2006b, Herndl et al. 2005). Information regarding the activity of deep-water prokaryotic communities is rather scarce compared to that of surface-water prokaryotic assemblages (Reinthaler et al. 2006). Measurements on depressurized samples indicate a decrease in picoplankton production of 1 to 2 orders of magnitude from the euphotic zone to the bathypelagic waters (Reinthaler et al. 2006), declining with depth slightly more than picoplankton abundance.

Variations in bulk prokaryotic activity may be related to changes in the abundance of specific groups. Some studies have shown a clear link between the distribution of the major prokaryotic groups to bulk (Massana et al. 1998) and group-specific prokaryotic activity in the dark ocean (Teira et al. 2004, 2006b, Herndl et al. 2005). This relationship might be more evident in regions with strong environmental gradients and relatively high picoplankton activity. Arístegui et al. (2005) measured

high prokaryotic activities in the mesopelagic waters of the subtropical NE Atlantic, a region characterized by strong hydrological variability, with most pronounced gradients close to the NW African coastal upwelling (Arístegui et al. 2003).

In the present study, we report the distribution of both the bulk prokaryotic activity and the abundance of major prokaryotic groups (marine *Crenarchaeota* Group I, marine *Euryarchaeota* Group II, SAR 11, *Roseobacter*, *Gammaproteobacteria* and *Bacteroidetes*) along a transect from the coastal waters of the NW African upwelling to the offshore waters of the Canary region. We used CARD-FISH to determine the composition of the prokaryotic community and relate it to bulk leucine incorporation. We hypothesized that the contribution of marine Crenarchaeota Group I and marine Euryarchaeota Group II to total picoplankton abundance increases along the coastal-ocean transect in the mesopelagic waters and, overall, that the surface-depth patterns of abundance and metabolism of Bacteria and Archaea are related to local hydrographic conditions.

Materials and methods

Location and sampling

A zonal section of 10 stations was sampled from the coastal waters of the NW Africa upwelling (27.9°N, 13.1°W) to the offshore waters of the Canary Coastal Transition Zone (CTZ) region (27.5° N, 15.3° W) (Fig. 1) during the CONAFRICA cruise (22 March to 7 April 2006) on board the RV 'Hespérides'. At each station, temperature, salinity and fluorescence were recorded down to 2000 m depth using a SeaBird 911 plus CTD system, mounted on a General Oceanics rosette sampler, equipped with twenty-four 12 l Niskin bottles. Samples for prokaryotic heterotrophic production and abundance were collected at each station from 5 depths ranging from 5 to 2000 m, including the deep chlorophyll maximum (DCM; 30 to 82 m), 200 m and the oxygen minimum zone (OMZ; 740 to 760 m). Samples for CARD-FISH were only collected at 6 selected stations and at 3 depths (DCM, OMZ and 2000 m).

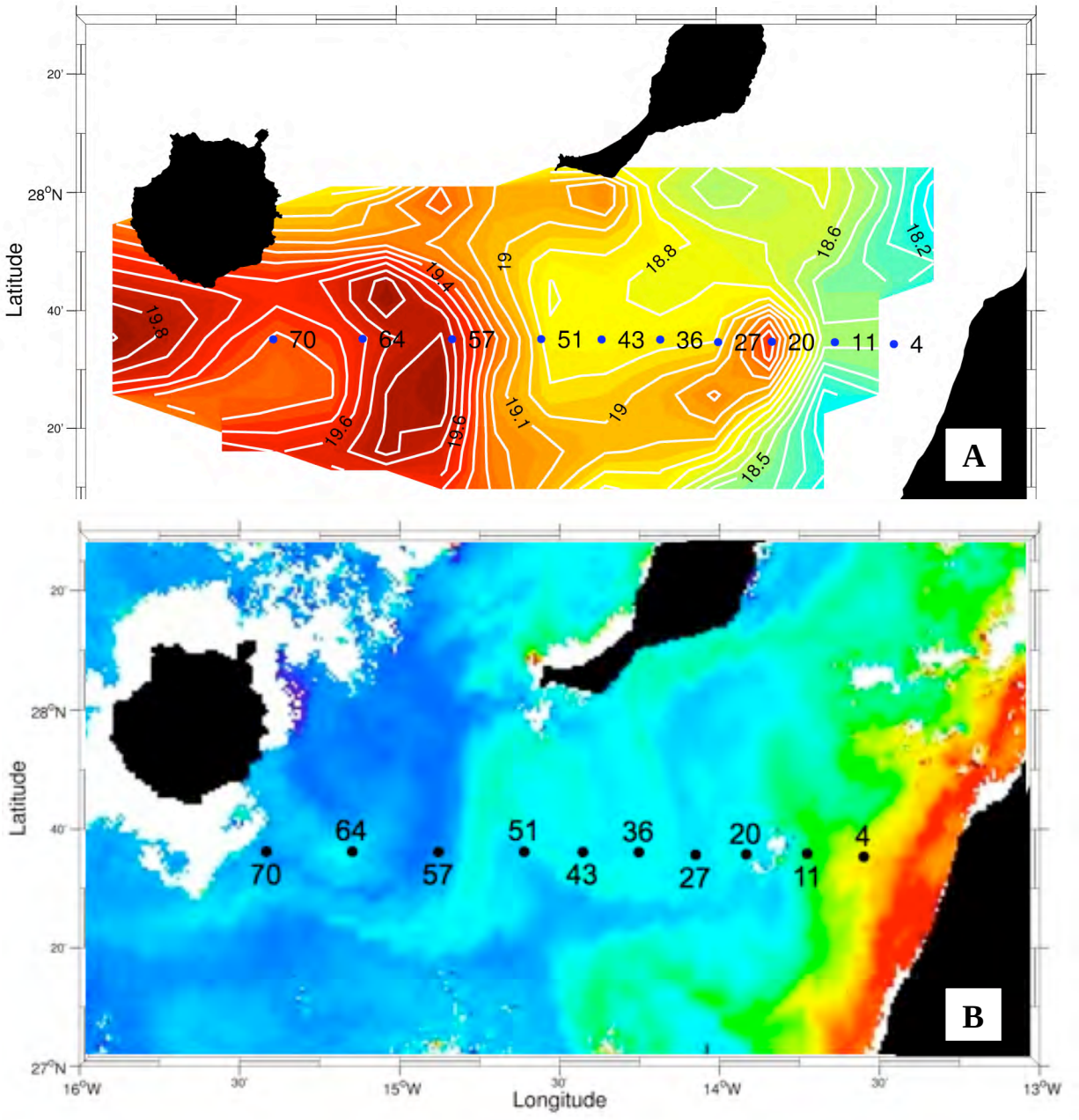


Fig. 1. (A) Near sea-surface temperature, and (B) Sea-viewing Wide Field-of-view Sensor (SeaWiFS) chlorophyll image from 7 April 2006. Station positions (4 to 70) overlaid on maps. Note the upwelling filament extending offshore from the coast

Prokaryotic abundance and biomass

Prokaryotic abundance was determined by flow cytometry (FCM) using a FACSCalibur (Becton Dickinson) with a laser emitting at 488 nm wavelength. Samples (1.5 ml) were fixed with paraformaldehyde (1% final concentration), incubated at 4°C for 15 to 30 min and then stored frozen in liquid nitrogen until analysis. Prior to counting the cells by FCM, 200 µl of sample was stained with a DMS-diluted SYTO-13 (Molecular Probes) stock (10:1) at 2.5 µM final concentration. Prokaryotes were identified by their signatures in a plot of side scatter (SSC) versus green fluorescence (FL1). High and low nucleic acid cells (H1-NA, H2-NA, L-NA) were separated in the scatter plot (Gasol et al. 1999). H1-NA cells exhibited slightly lower SSC and higher FL1 than L-NA cells. H2-NA cells showed high SSC and high FL1 (see Fig. 2). Picocyanobacteria were discriminated in a plot of FL1 versus red fluorescence (FL3). When the fluorescence signal of the prochlorophytes was very low, they were first enumerated in an unstained sample and their abundance subtracted from the total prokaryotic abundance determined by SYTO-13 staining. Prokaryotic carbon biomass was estimated from prokaryotic abundance assuming a conversion factor of 12 fg C cell^{-1} (Fukuda et al. 1998). A suspension of yellow-green 1 µm latex beads (10^6 beads ml^{-1}) was added as an internal standard (Polysciences). The concentration of beads was determined under an epifluorescence microscope. Overall, picoplankton abundance determined by FCM and by DAPI staining and epifluorescence microscopy gave very similar abundance values (FCM = 0.99 · DAPI – 0.226, $R^2 = 0.84$, $p < 0.001$, $n = 14$).

Prokaryotic heterotrophic production

Prokaryotic heterotrophic production was estimated from the rates of protein synthesis determined by the incorporation of tritiated leucine using the centrifugation method (Smith & Azam 1992). ^{3}H-Leucine (Leu, Amersham, specific activity = 171 Ci mmol^{-1}) was added at saturating concentration (40 nmol l^{-1}) to 4 replicate subsamples of 1.2 ml. Duplicate controls were established by adding 120 µl of 50% trichloroacetic acid (TCA) 10 min prior to isotope addition. The Eppendorf tubes were incubated at *in situ* temperature in temperature-controlled chambers for 2 to 7 h. Incorporation of leucine in the quadruplicate sample was stopped by adding 120 µl ice-cold 50% TCA. Subsequently, the subsamples and the controls were kept at –20°C until centrifugation (at ca. 12 000 · *g*) for 20 min, followed by aspiration of the water. Finally, 1 ml of scintillation cocktail was added to the Eppendorf tubes before determining the

incorporated radioactivity after 24 to 48 h on a Wallac scintillation counter with quenching correction using an external standard. Leucine incorporated into prokaryotic biomass was converted to carbon production using the theoretical conversion factor of 1.5 kg C mol^{-1} Leu assuming no isotope dilution (Simon & Azam 1989). Since both *Bacteria* and *Archaea* incorporate leucine, it is more appropriate to refer to the term 'prokaryotic heterotrophic production' instead of the commonly used 'bacterial production' (Herndl et al. 2005).

CARD-FISH

Immediately after collecting the samples from the Niskin bottles, 10 to 40 ml subsamples were fixed with paraformaldehyde (2% final concentration) and stored at 4°C in the dark for 12 to 18 h. The cells were collected on a 0.2 μm polycarbonate filter (Millipore, GTTP, 25 mm filter diameter) supported by a cellulose nitrate filter (Millipore, HAWP, 0.45 μm), washed twice with 0.2 μm filtered Milli-Q water, dried and stored in a microfuge vial at –20°C until further processing in the laboratory. Filters for CARD-FISH were embedded in low-gelling-point agarose and incubated either with lysozyme for the *Bacteria* probes Eub338-III (mixture of probes Eub338, Eub II and Eub III; Amann et al. 1990, Daims et al. 1999), for *Roseobacter* using Ros537 (Eilers et al. 2001), for *Gammaproteobacteria* Gam42a (Manz et al. 1992), for *Bacteroidetes* CF319a (Amann et al. 1990) and for SAR11 using SAR11-441R (Morris et al. 2002), or Proteinase-K for the marine *Euryarchaeota* Group II probe Eury806 and for the marine Crenarchaeota Group I probe Cren537 (Teira et al. 2004). Filters were cut in sections and hybridized with horseradish peroxidase (HRP)-labeled oligonucleotide probes and tyramide-Alexa488 for signal amplification, following the protocol described in Teira et al. (2004). Cells were counterstained with a DAPI mix: 5.5 parts Citifluor, 1 part Vectashield (Vector Laboratories) and 0.5 parts phosphate-buffered saline (PBS) with DAPI (final concentration 1 μg ml^{-1}). The slides were examined under a Zeiss Axioplan 2 microscope equipped with a 100 W Hg lamp and appropriate filter sets for DAPI and Alexa488. More than 800 DAPI-stained cells were counted per sample in a minimum of 30 fields of view. For each microscopic field, 2 different categories were enumerated: (1) total DAPI-stained cells, (2) cells stained with the specific probe. The counting error, expressed as the percentage of the standard error between replicates, was 2% for DAPI counts.

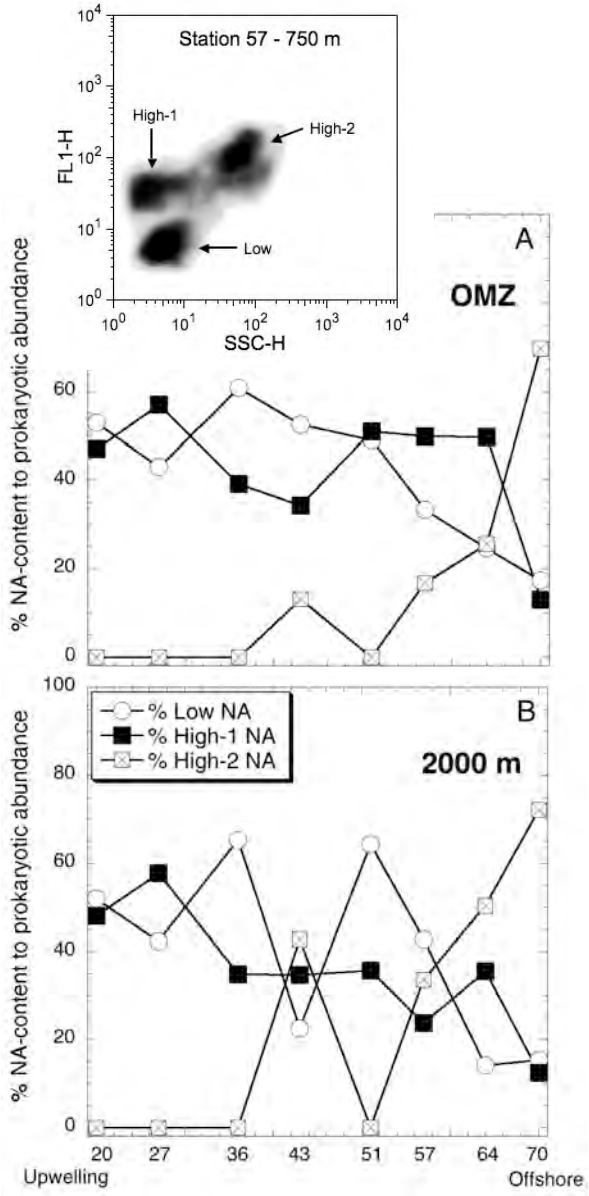


Fig. 2. Abundance of low and high nucleic-acid (NA) content cells as percentage of total prokaryotic abundance, at (A) the oxygen minimum zone (OMZ), and (B) 2000 m depth. The inset in (A) shows the 3 prokaryotic populations (Low, High-1 and High-2) according to their size (side scatter; SSC-H) and NA content (green fluorescence; FL1-H)

Results

Regional oceanographic settings

The stations were placed along a coast–ocean transect crossing an upwelling filament and an offshore anticyclonic eddy centered at Stn 57 (Fig. 1A). Filament-eddy systems have been described in previous studies for the same region (Barton et al. 2004). The eddy entrained filament-water with a higher chlorophyll content in a meandering fashion, giving rise to sharp fronts in chlorophyll and temperature. Fig. 1B (from 7 April 2006) illustrates the advection of high chlorophyll-containing water along

the filament, coinciding with a low surface temperature field (Fig. 1A) recorded during the course of the study (22 March to 7 April 2006). Fig. 3A shows the vertical cross-section of density and chlorophyll concentrations. The DCM was located between 20 and 40 m depth near the upwelling region and deepened slightly towards the open ocean, with the most pronounced deepening of the DCM in the eddy center at Stn 57. The sharpest gradients in density and chlorophyll were found between Stns 11 and 20 (upwelling front), Stns 57 and 64 (eddy-filament front) and Stns 64 and 70 (eddy-open ocean water front).

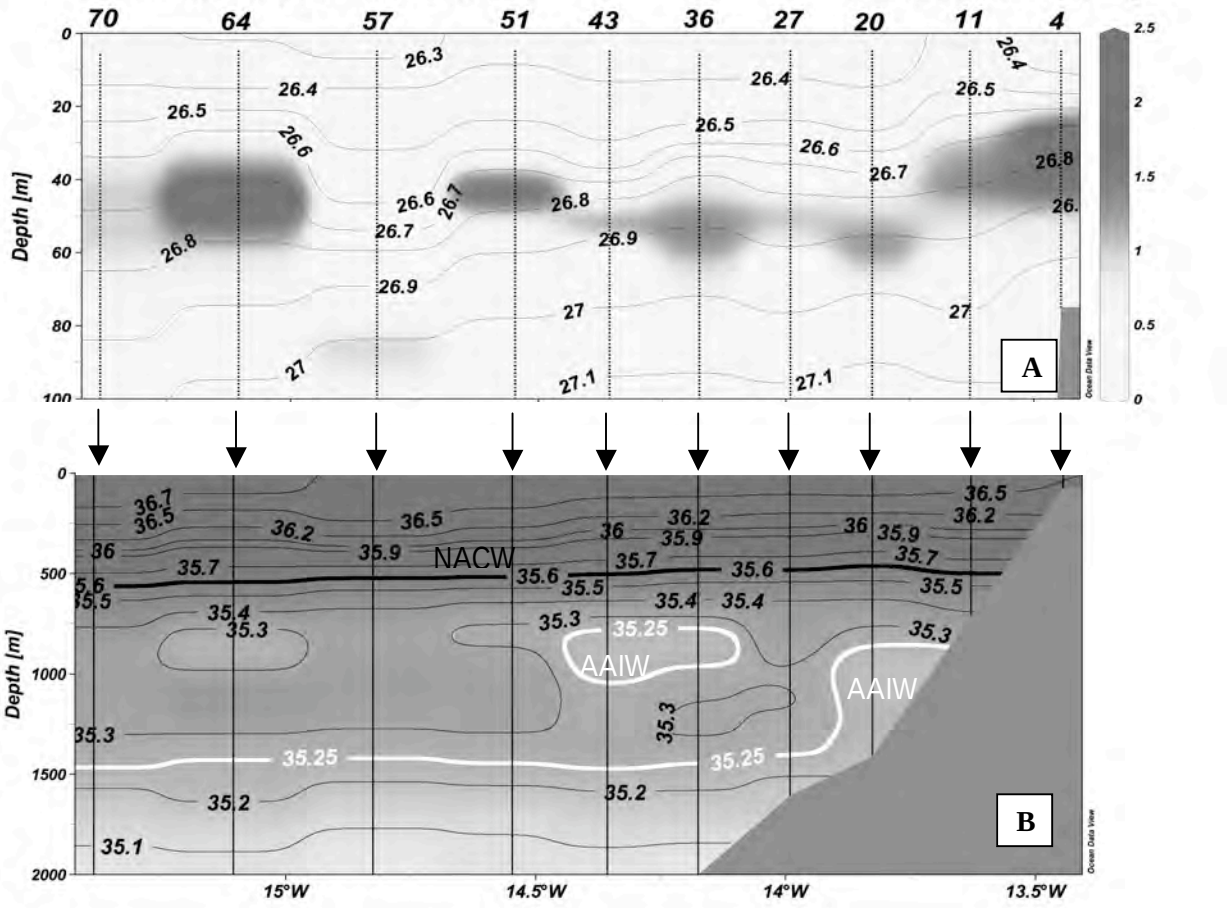


Fig. 3. (A) potential density (kg m^{-3}, grey lines) and chlorophyll fluorescence (relative units, in grey scale), and (B) salinity. AAIW, Antarctic Intermediate Water; NACW, North Atlantic Central Water

The North Atlantic Central Water (NACW) occupied the main thermocline (Fig. 3B). Below the NACW, 2 intermediate water masses dominated the 600 to 1500 m depth range: the Antarctic Intermediate Water (AAIW) with its fresh anomaly values (salinity values <35.3), and the warm, high-salinity Mediterranean Sea Outflow Water (MSOW). The AAIW was manifested as tongues of lower-salinity water, centered at 750 to 900 m depth, in the eastern part of the section (Stns 20, 36 and 43; Fig. 3B). The AAIW is transported northward by a current wider than the usual along-shore poleward undercurrent (Hernández-Guerra et al. 2001). All stations from the western part of the section (Stns 70, 64, 57, 51) as well as Stn 27 were dominated by MSOW. The water masses below the intermediate waters represented a mixture between North Atlantic Deep Water (NADW) and intermediate waters. The presence of the different hydrographic structures or water masses clearly influenced the distribution and activity of the prokaryotic community.

Prokaryotic abundance and activity

Generally, prokaryotic abundance in the surface waters and the DCM decreased from the upwelling region to the open ocean while for the 200 m depth horizon, the OMZ and the 2000 m depth horizon, no distinct lateral trend was detectable (Fig. 4A). Prokaryotic abundance in the surface and the DCM ranged from 1.5 to 8.3 · 10^5 cells ml^{-1}, decreasing exponentially to 0.8 to 3.7 · 10^4 cells ml^{-1} at 2000 m depth (Fig. 4A). Similar to prokaryotic abundance, leucine incorporation rates were highest in the surface waters and the DCM in the coastal upwelling region (398 pmol Leu l^{-1} h^{-1}), decreasing more than 20-fold towards the oceanic stations (16.7 pmol Leu l^{-1} h^{-1}) and by 3 orders of magnitude with depth (Fig. 4B). The highest values of cell-specific production were also found at the stations close to the upwelling region in the surface and the DCM layer (3.8 and 3.6 fmol C $cell^{-1}$ d^{-1}, respectively), decreasing towards the open ocean stations (Stn 70: 0.3 fmol C $cell^{-1}$ d^{-1}) (Fig. 4C). Interestingly, leucine incorporation and cell-specific production followed a similar pattern in the OMZ and at 2000 m depth, albeit 1 order of magnitude lower than in the epipelagic zone (Fig. 4B,C). Cell-specific leucine incorporation between the 200 m layer and the OMZ was not significantly different (Student's *t*-test; p = 0.24, n = 8) (Table 1); however, it was only half at 2000 m depth (Table 1, Fig. 4C). The prokaryotic biomass turnover time (prokaryotic abundance divided by production; Kirchman 2002) increased with depth (from <2 d in the surface waters to >30 d at 2000 m depth; Table 1). The longest

turnover times were observed at Stn 70, where prokaryotic activity was very low at all depths but cell numbers were higher in the deeper layers compared to the other stations, coinciding with the presence of large prokaryotic populations with H-NA content (Fig. 2).

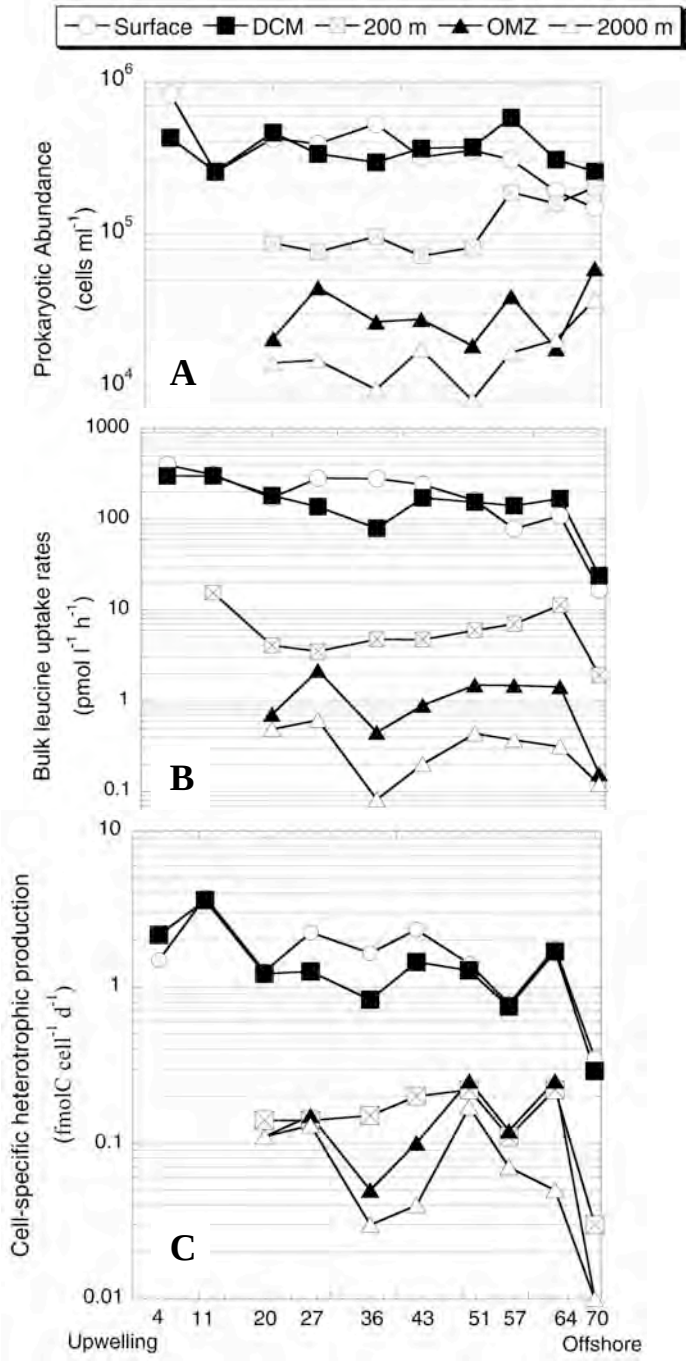


Fig. 4. (A) Prokaryotic abundance, (B) bulk leucine incorporation rates, and (C) cell-specific heterotrophic production along the coast–offshore transect in the surface waters (5 m), deep chlorophyll maximum (DCM), 200 m depth, oxygen minimum zone (OMZ) and 2000 m depth. Standard errors are not visible since they are smaller than the symbols. Note that the coastal stations have no deep samples

At this station, a pronounced decrease in the percentage of L-NA cells and in one of the groups of the H-NA (H1-NA) was observed compared to the same depth

layer of other stations. This decrease in the percentage of L-NA coincided with the highest relative abundance of the other H-NA group (H2-NA). Remarkably, the percentage of the H2-NA cells was higher in the 2000 m layer than in the OMZ (Fig. 2). As a general pattern, the dynamics of the individual NA groups followed a similar trend in the OMZ and the 2000 m layer along the transect.

Table 1. Mean (±SE) prokaryotic activity along the coast–offshore transect. CSHP: cell-specific heterotrophic production; PGR: prokaryotic growth rate; H-NA: high nucleic acid content prokaryotes; DCM: deep chlorophyll maximum; OMZ: oxygen minimum zone. Number of measurements in parentheses. Ten measurements were made at 5 m (surface) depth; 8 measurements were made at all other depths

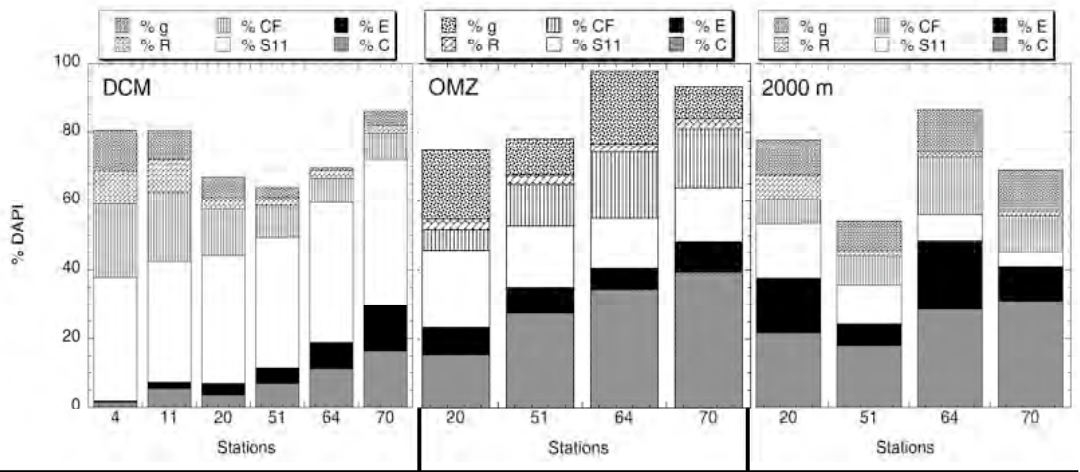
Depth	CSHP (fmol C cell^{-1} d^{-1})	PGR (d^{-1})	Turnover time (d)	% H-NA
5 m	1.71± 0.30	1.02 ± 0.18	1.4 ± 0.4	23 ± 3
DCM	1.45 ± 0.29	1.34 ± 0.27	1.1 ± 0.3	27.8 ± 4
200 m	0.15 ± 0.02	0.14 ± 0.02	10.5 ± 4	36.6 ± 4
OMZ	0.13 ± 0.03	0.12 ± 0.03	24.9 ± 15	66.5 ± 17
2000 m	0.08 ± 0.02	0.07 ± 0.02	30.2 ± 12	67.6 ± 19

Prokaryotic assemblage structure

The recovery efficiency of the prokaryotic community measured using CARD-FISH, i.e. the sum of the relative abundance of *Bacteria*, *Crenarchaeota* and *Euryarchaeota*, was 77 ± 3% (mean ± SD, n = 14) of total DAPI-stainable cells. On average, *Bacteria* accounted for 54 ± 2% (n = 6) in the DCM and 45 ± 2% (n = 8) at 2000 m depth of DAPI-stained cells.

Bacteria were relatively more abundant in the upwelling region and decreased in contribution to total prokaryotic abundance towards the oceanic stations, particularly in the layers between the DCM and the OMZ, while the archaeal contribution increased towards the open ocean (Fig. 5). The most abundant prokaryotic group in the DCM was SAR11, with a relative contribution to total DAPI-stainable cells ranging from 36 to 42% (Fig. 5). In the OMZ, the relative contribution of marine *Crenarchaeota* Group I and *Bacteroidetes* increased from the upwelling region towards the open ocean, while

the contribution of SAR11 decreased and marine *Euryarchaeota* Group II and *Roseobacter* remained almost invariant along the transect (Fig. 5). The abundance of SAR11 declined steeply with depth, reaching the lowest relative abundance at 2000 m depth (Fig. 5). Marine *Crenarchaeota* Group I showed a contrasting trend with a low relative contribution in the DCM and the highest relative abundance in deep waters, where they accounted for up to 39.5% of the DAPI-stained cells. The highest abundance of marine *Crenarchaeota* Group I, *Bacteroidetes* (with the exception of Stn 20) and *Gammaproteobacteria* were found in the OMZ. Marine *Euryarchaeota* Group II contributed up to 13 and 20% to the DAPI-stained cells in the DCM and 2000 m depth layer, respectively, without a clear vertical distribution pattern. *Roseobacter*, however, exhibited highest relative abundance in the DCM, particularly in the upwelling region, and lowest in the deep waters.



Stations	4	11	20	51	64	70
% g	12	8.4	5.9	3.1	0.7	4.2
% R	9.5	9.5	3.3	2.3	2.2	2.1
% CF	21.3	20	13.5	9.0	7.1	7.7
% S11	35.8	35.2	37.2	38.1	40.5	42.3
% E	0.4	1.9	3.3	4.6	7.7	13.1
% C	1.4	5.3	3.6	6.8	11.1	16.6

Stations	20	51	64	70
% g	19.9	10.3	21.1	9.5
% R	3.1	2.8	2.4	2.7
% CF	5.7	11.9	19.2	17.3
% S11	22.7	17.8	14.4	15.5
% E	7.7	7.4	6.2	8.8
% C	15.6	27.6	34.4	39.5

Stations	20	51	64	70
% g	10.5	8.4	12.4	11.9
% R	6.9	1.6	1.5	1.3
% CF	7.1	8.5	16.5	10.4
% S11	15.7	11.2	7.6	4.2
% E	16.1	6.3	19.9	9.9
% C	21.6	18.1	28.6	31

Fig. 5. Relative abundances of prokaryotic groups detected by horseradish peroxidase-oligonucleotide probes and CARD-FISH scaled to DAPI counts at the deep chlorophyll maximum (DCM), the oxygen minimum zone (OMZ) and at 2000 m depth. C: marine *Crenarchaeota* Group I; E: marine *Euryarchaeota* Group II; S11: SAR11; CF: *Bacteroidetes*; R: *Roseobacter*; g: *Gammaproteobacteria*

Discussion

Variability in bulk prokaryotic abundance and heterotrophic production

The range of variability in leucine incorporation rates (16.7 to 398 pmol Leu l^{-1} h^{-1}) measured in the surface waters along the coast–ocean transect (13 to 15.3°W) was similar to other studies covering much larger spatial scales. For instance, Alonso-Sáez et al. (2007b) found leucine incorporation rates from 15 to 331 pmol Leu l^{-1} h^{-1} in the eastern boundary region of the North Atlantic Ocean (14.5 to 26°W), and Hoppe et al. (2006) reported a range of 2 to 141 pmol Leu l^{-1} h^{-1} along a meridional transect across the central Atlantic Ocean (53°N to 65°S). The highest prokaryotic activities in the present study were measured in the upwelling stations (Fig. 4B), while the lowest activities were measured in the most oceanic station. Leucine incorporation at the 200 m depth horizon was highest in the upwelling region and decreased to Stn 20, remarkably increasing thereafter towards Stn 64 (Fig. 4B), probably reflecting the accumulation of organic matter in the core of the anticyclonic eddy. Arístegui et al. (2003) observed large accumulations of dissolved organic carbon in the center of anticyclonic eddies in this region.

The most pronounced changes in the incorporation rate of leucine and cell-specific production were the decrease observed from Stn 64 to Stn 70 (Fig. 4B,C). In contrast, prokaryotic abundance did not decrease significantly (in the DCM), or it increased (in the OMZ and at 2000 m depth) (Fig. 4A). As indicated in Fig. 1, Stn 64 is located within the offshore extension of the filament while Stn 70 represents open ocean waters. Arístegui & Montero (2005) reported high abundance of prokaryotes in the epipelagic zone at frontal regions between filaments and eddies. In their study, an increase in prokaryotic abundance was noticed at the 200 m depth horizon and deeper, but not in shallower waters. Excursions of isopycnals down to 800 m depth in eddy-pair fronts have been reported previously (e.g. Arístegui et al. 2003). This would explain the high abundance of prokaryotes at Stn 70, although surprisingly, this increase is observed even at 2000 m depth (Fig. 4A). None of the prokaryotic groups enumerated by CARD-FISH seemed to contribute specifically to this increase in prokaryotic abundance. However, it is evident that large cells with H-NA content contributed to the increase in prokaryotic abundance in the OMZ and 2000 m depth (Fig. 2).

These patterns may be related to the differences in the water masses. In intermediate waters such as the OMZ, prokaryotic activity was lower in the AAIW than

in the MSOW, except at Stn 70, which exhibited the lowest prokaryotic activity in almost all depth layers. The lower activity in the AAIW is presumably the result of lower (or more refractory) organic carbon concentrations in this old water mass. At 2000 m depth, the prokaryotic activity pattern paralleled that of the OMZ, suggesting mixing of the NADW with the intermediate waters (Fig. 4B).

Cell-specific prokaryotic production in surface waters was about 1 order of magnitude higher than in deep waters (Table 1), resulting in an average prokaryotic growth rate of 1.0 ± 0.2 d^{-1} for surface waters. Our growth rates are substantially higher than the 0.4 ± 0.1 d^{-1} reported by Arístegui et al. (2005) for the Canary Current region using the same approach as in the present study, although their stations were more oceanic. For Stn 70, the most oceanic station occupied in the present study, prokaryotic growth rate in surface waters was 0.26 d^{-1}, comparable to those reported by Arístegui et al. (2005).

The average cell-specific prokaryotic production in the mesopelagic (~0.14 fmol C $cell^{-1}$ d^{-1}) and bathypelagic (0.08 fmol C $cell^{-1}$ d^{-1}) zones is considerably higher than the range of 0.01 to 0.06 fmol C $cell^{-1}$ d^{-1} obtained by Reinthaler et al. (2006) for the meso- and bathypelagic realms of the eastern and western North Atlantic basin. Consequently, their estimated turnover times of 34 to 54 d are longer than the turnover times of 24 and 30 d obtained in the present study for the OMZ and 2000 m depth, respectively (Table 1). Moreover, Reinthaler et al. (2006) used a conversion factor of 3.1 kg C mol^{-1} Leu (assuming a 2-fold isotopic dilution), whereas we used 1.5 kg C mol^{-1} Leu (assuming no isotopic dilution). If we recalculate their data with 1.5 kg C mol^{-1} Leu, the cell-specific prokaryotic production would be even lower and, consequently, the turnover times longer, increasing the differences with our results even more. Hence, the variability in the metabolic rates between these 2 studies must be caused by factors other than the carbon-to-leucine conversion factors used to calculate prokaryotic production.

Differences in prokaryotic activity between the deep waters of the central North Atlantic and the eastern boundary region of the subtropical North Atlantic are likely caused by the greater supply of organic matter to prokaryotic communities closer to the continental margin. Arístegui et al. (2005) estimated an average prokaryotic growth rate of 0.13 ± 0.02 d^{-1} for the mesopelagic zone of the Canary Current, which is in agreement with the growth rate obtained in the present study for the 200 m horizon (0.14 ± 0.02 d^{-1}) and the OMZ (0.12 ± 0.03 d^{-1}). Arístegui et al. (2005) concluded, after

analyzing a set of metabolic indices, that prokaryotic communities in the mesopelagic zone are very active, acting as major sinks for organic carbon in the subtropical NE Atlantic Ocean.

Prokaryotic assemblage variability

One of the main goals of our study was to decipher patterns in distribution in bulk prokaryotic properties and assemblage structure linked to water mass characteristics and hydrographic regimes, particularly in the deep ocean. Previous studies using PCR-independent methods have identified and quantified the dominant bacterial groups in surface waters (Eilers et al. 2001, Fuchs et al. 2005); however, only rather rudimentary information is available on the abundance of the major bacterial groups in the meso- and bathypelagic zones using FISH (see 'Introduction'). In some of the surface-water studies, minor differences in the proportion of major phylogenetic groups, but larger differences in the proportion of more specific groups such as SAR86, SAR11, SAR116 (Fuchs et al. 2005) and SAR202 (Morris et al. 2004), were found. Alonso-Sáez et al. (2007a), using CARD-FISH, found that bacterial assemblage structure in surface waters of the subtropical NE Atlantic was highly influenced by environmental factors, with a distinct community in the upwelling region different from the more oceanic waters. In the present study, we observed a large variability in the prokaryotic assemblage along the coast–ocean transect. At the DCM, the relative contributions of SAR11, marine *Euryarchaeota* Group II and marine *Crenarchaeota* Group I to DAPI-stained cells increased towards the open ocean, while *Gammaproteobacteria*, *Roseobacter* and *Bacteroidetes* were more abundant towards the upwelling region (Fig. 5), where bulk prokaryotic activity was generally higher (Fig. 4B,C). This is in agreement with other studies, which also report high contributions of the *Bacteroidetes* cluster to total prokaryotic abundance in upwelling systems (Fandino et al. 2001, Alonso-Sáez et al. 2007a). *Roseobacter* was also related previously to the presence of high phytoplankton biomass (González & Moran 1997, Eilers et al. 2001), while the *Gammaproteobacteria* were associated with nutrient pulses (Eilers et al. 2000, Alonso-Sáez et al. 2007a). SAR11 was the most abundant group in the DCM, as also described in previous studies (Morris et al. 2002, Alonso-Sáez et al. 2007a). This cosmopolitan group represents >50% of the prokaryotic abundance in the surface waters of the NW Sargasso Sea and 25% of the mesopelagic microbial assemblages (Morris et

al. 2002), declining in its contribution to prokaryotic abundance with depth, however (Morris et al. 2004; our Fig. 5).

In the OMZ, the SAR11 clade showed a different distribution pattern than in the DCM, being more abundant at Stn 20 affected by upwelling AAIW (Fig. 5). Marine *Crenarchaeota* Group I and *Bacteroidetes* increased in their relative abundance towards the open ocean, with Stns 51 to 70 (affected by MSOW) exhibiting very similar community composition (Fig. 5). At 2000 m depth, no clear gradient in prokaryotic community composition was discernable, although *Bacteroidetes* and the marine *Crenarchaeota* Group I were more abundant in the more oceanic waters (Fig. 5). The estimated relative abundance of marine *Euryarchaeota* Group II (4 to 13% in the DCM and 6 to 20% in deep waters) is in agreement with the observations reported by Teira et al. (2006b) for the North Atlantic Ocean, where marine *Euryarchaeota* Group II ranged from <10 to >30% in subsurface waters and <15% of the total picoplankton community at the 100 m layer. While SAR11 decreased in relative abundance with depth, marine *Crenarchaeota* Group I increased with depth (Fig. 5). The highest relative abundance of marine *Crenarchaeota* Group I, *Bacteroidetes* and *Gammaproteobacteria* were found in the OMZ, except for Stn 20, where the OMZ was influenced by AAIW. Teira et al. (2006a) also found the highest absolute abundance of marine *Crenarchaeota* Group I (accounting for >40% of DAPI-stained cells) in the OMZ of the North Atlantic. The increase of marine *Crenarchaeota* Group I and *Gammaproteobacteria* in the OMZ might be related to nitrification processes in the OMZ. Marine nitrification is mediated by 3 different groups of prokaryotes belonging to the *Beta-* and *Gammaproteobacteria* (Ward 2002), although recently, evidence has been accumulating that *Crenarchaeota* also contribute to marine nitrification (Könneke et al. 2005, Wuchter et al. 2006). It is noteworthy, however, that Wuchter et al. (2006) did not find any gamma-proteobacterial *amo*A genes, a gene indicative for the potential to oxidize ammonia. Only betaproteobacterial and marine crenarchaeotal Group I.1a *amo*A genes were retrieved (Wuchter et al. 2006). It is worth noting that crenarchaeal *amo*A gene copy numbers determined by q-PCR dominated over betaproteobacterial *amo*A gene copy numbers throughout the North Atlantic mesopelagic waters (Wuchter et al. 2006). In soils, the copy numbers of crenarchaeal *amo*A genes also outnumber beta-proteobacterial *amo*A genes (Leininger et al. 2006). *Crenarchaeota* oxidizing ammonia could play an important, previously unrecognized, role in the biogeochemical cycling of

nitrogen in the ocean, although their actual nitrification rates in natural environments remain to be determined.

Bacteria versus *Archaea*

Recent reports on the distribution of *Bacteria* and *Archaea* in the oceans indicate that planktonic marine *Crenarchaeota* Group I increase in their relative contribution to prokaryotic abundance with depth, reaching cell numbers similar to or higher than those of *Bacteria* in the mesopelagic zone (Karner et al. 2001, Teira et al. 2006b). Marine *Euryarchaeota* Group II, the other major archaeal group, is, however, commonly more abundant in surface than deep waters (Massana et al. 2000, Karner et al. 2001, Church et al. 2003), comprising up to 10 to 30% of picoplankton abundance in subsurface waters (Herndl et al. 2005, Teira et al. 2006b). Some *Euryarchaeota* in surface waters harbor proteorhodopsin, a light-harvesting pigment that allows light to be used as an additional energy source, while deep-water *Euryarchaeaota* lack proteorhodopsin (Frigaard et al. 2006).

The fact that marine *Crenarchaeota* Group I increase in their relative abundance with depth while bacterial abundance decreases (e.g. Massana et al. 1997, Herndl et al. 2005, Teira et al. 2006a) suggests that they occupy different ecological niches, likely caused by environmental conditions and different substrate requirements. These different ecological niches, however, cannot be deciphered at the moment, as both marine *Crenarchaeota* Group I and *Bacteria* are capable of utilizing similar model substrates (Teira et al. 2006a, Kirchman et al. 2007). Our results show a relative increase with depth (Fig. 5) of both marine *Crenarchaeota* Group I and marine *Euryarchaeota* Group II, although absolute archaeal abundances were always higher at the DCM. In all cases the ratio of total *Archaea* : *Bacteria* was ≤ 1 (average ± SD: 0.24 ± 0.08 for the DCM, 0.82 ± 0.11 for the OMZ and 0.68 ± 0.17 for the 2000 m depth layer).

An even more pronounced trend in the distribution of *Bacteria* versus *Archaea* than over depth was observed laterally along the coast–offshore gradient in the DCM (Fig. 5). Although *Archaea* were always 2 to 7 times lower in abundance than *Bacteria*, the decrease in bacterial abundance from the upwelling region towards the open ocean coincided with a concomitant increase in *Archaea* as described by the equation: *Bacteria* (cells ml^{-1}) = 0.61 · *Archaea* (cells ml^{-1}) – 60255 ($R^2 = 0.98$, $p < 0.00001$). A similar, although less pronounced, contrasting abundance pattern of archaeal and

bacterial abundance was found for the OMZ (data not shown). Marine *Crenarchaeota* Group I constituted 79% of the total abundance of *Archaea* in the OMZ, while in the DCM both archaeal groups contributed roughly equally. Lateral gradients in the relative abundance of *Bacteria* versus *Archaea* have not been reported thus far. The decrease in bulk leucine uptake along the coast–ocean gradient was positively related ($R^2 = 0.72$, n = 6) to the decrease in bacterial abundance in the DCM, and negatively related ($R^2 = -0.76$, n = 6) to the increase in archaeal abundance. This suggests that most of the bulk prokaryotic heterotrophic production, as estimated by leucine incorporation, was due to bacterial activity. In addition, SAR11 was the most abundant prokaryotic group in the DCM, decreasing 2-fold from Stn 4 to Stn 70, although increasing in the relative contribution to prokaryotic abundance towards the oceanic stations. Thus, this group is probably the major contributor to the bulk prokaryotic metabolism in the open ocean's surface waters.

Conclusions

A high variability was observed in bulk prokaryotic abundance and metabolism, as well as in changes in community structure, in the surface waters from the NW Africa upwelling region towards the open ocean. This variability was comparable to the variability reported for ocean-basin studies. *Bacteria* (SAR11, *Roseobacter*, *Gammaproteobacteria* and *Bacteroidetes*) were relatively more abundant in coastal than in oceanic regions, in contrast to the distribution of marine *Euryarchaeota* Group II and marine *Crenarchaeota* Group I. SAR11 was mainly responsible for the surface-water variability in bulk prokaryotic abundance. Prokaryotic abundance decreased about 2 orders of magnitude from surface to 2000 m deep waters, whereas prokaryotic production decreased by 3 orders of magnitude. Both archaeal groups contributed up to 50% to the total prokaryotic abundance in the dark ocean. AAIW exhibits lower prokaryotic activity than MSOW, presumably due to lower organic matter content of the AAIW. Prokaryotic growth rates in the deep ocean were considerably higher than those reported for the central Atlantic, but comparable to previous studies from the same region. This supports the view that prokaryotic communities in the deep waters of the subtropical NE Atlantic maintain higher metabolic rates than in the central Atlantic due to lateral input of organic matter from the continental margins.

Acknowledgements

Fieldwork was supported by the CONAFRICA (CTM2004-02319) and RODA (CTM 2004-06842-C03/MAR) projects. This is a contribution to the MarBEF European Network of Excellence funded by the European Union (GOCE-CT-2003-505446) and to EUR-OCEANS, a European Network of Excellence funded by the European Commission (6th Framework Programme, contract no. 511106). Financial support was provided by a PhD fellowship from the Spanish government to F.B. We thank the crew and other scientists aboard the RV 'BIO-Hesperides' for their help during the cruise. The authors thank 2 anonymous reviewers whose comments and suggestions helped improve the manuscript.

References

Alonso-Sáez L, Arístegui J, Pinhassi J, Gómez-Consarnau L, González LM, Vaqué, D, Agustí S, Gasol JM (2007a) Bacterial assemblage structure and carbon metabolism along a productivity gradient in the NE Atlantic Ocean. Aquat. Microb. Ecol. 46:43–53

Alonso-Sáez L, Gasol JM, Arístegui J, Vilas JC, Vaqué D, Duarte CM, Agustí S (2007b) Large-scale variability in surface bacterial carbon demand and growth efficiency in the subtropical northeast Atlantic Ocean. Limnol. Oceanogr. 52:533–546

Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microb. 56:1919-1925

Arístegui J, and Montero MF (2005) Temporal and spatial changes in plankton respiration and biomass in the Canary Islands region: the effect of mesoscale variability. J. Marine Syst. 54:65-82

Arístegui J, Barton ED, Montero MF, García-Muñoz M, Escánez J (2003) Organic carbon distribution and water column respiration in the NW Africa-Canaries Coastal Transition Zone. Aquat. Microb. Ecol. 33:289-301

Arístegui J, Duarte C, Gasol JM, Alonso-Sáez L (2005) Active mesopelagic prokaryotes support high respiration in the subtropical northeast Atlantic Ocean, Geophys. Res. Lett., 32, L03608, doi: 10.1029/2004GL021863

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water column microbes in the sea. Mar. Ecol. Prog. Ser. 10:257-263

Barton ED, Arístegui J, Tett P, Navarro-Pérez E (2004) Variability in the Canary Islands area of filament-eddy exchanges. Progr. Oceanogr. 62:71–94

Church MJ, DeLong EF, Ducklow HW, Karner MB, Preston CM, Karl DM (2003) Abundance and distribution of planktonic Archaea and Bacteria in the waters west of the Antarctic Peninsula. Limnol. Oceanogr. 48:1893–1902

Cottrell MT, Kirchman DL (2000) Natural assemblages of marine Proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. Appl. Environ. Microbiol. 66:1692-1697

Daims H, Bruhl A, Amann R, Schleifer KH, Wagner M (1999) The domain-specific probe EUB338 is insufficient for the detection of all bacteria: development and evaluation of a more comprehensive probe set. Syst. Appl. Microbiol. 22:434-444

DeLong EF, Wu KY, Prezelin BB, Jovine RVM (1994) High abundance of Archaea in Antarctic marine picoplankton. Nature 371:695-697.

Eilers H, Pernthaler J, Amann R (2000) Succession of pelagic marine bacteria during enrichment: a close look at cultivation-induced shifts. Appl. Environ. Microbiol. 66:4634- 4640

Eilers H, Pernthaler J, Peplies J, Glöckner FO, Gerdts G, Amann R (2001) Isolation of novel pelagic bacteria from the German Bight and their seasonal contributions to surface picoplankton. Appl. Environ. Microbiol. 67:5134-5142

Fandino LB, Riemann L, Steward GF, Long RA, Azam F (2001) Variations in bacterial community structure during a dinoflagellate bloom analyzed by DGGE and 16S rDNA sequencing. Aquat. Microb. Ecol. 23:119–130

Frigaard N-U, Martínez A, Mincer TJ, DeLong EF (2006) Proteorhodopsin lateral gene transfer between marine planktonic Bacteria and Archaea. Nature 439:847-850

Fuchs BM, Woebken D, Zubkov MV, Burkill P, Amann R (2005) Molecular identification of picoplankton populations in contrasting waters of the Arabian Sea. Aquat. Microb. Ecol. 39:145–157

Fukuda R, Ogawa H, Nagata T, Koike I (1998) Direct determination of carbon and nitrogen content of natural bacteria assemblages in marine environments. Appl. Environ. Microbiol. 64:3352-3358

Gasol JM, del Giorgio PA, Duarte CM (1997) Biomass distribution in marine planktonic communities. Limnol. Oceanogr. 42: 1353-1363

Gasol JM, Zweifel UL, Peters F, Fuhrman JA, Hagström Å (1999) Significance of size and nucleic acid content in heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl. Environ. Microbiol. 65:4475–4483

Giovannoni SJ, Rappé MS (2000) Evolution, diversity and molecular ecology of marine prokaryotes. In: Kirchman DL (ed) Microbial Ecology of the Oceans. Wiley-Liss, New York, p 47–85

Giovannoni SJ, Britschgi TB, Moyer CL, Field KG (1990) Genetic diversity in Sargasso Sea bacterioplankton. Nature 345:60–63

Gleason HA (1926) The individualistic concept of the plant association. Bull Torrey Bot Club 53:7–26

González JM, Moran MA (1997) Numerical dominance of a group of marine bacteria in the alpha-subclass of the class Proteobacteria in coastal seawater. Appl. Environ. Microbiol. 63:4237–4242

Hernández-Guerra A, López-Laatzen F, Machín F, De Armas D, Pelegrí JL (2001) Water masses, circulation and transport in the eastern boundary current of the North Atlantic subtropical gyre. Sci. Mar. 65 (Sppl.1):177-186

Hewson I, Steele JA, Capone DG, Fuhrman JA (2006) Remarkable heterogeneity in meso- and bathypelagic bacterioplankton assemblage composition. Limnol Oceanogr 51(3):1274-1283

Herndl GJ, Reinthaler T, Teira E, Van Aken H, Veth C, Pernthaler A, Pernthaler J (2005) Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean. Appl. Environ. Microbiol. 71:2303–2309

Hoppe HG, Gocke K, Koppe R, Kraus G (2006) Changing bacterioplankton growth characteristics on a large spatial scale: oligotrophic versus mesotrophic ocean. Mar. Ecol. Prog. Ser. 323:21–33

Karner MB, DeLong EF, Karl DM (2001) Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature 409:507–510

Könneke M, Bernhard AE, Torre JR de la, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437:543-546

Kirchman DL (2002) Calculation microbial growth rates from data on production and standing stocks. Mar. Ecol. Prog. Ser. 233:303-306

Kirchman DL, Elifantz H, Dittel AI, Malmstrom RR, Cottrell MT (2007) Standing stock and activity of Archaea and Bacteria in the western Arctic Ocean. Limnol. Oceanogr. 52:495-507

Leininger S, Ulrich T, Schloter M, Schwark L, Qi J, Nicol G, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806-809

Malmstrom RR, Kiene RP, Cottrell MT, Kirchman DL (2004a) Contribution of SAR11 bacteria to dissolved dimethylsulfoniopropionate and amino acid uptake in the North Atlantic Ocean. Appl. Environ. Microbiol. 70:4129–4135

Malmstrom RR, Kiene RP, Kirchman DL (2004b) Identification and enumeration of bacteria assimilating dimethylsulfoniopropionate (DMSP) in the North Atlantic and Gulf of Mexico. Limnol. Oceanogr. 49:597–606

Massana R, Murray AE, Preston CM, DeLong EF (1997) Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. Appl. Environ. Microbiol. 63:50–56

Massana R, LT Taylor, AE Murray, Wu KY, Jeffrey WH, DeLong EF (1998) Vertical distribution and temporal variation of marine planktonic archaea in the Gerlache Strait, Antarctica, during early spring. Limnol. Oceanogr. 43:607–617

Massana R, DeLong EF, Pedrós-Alió C (2000) A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. Appl. Environ. Microbiol. 66:1777-1787

Manz W, Amann R, Ludwig W, Wagner M, Schleifer KH (1992) Phylogenetic oligodeoxynucleotide probes for the major subclasses of Proteobacteria-Problems and solutions. Syst. Appl. Microbiol. 15:593-600

Morris RM, Rappé MS, Connon SA, Vergin KL, Slebold WA, Carlson CA, Giovanonni SJ (2002) SAR11 clade dominates ocean surface bacterioplankton communities. Nature 420:806-810

Morris RM, Rappe MS, Urbach E, Connon SA, Giovannoni SJ (2004) Prevalence of the chloroflexi-related SAR202 bacterioplankton cluster throughout the mesopelagic zone and deep ocean. Appl. Environ. Microbiol. 70:2836–2842

Mullins TD, Britschgi TB, Krest RL, Giovannoni SJ (1995) Genetic comparisons reveal the same unknown bacterial lineages in Atlantic and Pacific bacterioplankton communities. Limnol. Oceanogr. 40:148–158

Pedrós-Alió C (2006) Marine microbial diversity: can it be determined?. TRENDS in Microbiology 14 (6):257-263

Pernthaler A, Pernthaler J, Amann R (2002) Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Appl. Environ. Microbiol. 68:3094–3101

Rappe MS, Vergin K, Giovannoni SJ (2000) Phylogenetic comparisons of a coastal bacterioplankton community with its counterparts in open ocean and freshwater systems. FEMS Microbiol. Ecol. 33:219–232

Reinthaler T, Van Aken H, Veth C, Lebaron P, Arístegui J, Robinson C, Williams PJ Le B, Herndl GJ (2006) Prokaryotic respiration and production in the meso- and bathypelagic realm of the eastern and western North Atlantic basin. Limnol. Oceanogr. 51(3):1262–1273

Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol. Prog. Ser. 51:201–213

Smith DC, Azam F (1992) A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. Mar. Microb. Food. Webs 6:107-114

Teira E, Reinthaler T, Pernthaler A, Pernthaler J, Herndl, G (2004) Combining catalyzed reported deposition-fluorescence in situ hybridization and

microautoradiography to detect substrate utilization by Bacteria and Archaea in the deep ocean. Appl. Environ. Microbiol. 70:4411-4414

Teira E, Van Aken HM, Veth C, Herndl GJ (2006a) Archaeal uptake of enantiomeric amino acids in meso- and bathypelagic waters of the North Atlantic. Limnol. Oceanogr. 51:60–69

Teira E, Lebaron P, Van Aken HM, Herndl GJ (2006b) Distribution and activity of Bacteria and Archaea in the deep water masses of the North Atlantic. Limnol. Oceanogr. 51:2131-2144

Ward BB (2002) Nitrification in aquatic systems. In: Capone DA (ed) Encyclopedia of Environmental Microbiology. Wiley, New York, p 2144 –2167

Wuchter C, Abbas B, Coolen MJL, Herfort L, van Bleijswijk J, Timmers P, Strous M, Teira E, Herndl GJ, Middelburg JJ, Schouten S, Sinninghe Damste JS (2006) Archaeal nitrification in the ocean. Proc. Nat. Acad. Sci. USA 103 (33):12317-12322

Chapter II

Evidence of prokaryotic metabolism on suspended particulate organic matter in the dark waters of the (sub)tropical North Atlantic

F. Baltar, J. Arístegui, J. M. Gasol, E. Sintes, G. J. Herndl

Limnology and Oceanography 54 (1), 182-193 (2009)

Abstract

The distribution of prokaryotic abundance (PA), respiratory activity (ETS), heterotrophic production (PHP), and suspended particulate (POM) and dissolved (DOM) organic matter was determined in the meso- and bathypelagic waters of the (sub)tropical North Atlantic. PA decreased by one order of magnitude from the lower euphotic zone to the bathypelagic waters, while ETS decreased by two and PHP by three orders of magnitude. On a section following the Mid-Atlantic Ridge from 35°N to 5ºN, ETS below 1000 m depth increased southwards up to three-fold. This latitudinal gradient in the deep-waters was paralleled by a six-fold increase in Particulate Organic Carbon (POC), whereas no trend was apparent in the DOM distribution. Significant correlations between POM and ETS were obtained in the water masses between 1000 m and 3000 m depth, the Antarctic Intermediate Water and the North East Atlantic Deep Water. A strong imbalance in the dark ocean was found between prokaryotic carbon demand (estimated through two different approaches) and the carbon sinking flux derived from sediment trap records corrected with ^{230}Th. The imbalance was greater when deeper in the water column, suggesting that the suspended carbon pool must account for most of the carbon deficit. Our results, together with other recent findings discussed in this chapter, indicate that microbial life in the dark ocean is likely more dependent on slowly sinking or buoyant, laterally advected suspended particles than hitherto assumed.

Introduction

For a long time, the dark ocean was considered to be a site of almost negligible biological activity due to the harsh environmental conditions (Morita 1984). Over the last two decades, however, the relatively intense microbial activity taking place in the dark ocean has been recognized (Cho & Azam 1988, Karl et al. 1988, Nagata et al. 2000). In particular, deep ocean respiration has been estimated to account for one third of the biological CO_2 production in the ocean (del Giorgio & Duarte 2002, Arístegui et al. 2005a). These high respiration rates, however, largely exceed the downward export flux of dissolved (DOC) and sinking particulate organic carbon (POC) (Reinthaler et al.

2006). Arístegui et al. (2002) showed, based on the relation between DOC and apparent oxygen utilization (AOU) in a large global-ocean data set, that DOC accounts for only 10-20% of oxygen consumption in the dark ocean, supporting the view that the bulk of dark ocean respiration is driven by particulate material (McCave 1975, Honjo 1980, 1986, Arístegui et al. 2005a). Their results agree with the study of Hansell & Carlson (1998), who inferred extremely low remineralization rates from basin-scale gradients of DOC in the deep ocean. However, the sinking POC supply collected with sediment traps, commonly used for inferring remineralization rates in the ocean, does not explain the remaining oxygen consumption in the dark waters (Arístegui et al. 2005a; Reinthaler et al. 2006). One explanation of this discrepancy could be that the suspended (or low-buoyancy) fraction of the POM pool is underestimated (or simply not collected) with sediment traps. This pool is quantitatively far larger than the sinking pool (McCave 1984; Verdugo et al. 2004), and might account for the excess oxygen consumption rates. This suspended POM might be laterally transported through intermediate and deep waters while being remineralized. Bauer & Druffel (1998) concluded from a study comparing the natural radiocarbon abundance in two coastal and open ocean profiles in the North Atlantic and North Pacific Oceans, that the POC inputs from ocean margins to the ocean interior could be more than one order of magnitude higher than the input of recently produced organic carbon derived from the surface ocean. Regardless, whether the suspended carbon in the dark ocean is transported from ocean margins or originates from sinking particles, this pool has been largely ignored in ocean carbon budgets.

In this study we investigated regional gradients and the depth distribution of the dissolved (DOM) and particulate organic matter (POM) pools, as well as the microbial metabolism in the dark ocean to determine the relation between changes in the OM pools and the activity of deep-water microbes in the (sub)tropical North Atlantic. To address this question, we determined the potential respiration via electron transport system (ETS) measurements along with particulate and dissolved organic carbon (POC and DOC) and nitrogen (PON and DON), as well as prokaryotic abundance and production in the mixed layer (≈ 50 m depth), the upper thermocline (≈ 100 m depth), the mesopelagic (200-1000 m depth) and bathypelagic (1000-4500 m depth) waters of the (sub)tropical North Atlantic. DOC is largely refractory below the mixed layer of the (sub)tropical regions (Hansell & Carlson 2002) where overturning circulation is greatly restricted, and the sinking fluxes are low in open ocean waters. Thus, we hypothesized that the metabolism of prokaryotes in the dark ocean largely relies on suspended

particles, which we would be able to measure as POM. Hence, variations in the distribution of POM collected with oceanographic bottles, considered to be mostly suspended detrital material, should be related to variations in prokaryotic metabolism in the dark ocean.

Material and methods

Study site and sampling

To resolve the areal variability in organic matter and prokaryotic activity in the meso- and bathypelagic waters of the eastern North Atlantic, more than 9000 km were covered comprising the subtropical gyre region (SG) and the North Equatorial Counter Current region (NEqCC) (Fig. 1) on board RV *Pelagia* (November-December 2005). The study area was divided into two regions (SG and NEqCC) (Varela et al. 2008) separated by the latitudinal front of the intermediate and central waters (*see* Fig. 1). The circulation pattern of the bathypelagic water masses, however, did not differ across the two regions. Samples were taken at 25 out of 43 stations occupied during the ARCHIMEDES-I cruise from seven depths: the surface mixed layer (SML, average depth 50 m) for POM and ETS only, the base of the mixed layer ("subsurface", 100 m layer), the oxygen minimum layer (OML; average depth 400 m), the Mediterranean Sea Overflow Water (MSOW; average depth 900 m) in the SG, the Antarctic Intermediate Water (AAIW; average depth 900 m) in the NEqCC, the North East Atlantic Deep Water (NEADW; average depth 2750 m) and the Lower Deep Water (LDW; average depth 4000 m). The specific water masses were identified based on their distinct temperature-salinity characteristics, oxygen content and inorganic nutrient signatures (Table 1). The LDW, characterized by low salinity (34.9) and temperature (2.3-2.6ºC), consisted mainly of Antarctic Bottom Water coming from the south and mixed with some NEADW. Iceland Scotland Overflow Water and Labrador Sea Water principally form the NEADW (Varela et al. 2008). Samples from the distinct water masses were collected with 12 L NOEX (no oxygen exchange) bottles mounted on a CTD

(conductivity, temperature, depth) frame to determine total picoplankton abundance, leucine incorporation, POM, DOM, and ETS as described below.

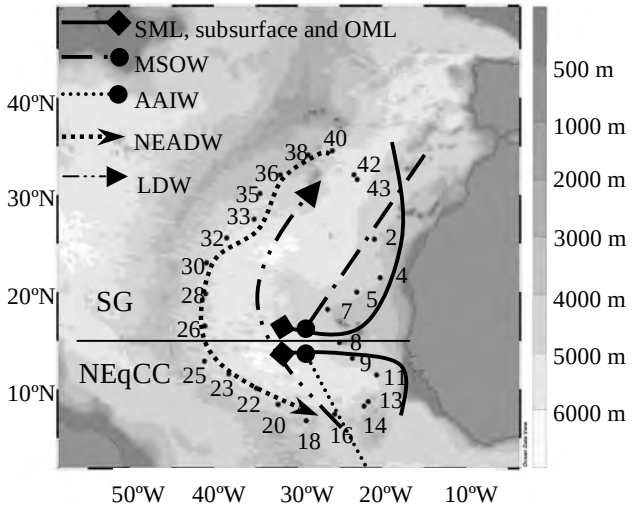


Fig. 1. Stations occupied during ARCHIMEDES-I. Sampled stations are indicated by numbered dots. Horizontal line at about 15°N separates the stations of the subtropical gyre (SG) from those in the North Equatorial Counter Current (NEqCC). The section plotted in Fig. 4 extends from Sta. 40 to Sta. 16. Lines indicate the flow pattern of the main water masses. For water mass abbreviation see Table 1

Prokaryotic abundance (PA) determined by flow cytometry

Picoplankton collected from the different depth layers of the water column were enumerated using flow cytometry. Samples (2 mL) were fixed with 1% paraformaldehyde (final concentration), shock-frozen in liquid nitrogen for 5 min and stored at -80°C. Picoplankton cells were stained with SYBR-Green I and enumerated within 3 months with a FACSCalibur flow cytometer (Becton Dickinson) equipped with a 488 nm laser. Immediately before analysis, the thawed picoplankton samples were diluted 5- to 10-fold in TE buffer (10 mmol L^{-1} Tris, 1 mmol L^{-1} EDTA, pH 8) and stained with SYBR-Green I at room temperature in the dark for 15 min. Fluorescent microspheres (Molecular Probes) with a diameter of 1 μm were added to all samples as an internal standard. Picoplankton cells were differentiated according to their right angle scatter and green fluorescence. The counting window of the flow cytometer was set to exclude the occasionally present eukaryotic picoplankton. Prokaryotic carbon

biomass was calculated assuming a carbon content of 10 fg C per cell, which seems to be most suitable for dark ocean prokaryotes (Ducklow et al. 2000).

Table 1. Characteristics of the main water masses sampled during the ARCHIMEDES-I cruise based on CTD profiles at individual stations. Ranges are given for each water mass where samples were collected. SG: Subtropical Gyre, NEqCC: North Equatorial Counter Current, SML: surface mixed layer, OML: oxygen minimum layer, MSOW: Mediterranean Sea Overflow Water, NEADW: North East Atlantic Deep Water, LDW: Lower Deep Water, AAIW: Antarctic Intermediate Water

Zone	Water mass	Depth (m)	Temperature (ºC)	Salinity	Oxygen (μmol kg^{-1})
SG	SML	50	20.1-26.8	36.5-37.2	178.5-213.1
	subsurface	100	17.7-24.1	36.4-37.5	138.9-221.5
	OML	250-500	10.1-17.9	35.3-36.5	87.5-207.2
	MSOW	900-1000	7.6-9.2	35.1-35.6	121.2-197.7
	NEADW	2750	2.8-3.0	34.9	223.8-250.8
	LDW	3500-5000	2.4-2.6	34.9	223.7-242-5
NEqCC	SML	50	19.4-27.8	35.9-36.1	118-184.5
	subsurface	100	14.2-18.5	35.5-36.4	79.6-132.7
	OML	250-500	7.9-12.0	34.7-35.3	44.0-122.3
	AAIW	900-1000	5.1-6.5	34.6-34.7	102.4-129.5
	NEADW	2750	2.8-3.0	34.9	234.3-247.1
	LDW	3750-4000	2.3-2.4	34.9	227.7-246.8

Prokaryotic heterotrophic production (PHP) estimated by [^{3}H] leucine incorporation

Bulk picoplankton heterotrophic production was measured by incubating triplicate 10–40 mL of samples and formaldehyde killed blanks (2% final concentration) with 10

nmol L^{-1} [^{3}H]leucine (final concentration, specific activity 157 Ci $mmol^{-1}$; Amersham) in the dark at in situ temperature for 1-12 h depending on the water mass and the expected prokaryotic activity (Kirchman et al. 1985). Thereafter, the incubation was terminated by adding formaldehyde (2% final concentration) to the samples. The fixed samples were filtered through 0.2 μm polycarbonate filters (25 mm filter diameter; Millipore) supported by Millipore HAWP filters. Subsequently, the filters were rinsed three times with 10 mL of 5% ice-cold trichloroacetic acid, dried and placed in scintillation vials. Scintillation cocktail (8 mL Canberra-Packard Filter Count) was added and after 18 h, counted in a liquid scintillation counter (LKB Wallac Model 1212). The mean disintegrations per minute (DPM) of the formaldehyde-fixed blanks were subtracted from the mean DPM of the respective samples and the resulting DPM converted into leucine incorporation rates. Prokaryotic carbon biomass production was estimated using the conservative theoretical conversion factor of 1.55 kg C mol^{-1} Leu incorporated assuming no internal isotope dilution (Kirchman & Ducklow 1993).

Respiratory activity of the electron transport system (ETS)

ETS activity was measured following the modifications of the tetrazolium reduction technique as described in Arístegui & Montero (1995). Some minor modifications of the method were made to increase its sensitivity. Briefly, about 10 L of sample was filtered through a Whatman GF/F filter (47-mm diameter). Filters were folded into cryovials and immediately stored in liquid nitrogen until analysis in the laboratory. Back in the laboratory, the filters with the collected material were homogenized in 2.5 mL phosphate buffer with a Teflon-glass tissue grinder at 0–4ºC for 1.5 min. A 0.9 mL aliquot of the crude homogenate was incubated in duplicate with 0.5 mL of substrate solution and 0.35 mL of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) at 18ºC for 20 min. The reaction was quenched by adding 0.25 mL of a mixture of formalin and phosphoric acid. The quenched reaction mixture was centrifuged at 4000 *g* at 4ºC for 20 min and the absorbance of the particle-free solution measured in a Beckman DU-650 spectrophotometer at 490 and 750 nm wavelength after adjusting the sample to room temperature. Readings at 750 nm, to correct for turbidity, were always negligible. In addition to the samples, duplicate controls were run by replacing the crude extract with a clean Whatman GF/F filter homogenized in phosphate buffer. ETS activity was calculated using the equation given in Packard & Williams (1981):

$$ETS_{ASSAY}\ (mmol\ O_2\ m^{-3}\ h^{-1}) = H \times S \times (OD_{corr}) / (1.42 \times V \times f \times t/60) \times 22.4 \quad (1)$$

where H is the volume of the homogenate (in mL), S is the volume of the quenched reaction mixture (in mL), OD_{corr} is the absorbance of the sample measured at 490 nm wavelength and corrected for blank absorbance, V is the volume (in L) of the seawater filtered through the Whatman GF/F filter, f is the volume of the homogenate used in the assay (in mL), t is the incubation time (in min), 60 converts minutes to hours, the factor 1.42 converts the INT-formazan formed to oxygen units (in μL) and 22.4 converts the μL O_2 to μmol O_2. ETS activity was corrected to in situ temperature using the following equation:

$$ETS_{IN\ SITU} = ETS_{ASSAY} \times e^{(Ea/R \times (1/Tass - 1/Tis))} \quad (2)$$

where Ea is the Arrhenius activation energy (in kJ mol^{-1}), R is the gas constant, and Tass and Tis are the temperatures (in degrees Kelvin) in the assay and in situ, respectively. A calculated activation energy of 67 kJ mol^{-1} was used (Arístegui & Montero 1995). Actual rates of oxygen consumption in carbon units (R) were calculated assuming a R:ETS = 0.09 (*see* details in the discussion section) and a respiratory quotient of 1.

Particulate organic carbon (POC) and organic nitrogen (PON)

Samples (4-6 L) for POC and PON were filtered through combusted (450ºC for 12 h) 25-mm Whatman GF/F filters. The filters were wrapped in combusted aluminum foil and kept frozen at -20ºC until processed. In the laboratory, the filters were thawed and dried overnight at 65ºC in a desiccator under HCl fumes to remove carbonates and finally, dried overnight in a desiccator with silica gel. Prior to analysis, samples were packed into ultraclean tin caps. The carbon analyses were performed on a Perkin-Elmer 2400 CHN (carbon, hydrogen, nitrogen) elemental analyzer according to the JGOFS (Joint Global Ocean Flux Study) protocol (UNESCO 1994). Measurements of organic material from low volume filtrations may be questionable due to the possible sorption of DOM on the filter material (Moran et al. 1999, Turnewitsch et al. 2007). Hence, sorption blanks were determined for each station by placing 2 Whatman GF/F filters together on the filter holder while filtering the water sample. The adsorbed organic C

and N collected by these filters was measured as described above and subtracted from the POC- and PON-content of the corresponding particulate samples.

Total organic carbon (TOC)

For TOC determinations, 8 mL of the unfiltered sample was pipetted into combusted (450°C for 4 h) glass ampoules, immediately acidified with 3 – 4 drops of 45 % H_3PO_4 and the ampoule sealed. Samples were stored at –20°C until analysis. TOC analysis was performed using the high temperature combustion method on a modified Shimadzu TOC-5000A. The absorbance of the CO_2 evolving from the combusted TOC was detected with an external infrared cell (LiCor Model LI-6252) and quantified as peak area by the Shimadzu integrator. Per sample, 100 μL was automatically injected in quadruplicate with a Shimadzu ASI-5000A autosampler on a platinized aluminum catalyst (Elemental Microanalysis) at a combustion temperature of 680°C (Benner & Strom 1993). Standards were prepared with potassium hydrogen phthalate (Baker) in Milli-Q water. The overall analytical precision was always < 3 %. The reported TOC concentrations resemble DOC (water filtered through rinsed 0.2 μm polycarbonate filters) since there was no significant difference between corresponding TOC and DOC samples (data not shown). Occasionally, the DOC samples were even slightly higher than the corresponding TOC values.

Dissolved organic nitrogen (DON)

For DON determinations, 10-100 mL of filtered (through combusted Whatman GF/F glass fiber filters) water was collected in acid-rinsed polyethylene bottles. The DON and DOP (data not shown) analyses were performed simultaneously according to the method of Valderrama (1981). This procedure is based on an alkaline persulfate digestion (at 120°C in an autoclave for 90 min) over a wide pH range starting at pH 9 and ending at pH 4 using boric acid and sodium hydroxide. The resulting NH_4^+ was measured using an autoanalyzer (TRAACS). DON concentrations were obtained by subtracting the sum of the concentration of the inorganic nitrogen species (NH_4^+ + NO_3^- + NO_2^-) from the corresponding total dissolved N concentrations. To check the efficiency of DON determinations, 10 different compounds containing organic N were measured in parallel with the samples. For this reference material, the recovery efficiency of organic nitrogen was 92 % of the calculated organic nitrogen concentration.

Statistical analysis

To check the normality of the individual data sets, the Kolmogorov–Smirnoff test was used. If normality was not attained, the non-parametric Mann–Whitney test (for comparison of two independent variables) was applied. Data were log transformed to attain normality before Spearman's rank correlation coefficients were calculated.

Results

Prokaryotic abundance (PA) and prokaryotic heterotrophic production (PHP)

PA ranged between 2.2 – 3.9 x 10^5 cells mL^{-1} at 100 m depth and from 0.1 – 0.8 x 10^5 cells mL^{-1} in the meso- and bathypelagic waters, decreasing exponentially with depth (Fig. 2A). No significant differences were found in the PA of the subsurface layer (100 m depth) between the SG and the NEqCC (Mann–Whitney, $p > 0.05$, $n = 42$, Fig. 2A). In the OML, intermediate and deep-water masses, PA was, however, significantly higher in the NEqCC than in the corresponding water masses of the SG (Mann–Whitney tests; OML: $p < 0.01$, $n = 44$; AAIW: $p < 0.001$, $n = 22$; NEADW: $p < 0.001$, $n = 22$; LDW: $p < 0.001$, $n = 43$, Fig. 2A).

PHP decreased with depth by three orders of magnitude (Fig. 2B) and followed the same vertical distribution as PA. The difference in PHP between both regions was not significant in the NEADW even though PHP was about twice as high in the NEqCC than in the SG. Cell-specific PHP decreased only by one order of magnitude (Fig. 2C) following the same pattern as bulk PHP. Cell-specific PHP was higher in the subsurface waters of the SG than in the NEqCC (Mann–Whitney, $p < 0.01$, $n = 22$), but not significantly different in the intermediate water masses and NEADW (Mann–Whitney, $p > 0.05$, $n = 22$ and $n= 19$ for intermediate water masses and NEADW, respectively). Remarkably, cell-specific PHP was about three times higher in the LDW of the SG than of the NEqCC and higher than the cell-specific PHP of NEADW and intermediate waters (Fig. 2C). Prokaryotic turnover time (C-biomass/PHP) increased from 22 ± 3 d in the subsurface layer to 798 ± 193 d in the LDW with no significant differences between the water masses in the NEqCC and the SG (Fig. 2D).

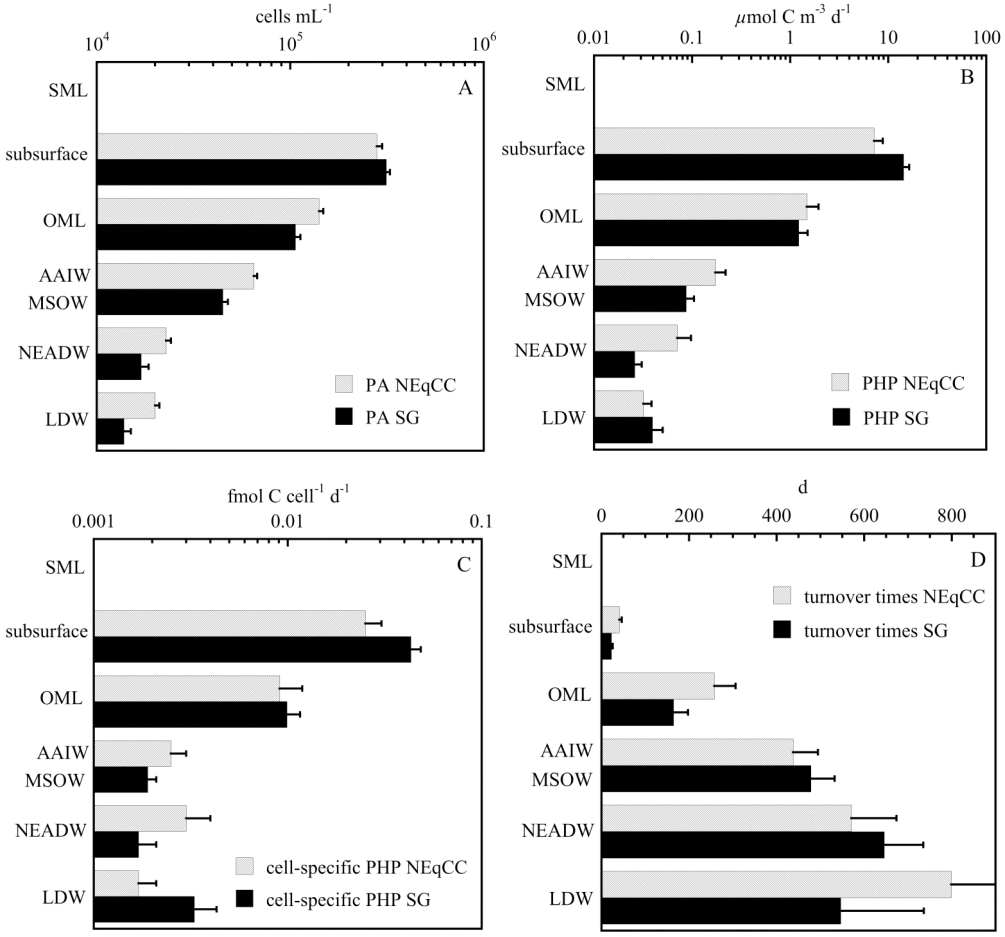


Fig. 2. Distribution of basic prokaryotic parameters in the different water masses in the subtropical gyre (SG) and North Equatorial Counter Current (NEqCC) zones (*see* Table 1 for depths of each water mass). (A) prokaryotic abundance (cell^{-1} mL^{-1}), (B) prokaryotic heterotrophic production (μmol C m^{-3} d^{-1}), (C) cell-specific prokaryotic heterotrophic production (fmol C cell^{-1} d^{-1}), (D) turnover time (d). Bars represent means ± SE

Potential respiration estimated via ETS measurements

Potential respiration rates as determined by ETS measurements decreased over the sampled depth-range by two orders of magnitude (Fig. 3A). ETS values in the SML were significantly higher in the NEqCC than in the SG (Mann–Whitney, $p < 0.02$, $n = 21$). No significant differences were found, however, in the ETS of the subsurface and the OML between the SG and the NEqCC (Mann–Whitney, $p > 0.05$, $n = 24$ and $n = 46$ respectively, Fig. 3A). Remarkably, ETS, like PA and PHP (except in the LDW), was higher in all the deep-water masses of the NEqCC than in the SG (Mann–Whitney tests; AAIW: $p < 0.02$, $n = 21$; NADW: $p < 0.001$, $n = 23$; LDW: $p < 0.02$, $n = 21$, Fig. 3A). Although both, prokaryotic abundance (Fig. 2A) and potential respiration (Fig. 3A)

decreased with depth, cell-specific ETS showed a different trend: first decreasing with depth to reach the lowest values in the intermediate water masses (AAIW and MSOW) and then increasing again, reaching the highest cell-specific ETS of all the water masses in the LDW (Fig. 3B). Cell-specific ETS was significantly higher in the deep water masses (NEADW: Mann–Whitney, $p < 0.02$, $n = 23$; LDW: Mann–Whitney, $p < 0.05$, $n = 21$) of the NEqCC than in the SG, with a cell-specific ETS in the LDW of the NEqCC being two times higher than in the subsurface waters (Fig. 3B). A pronounced latitudinal gradient in ETS was detectable below 1000 m depth with increasing values towards the south, particularly in the NEADW (core around 2750 m depth) (Fig. 4A).

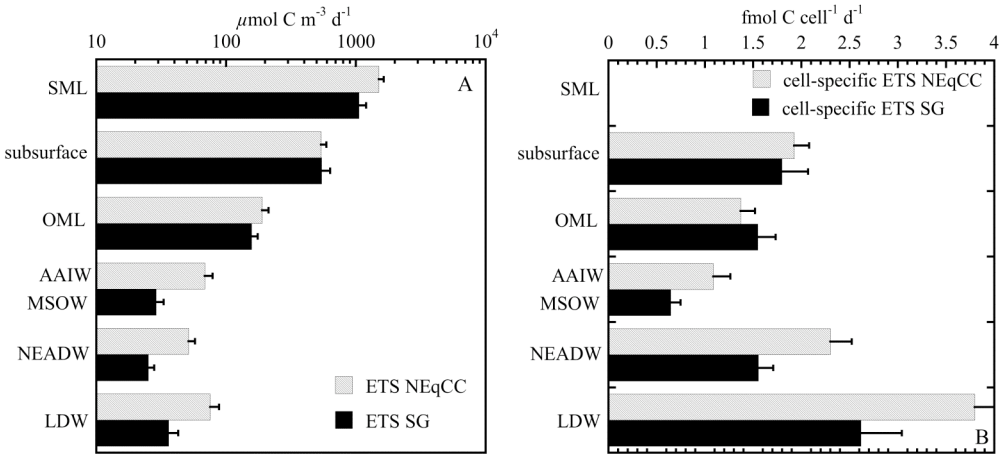


Fig. 3. Distribution of (A) ETS (μmol C m^{-3} d^{-1}) and (B) cell-specific ETS (fmol C $cell^{-1}$ d^{-1}) throughout the water column along the subtropical gyre (SG) and North Equatorial Counter Current (NEqCC) zones (*see* Table 1 for depths of each water mass). Bars represent mean ± SE

Particulate (POM) and dissolved organic matter (DOM) distribution in the water column

Like ETS, also POC but not DOC (compare Fig. 4B and C) increased towards the south along the Mid-Atlantic Ridge in the deep waters, except in the LDW, where the influence of POC resuspended from the ocean bottom (as inferred from the high C:N ratios) was apparent. Overall, POC concentrations were significantly higher in the water masses of the NEqCC than of the SG (Mann–Whitney, $p < 0.0001$, $n = 163$, Fig. 5A) except in the LDW (Mann–Whitney, $p > 0.05$, $n = 22$). Strikingly, POC concentrations were higher in the OML than in the water masses above and below (Fig. 5A), a fact that could be due to increasing density and reduced sinking rates in micro-density gradients between water masses.

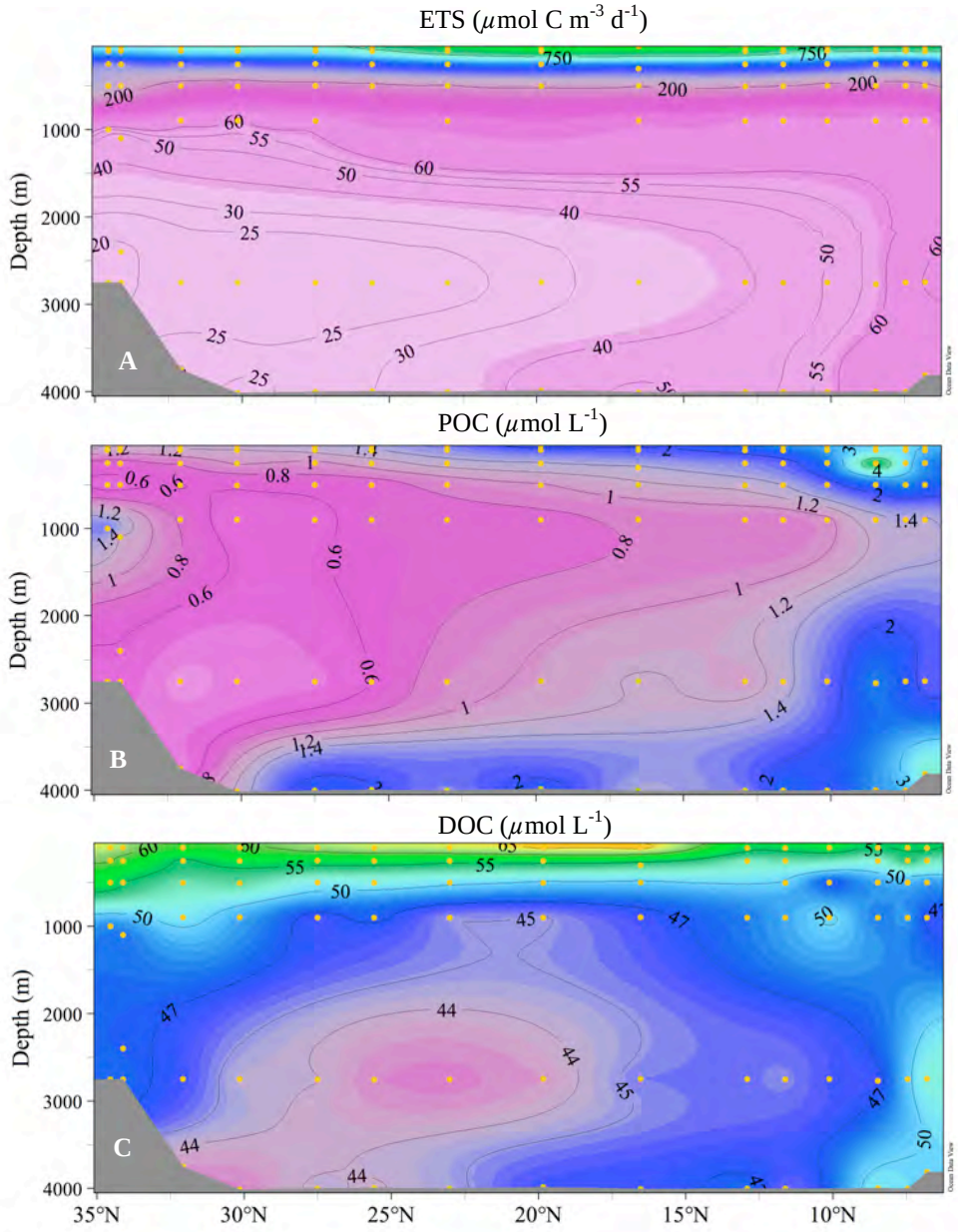


Fig. 4. Distribution of (A) ETS (μmol C m^{-3} d^{-1}), (B) POC (μmol L^{-1}), (C) and DOC (μmol L^{-1}) throughout the water column along a transect east of the Mid-Atlantic Ridge in the North Atlantic extending from stations 40 to 16 as shown in Fig. 1

PON concentrations were generally an order of magnitude lower than POC (Fig. 5B). In contrast to POC concentrations, PON was higher in almost all the water masses of the SG region (compare Fig. 5A and B). The molar C:N ratio of the POM clearly increased with depth (Fig. 5C). Throughout the entire water column, the mean C:N ratio of POM was significantly higher in the NEqCC than in the SG (Mann–Whitney, $p < 0.01$, $n = 157$), due to the higher POC and lower PON concentrations in the NEqCC as

compared to the SG.

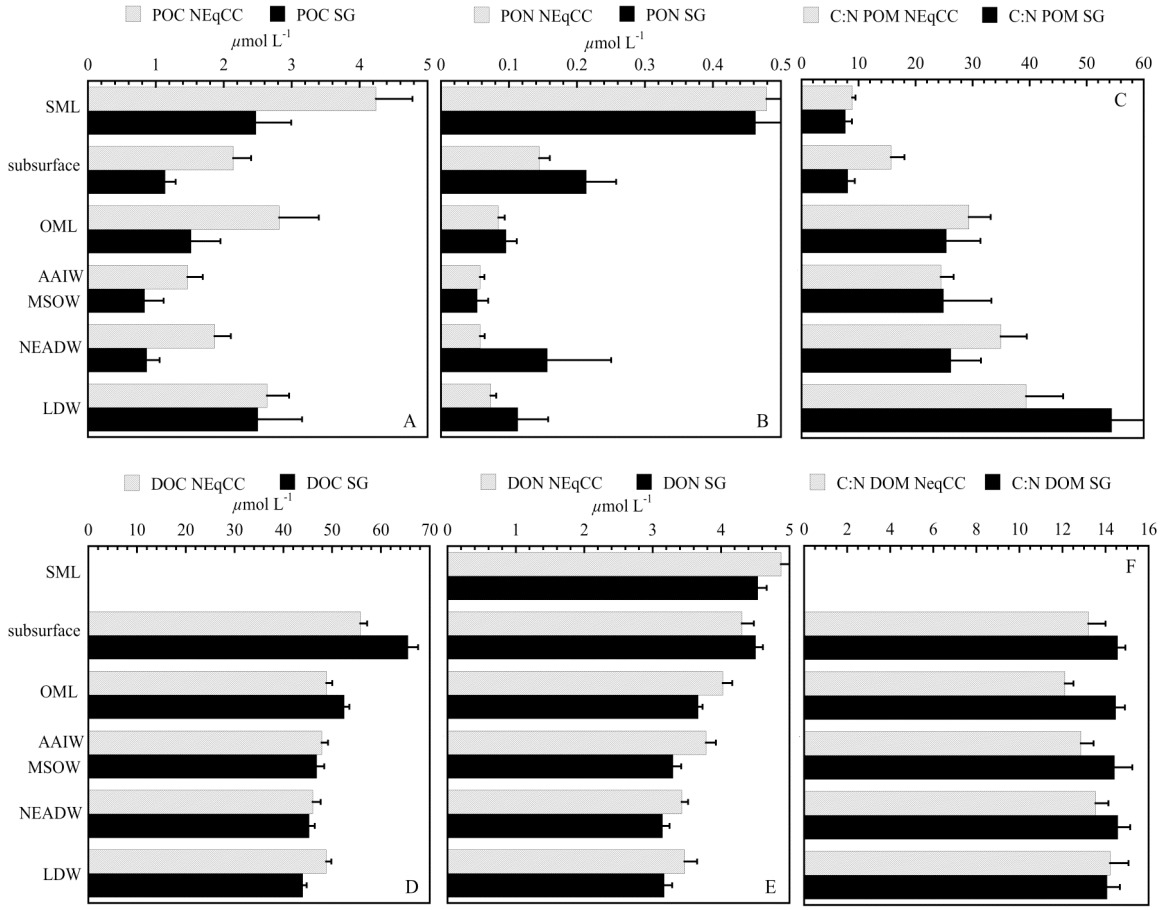


Fig. 5. Distribution of basic prokaryotic parameters in the different water masses of the subtropical gyre (SG) and North Equatorial Counter Current (NEqCC) zones. (A) POC (μmol L^{-1}), (B) PON (μmol L^{-1}), (C) POC:PON molar ratio, (D) DOC (μmol L^{-1}), (E) DON (μmol L^{-1}), (F) DOC:DON molar ratio. *See* Table 1 for depths of each water mass. Bars represent means ± SE

In contrast to POC, DOC concentrations remained rather stable throughout the water column (Fig. 5D). There was no significant difference in DOC concentrations between the NEqCC and SG water masses (Mann–Whitney, $p > 0.05$ for all water masses; Fig. 4C, Fig. 5D). DON concentrations were generally one order of magnitude lower than DOC values, slightly decreasing with depth (Fig. 5E). Higher DON concentrations were obtained for the deep-water masses of the NEqCC than of the SG, except in the LDW (Mann–Whitney tests; OML: $p < 0.02$, $n = 48$; AAIW: $p < 0.03$, $n = 24$; NADW: $p < 0.05$, $n = 26$; LDW: $p > 0.20$, $n = 22$). In contrast to the POM pool (Fig. 5C), the C:N molar ratio of the DOM pool (Fig. 5F) showed essentially no

variation with depth. The C:N of the DOM was significantly lower in the NEqCC than in the SG (Mann–Whitney, $p < 0.0002$, $n = 141$).

Discussion

The average depth distribution of the C:N molar ratio of the POM pool (Fig. 5C) shows that the ratio is substantially higher in the meso- and bathypelagic waters than in the upper 200 m layer, but without a clear depth gradient across these layers. Only the LDW of the Subtropical Gyre shows a significant increase in the C:N ratio due to a marked enhancement in the POC concentrations (but not in PON), possibly caused by lateral advection of N-depleted POM. The high C:N ratios of the POM pool in the dark ocean (about two times higher than the average C:N ratio of DOM; Fig. 5F) indicate a preferential utilization of PON by the microbial assemblages. However, the lack of a clear depth gradient in the C:N ratios suggests that the source of this POM may not be related to the sinking flux from the overlying surface ocean. Fig. 4B illustrates a latitudinal POC gradient, increasing from 35°N to 5°N, with higher POC concentrations in the deep-ocean near the equator. The gradient is more apparent below 1000 m depth, coinciding with a similar latitudinal trend in ETS (Fig. 4A). Taken together, this suggests a predominant lateral transport of suspended particles. Although the ranges of variability in POC and ETS are low, POC is strongly positively correlated with ETS (Spearman's $rho = 0.70$, $p < 0.02$, Fig. 6A) in the NEADW of the SG but not in the NEqCC. The lack of a correlation between POC and ETS in the NEqCC is probably a consequence of the more refractory POM compared to the SG (Fig. 5A-C). The AAIW also shows a strong correlation between POM and microbial activity. POC (Fig. 6B) and PON are both correlated with ETS (Spearman's $rho = 0.82$, $p < 0.0001$ for POC and 0.93, $p < 0.0001$ for PON) and cell-specific ETS (Spearman's $rho = 0.83$, $p < 0.002$ for POC and 0.66, $p < 0.02$ for PON). The fact that POM and ETS activity are strongly correlated along these water masses supports the hypothesis that deep-water prokaryotic activity depends to a large extent on suspended particles.

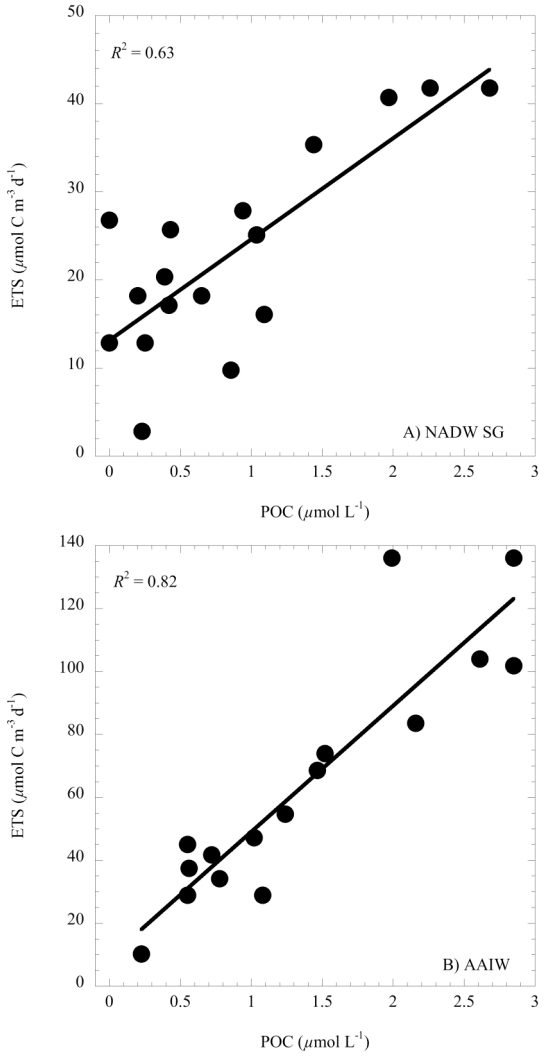


Fig. 6. Relation between ETS (μmol C m^{-3} d^{-1}) and POC (μmol L^{-1}) in the NEADW of the (A) SG and in the (B) AAIW. Data are derived from Fig. 5A (for POC) and Fig. 3A (for ETS)

There are very few published studies relating particle flux and deep-water microbial metabolism, and these report contradictory results. Nagata et al. (2000) concluded from a study in the subarctic Pacific that the deep-water microbial activities are related to the sinking POC fluxes. Also, Simon et al. (2004) found that prokaryotic production in the mesopelagic waters was significantly correlated to primary production and surface chlorophyll in the Southern Ocean. Moreover, Anderson et al. (2007) concluded for the oxygen minimum zone of the Arabian Sea, that the modeled vertical export flux of detritus was sufficient to account for the measured bacterial production below the euphotic zone in the Arabian Sea. Ducklow (1993) found that the rates of DOC release from the sinking particle flux is sufficient to support bacterial production in the Gulf of Oman, however, insufficient to meet the bacterial carbon demand in the

open Arabian Sea. Additionally, Hansell & Ducklow (2003) found that only prokaryotic abundance and not activity was related to the POC flux in the Arabian Sea, suggesting that deep-ocean prokaryotic abundance and activity might depend on the long-term averages of POC flux and not on the more immediate flux. Recently, Steinberg et al. (2008), found that the POC flux is too low to meet the bacterial and mesozooplankton carbon demand at two sites in the Pacific. These authors proposed diel vertical migration of zooplankton and the associated POC transfer as a mechanism to provide additional organic carbon for mesopelagic metabolism to resolve the discrepancy between the POC flux and the mesopelagic carbon demand. Taken together, these contradictory findings may reflect variability in the transfer efficiency of organic material from the euphotic layer to the deep sea (Francois et al. 2002) or differences in the magnitude of other carbon sources, like lateral transport of suspended POM and DOM. Arístegui et al. (2005b) invoked a coastal-ocean lateral transport of POM to explain the high prokaryotic metabolic rates measured in the mesopelagic zone of the Canary Current, where POM concentrations are several times higher than in the open ocean waters of the Subtropical Gyre (Neuer et al. 2007). A recent box-model study of the Canary Current region (Alonso-González et al. 2009) supports this view, providing evidence that suspended POM may account for up to 60 % of the remineralization rates in the mesopelagic zone.

In order to examine the balance between vertical carbon flux and the prokaryotic carbon demand (PCD = PHP + R), we estimated the sinking POC flux using the model of Antia et al. (2001), based on sediment trap data collected from the subpolar region to the oligotrophic subtropical North Atlantic. The POC available at a depth "z" (in m) is derived from the following equation:

$$POC\ (mmol\ C\ m^{-3}\ d^{-1}) = 0.068 \times PP^{1.77} \times z^{-1.68} \quad (3)$$

where PP is the primary production of the mixed-layer (in mmol C m^{-2} d^{-1}).

The potentially available POC is calculated using the lower and upper value of the range of primary production (12-90 mmol C m^{-2} d^{-1}) provided by Antia et al. (2001) for the NE Atlantic. We assume that a primary production of 12 mmol C m^{-2} d^{-1} is representative for the SG and 90 mmol C m^{-2} d^{-1} for the NEqCC region. Using these primary production rates, the available POC is only sufficient to support the prokaryotic carbon demand (PCD) in the euphotic zone (Table 2), whereas below the subsurface waters, the PCD exceeds POC availability. The gap between PCD and POC increases with depth. The main responsible for the increasing difference between PCD and POC

availability is microbial respiration, since PHP decreases 10 times more with depth than respiration (R). To further constrain prokaryotic carbon demand, a PGE of 0.04 was used as well (Table 2) based on a compilation of data of PHP and R from the global dark ocean (Arístegui et al. 2009). Also the OUR (oxygen utilization rates) for the mesopelagic zone in the North and South Atlantic are given in Table 2 (Jenkins 1982, Jenkins & Wallace 1992, Brea 2008). Generally, PCD estimated via ETS is similar to that obtained by using the prokaryotic growth efficiency in the mesopelagic zone, but higher in the bathypelagic. This may be caused by either overestimating the ETS in the bathypelagic (by using an inappropriate R:ETS ratio), or by underestimating the PCD by using a too high PGE or by underestimating PHP.

It is unlikely that the disparity between POC fluxes and PCD is caused by an overestimation of R. There is some uncertainty related to ETS measurements because ETS provides, as other enzymatic measurements, only potential rates, which need to be converted to actual rates by conversion factors. ETS has been shown, however, to be closely correlated to actual oxygen consumption measurements in lakes (del Giorgio 1992), the deep North Atlantic (Reinthaler et al. 2006) and the epipelagic ocean using a large global data set (Arístegui & Montero 1995). Christensen et al. (1980), working with marine bacteria cultures, found that the R:ETS of bacteria during the exponential growth phase ranges between 0.6 and 1.7, whereas in the senescent phase this ratio drops to 0.09. In the present study we have applied an R:ETS ratio of 0.09 to convert ETS measurements to respiration rates assuming that only a fraction of the bacterial community is metabolically active. However, this ratio (and hence R) might be somewhat higher at least in the mesopelagic zone. Recently, Arístegui et al. (2005b) measured R and ETS in the mesopelagic waters of the Canary Region obtaining a R:ETS of 0.68 ± 0.11, in agreement with a highly active prokaryotic assemblage, as confirmed by several other concomitantly measured proxies for bacterial activity. This latter ratio, however, may be not applicable to the bacterial assemblages in the present study, since the mesopelagic waters of the Canary region are characterized by much higher suspended POM concentrations than the water masses investigated in this study. Anyway, for the purpose of our study, the R derived from ETS (using a R:ETS ratio of 0.09) may represent an underestimation (but unlikely an overestimation) of the actual R in the dark ocean. Thus, we conclude that there is an imbalance between the POC available from the sinking flux and oxygen consumption in the dark ocean, particularly in the bathypelagic zone where ETS and POC are significantly correlated. Jenkins and

Table 2. Comparison of the variation in the different water masses and regions of prokaryotic heterotrophic production (PHP), respiration rates via ETS (R(ETS)), prokaryotic carbon demand (PCD) calculated using a prokaryotic growth efficiency (PGE) of 0.04 (PCD (PGE)) (*see* text for details) and PCD derived from ETS and PHP measurements (PCD(ETS)) assuming an R:ETS of 0.09 (*see* text for details), available sinking POC (POC Av) inferred from the POC flux model of Antia et al. (2001) assuming a surface primary production of 12 and 90 mmol C m^{-2} d^{-1} (*see* text for details), oxygen utilization rate (OUR) calculated by Jenkins (1982) for the North Atlantic Subtropical Gyre (OUR NAtl), and OUR estimated by Brea (2008) for the South Atlantic Subtropical Gyre (OUR SAtl) (*see* text for details). SG: Subtropical Gyre, NEqCC: North Equatorial Counter Current, SML: surface mixed layer, OML: oxygen minimum layer, MSOW: Mediterranean Sea Overflow Water, NEADW: North East Atlantic Deep Water, LDW: Lower Deep Water, AAIW: Antarctic Intermediate Water. All units in µmol C m^{-3} d^{-1}. SE in brackets

Water mass	Depth range (m)	PHP NEqCC	PHP SG	R (ETS) NEqCC	R(ETS) SG	PCD (PGE) NEqCC	PCD (PGE) SG	PCD (ETS) NEqCC	PCD (ETS) SG	POC Av PP=90	POC Av PP=12	OUR NAtl	OUR SAtl
LDW	3500-5000	0.0318 (0.007)	0.0394 (0.011)	6.50 (1.15)	3.09 (0.62)	0.80 (0.17)	0.99 (0.27)	6.53 (1.17)	3.13 (0.64)	0.18 (0.04)	0.01 (0.001)		
NEADW	2750	0.0704 (0.027)	0.026 (0.005)	4.42 (0.57)	2.16 (0.25)	1.76 (0.67)	0.65 (0.12)	4.49 (0.60)	2.19 (0.26)	0.33 (0.06)	0.01 (0.002)		
AAIW/MSOW	900-1000	0.1724 (0.047)	0.0869 (0.018)	5.90 (0.92)	2.49 (0.38)	4.31 (1.17)	2.17 (0.44)	6.07 (0.98)	2.58 (0.40)	2.07 (0.41)	0.06 (0.012)	6.7	11.8
OML	250-500	1.4748 (0.47)	1.2169 (0.30)	16.30 (2.07)	13.50 (1.62)	36.87 (11.9)	30.43 (7.3)	17.77 (2.49)	14.72 (1.91)	12.12 (1.75)	0.34 (0.05)	14.7	18.3
subsurface	100	7.1599 (1.62)	14.302 (2.11)	46.44 (4.86)	46.94 (8.1)	179.0 (40)	357.5 (52.8)	53.60 (6.48)	61.24 (10.2)	84.54 (16.9)	2.39 (0.478)		
SML	50			129.36 (12)	90.88 (13)					265.10 (56.5)	7.49 (1.59)		

Subtropical Gyres, respectively. This approach has its own inherent uncertainty, due to the fact that water masses might mix while aging. Our ETS-derived R rates are in good agreement with the biogeochemical OUR estimates (Table 2), although OUR is somewhat higher than the ETS-derived R, as expected if the latter represents most likely an underestimation. In any case, both, the OUR and ETS-derived R rates are higher than the estimated sinking POC reaching the lower mesopelagic zone. The gap between POC supply and respiration increases towards the bathypelagic ocean. In summary, although we are aware of the degree of uncertainty in R derived from ETS and also in the OUR (obtained from changes in the biogeochemical fields), the two independent approaches yield comparable estimates, which do not match with the calculated sinking fluxes of POC.

Besides the uncertainties in ETS measurements, decompression of samples prior to measuring prokaryotic production by leucine incorporation might introduce biases. It has been shown that metabolic rates of deep-sea bacterioplankton incubated under in situ pressure conditions were 4.5 ± 4.5 (mean ± SD; n = 19) times higher than under decompressed conditions (Tamburini et al. 2003). If this applies also to our deep-water samples, the actual prokaryotic carbon demand would be even higher and hence, the gap between POC supply and carbon demand wider.

Apart from these uncertainties in the carbon demand estimates in the meso- and bathypelagic realm, POC supply estimates might be biased as well. Sediment traps are known to preferentially collect fast-sinking particles leaving buoyant or slow-sinking particles underrepresented (Honjo et al. 1984, Asper 1987). Nevertheless, the POC flux derived from the model of Antia et al. (2001) is corrected for ^{230}Th hence, should not underestimate the actual flux. The thorium approach has been used to calibrate sediment traps (Scholten et al., 2001) and suggests that for any given site, the efficiency of the shallower (< 1500 m depth) traps is consistently lower than that of the deep traps at the same site. This lower collection efficiency at shallower depths in moored deep ocean conical traps is likely caused by a combination of effects. Higher turbulence at shallower depths than in deep-waters might disrupt fragile, marine snow-type particles around moored sediment traps. Also swimmers are commonly more abundant at shallower waters than in the bathypelagic region, which might feed on accumulating POM in the traps. The nature and hydrodynamic properties of the settling particles might also change with depth as particles are becoming more consolidated and denser through cycles of aggregation and disaggregation (Buesseler et al. 2007).

The slow-sinking or buoyant suspended POM might arise from partial remineralization of sinking particles or from lateral input of neutrally buoyant particles from distant sources, like ocean margins. A recent study in the Canary Current region indicates that POM collected with bottles largely contributes to the carbon flux in the dark ocean (Alonso-González et al. 2009). Thus, lateral transport of suspended particles might at least partly compensate for the observed imbalance between estimated prokaryotic carbon demands in the deep-ocean and export fluxes derived from sinking particles collected with sediment traps. However, since suspended POM concentrations are much smaller in the open ocean than in boundary currents or continental margins, other reasons must be invoked to account for the great deficit observed between the vertical carbon fluxes and oxygen consumption rates in open ocean regions (Reinthaler et al. 2006). Steinberg et al. (2008) suggested that particle transfer by migrating mesozooplankton feeding in the euphotic layer and residing in the mesopelagic waters during the day might provide additional POC not captured by sediment traps and fuel mesopelagic carbon demand.

Evidence has been accumulating recently, suggesting a preferential particle-associated life mode of prokaryotes in the deep ocean. DeLong et al. (2006) found that deep-water prokaryotes are enriched in transposases, polysaccharide and antibiotic synthesis genes and have high levels of chaperone-encoding genes, all suggesting a predominately particle-attached life mode. Arístegui et al. (2005b) and Reinthaler et al. (2006) reported an increasing nucleic acid content per cell with depth, indicative for a larger genome size which, in turn, might point to an opportunistic life style (Lauro & Bartlett 2007).

In summary, our data, together with other published evidence, clearly suggest that microbial life in the dark ocean is more closely related to suspended particles than hitherto assumed. Most of these particles are not collected with sediment traps. Thus, estimates of carbon availability derived exclusively from sinking fluxes lead to underestimations of the total carbon supply to prokaryotes, and hence to imbalances with the prokaryotic carbon demand. The correlation between the particulate fraction of the organic matter pool and the activity of prokaryotes could be expected a priori taking into account that cell-specific activities are higher in particle-associated than in free-living microbes, the latter thriving in a highly refractory organic matter environment. The notion that microbes grow associated with (colloidal) particles in the deep ocean, where they may maintain high metabolic activities, explains also the discrepancy found

between the PCD and the sinking POC flux. However, the collection of samples in the water column with standard methods frequently leads to the disruption of the size-continuum of organic matter, possibly altering the colloidal micro-environment where microbial assemblages develop. Thus, new sampling strategies are needed to selectively collect deep ocean particles to decipher the actual role of particle-attached versus free-living microbes in the deep ocean's biogeochemical cycles.

Acknowledgements

We thank the captain and crew of R/V *Pelagia* for their help during work at sea. S. Gonzalez and K. Bakker performed the Total Organic Carbon and nutrient analyses, respectively, and A. Smit the leucine incorporation measurements. M. F. Montero, and M. Espino helped with the Electron Transport System analyses and I. J. Alonso-González with the particulate organic matter measurements. Water masses were identified by H. M. van Aken. We also thank two anonymous reviewers for their comments and suggestions to improve the manuscript. This research was supported by a predoctoral Fellowship of the Spanish Ministry of Education and Science (AP2005-3932) to F.B., a grant of the Earth and Life Science Division of the Dutch Science Foundation (ALW-NWO; ARCHIMEDES project, 835.20.023) to G. J. H. and a grant of the Spanish Ministry of Education and Science to J. A. (Remolinos Oceánicos y Deposiciones Atmosféricas (RODA) project; CTM 2004-06842-C03/MAR). The work was carried out within the frame of the EU 'Networks of Excellence' MarBef and EurOceans.

References

Alonso-González IJ, Arístegui J, Vilas JC, Hernández-Guerra A (2009) Lateral transport and consumption in surface and deep waters of the Canary Current: A box model study. Glob. Biogeochem. Cy. 23: GB2007, doi: 10.1029/2008GB003185.

Antia AN, Koeve W, Fischer G, Blanz T, Schulz-Bull D, Scholten J, Neuer S, Kremling K, Kuss J, Peinert R, Hebbeln D, Bathmann U, Conte M, Fehner U, Zeitzschel B (2001) Basin-wide particulate carbon flux in the Atlantic Ocean: Regional export patterns and potential for atmospheric CO_2 sequestration. Glob. Biogeochem. Cy. 15:845-862

Arístegui J, Montero MF (1995) The relationship between community respiration and ETS activity in the ocean. J. Plankton Res. 17:1563-1571

Arístegui J, Agustí S, Middelburg JJ, Duarte CM (2005a) Respiration in the mesopelagic and bathypelagic zones of the ocean, In: Williams PJleB & del

Giorgio PA (eds) Respiration in Aquatic Ecosystems. Oxford University Press, New York

Arístegui J, Duarte CM, Gasol JM, Alonso-Sáez L (2005b) Active mesopelagic prokaryotes support high respiration in the subtropical northeast Atlantic Ocean. Geophys. Res. Lett. 32:L03608, doi:03610.01029/02004GL021863

Arístegui J, Gasol JM, Duarte CM, Herndl GJ (2009) Microbial oceanography of the dark ocean's pelagic realm. Limnol. Oceanogr. 54(5):1501-1529

Asper VL (1987) Measuring the flux and sinking speed of marine snow aggregates. Deep-Sea Res. 34:1-17

Bauer JE, Druffel ERM (1998) Ocean margins as a significant source of organic matter to the deep open ocean. Nature 392:482-485

Brea S (2008) Circulación, mezcla, ventilación y mineralización de las aguas del Atlántico Sur. Ph. D. thesis. Univ. of Vigo.

Cho BC, Azam F (1988) Major role of bacteria in biogeochemical fluxes in the ocean's interior. Nature 332:441-443

Christensen JP, Owens TG, Devol AH, Packard TT (1980) Respiration and physiological state in marine bacteria. Mar. Biol. 55:267-276

del Giorgio PA (1992) The relationship between ETS (electron transport system) activity and oxygen consumption in lake plankton: a cross-system calibration. J. Plankton Res. 14:1723-1741

del Giorgio PA, Duarte CM (2002) Respiration in the open ocean. Nature 420:379-384

DeLong EF, Preston CM, Mincer T, Rich V, Hallam SJ, Frigaard NU, Martínez A, Sullivan MB, Edwards R, Brito BR, Chisholm SW, Karl DM (2006) Community genomics among stratified microbial assemblages in the ocean's interior. Science 311:496-503

Francois R, S. Honjo, Krishfield R, Manganini S (2002) Factors controlling the flux of organic carbon to the bathypelagic zone of the ocean. Glob. Biogeochem. Cy. 16:1087, doi:1010.1029/2001GB001722

Hansell DA, Ducklow HW (2003) Bacterioplankton distribution and production in the bathypelagic ocean: Directly coupled to particulate organic carbon export? Limnol. Oceanogr. 48:150-156

Honjo S (1980) Material fluxes and modes of sedimentation in the mesopelagic and bathypelagic zones. J. Mar. Res. 38:53-97

Honjo S (1986) Oceanic particles and pelagic sedimentation in the western North Atlantic Ocean, Volume M, The western North Atlantic Region. Geological Society of America, Washington, D. C.

Honjo S, Doherty KW, Agrawal YC, Asper VL (1984) Direct optical assessment of large amorphous aggregates (marine snow) in the deep ocean. Deep-Sea Res. 31:67-76

Jenkins WJ (1982) Oxygen utilization rates in the North Atlantic Subtropical Gyre and primary production in oligotrophic systems. Nature 300:246-248

Jenkins WJ, Wallace DWR (1992) Tracer based inferences if new primary production in the Sea, Plenum, New York

Karl DM, Knauer GA, Martin JH (1988) Downward flux of particulate organic matter in the ocean: a particle decomposition paradox. Nature 332:438-441

Kirchman D, K'Ness E, Hodson R (1985) Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. 49:599-607

Kirchman DL, Ducklow HW (1993) Estimating conversion factors for thymidine and leucine methods for measuring bacterial production. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, p 513-517

Lauro F, Bartlett DH (2007) Prokaryotic lifestyles in deep sea habitats. Extremophiles 12:15-25

McCave IN (1975) Vertical fluxes of particles in the ocean. Deep-Sea Res. 22:491-502

Moran SB, Charette MA, Pike SM, Wicklund CA (1999) Differences in seawater particulate organic carbon concentrations in samples collected using small- and large-volume methods: the importance of DOC adsorption to the filter blank. Mar. Chem. 67:33-42

Nagata T, Fukuda H, Fukuda R, Koike I (2000) Bacterioplankton distribution and production in the deep Pacific waters: large-scale geographic variations and possible coupling with sinking particle fluxes. Limnol. Oceanogr. 45:426-435

Neuer S, A. Cianca, P. Helmke, T. Freudenthal, R. Davenport, H. Meggers, M. Knoll, J. M. Santana-Casiano, M. González-Davila, M. J. Rueda, Llinás O (2007) Biogeochemistry and hydrography in the eastern subtropical North Atlantic gyre. Results from the European time-series station ESTOC. Progr. Oceanogr. 72:1-29

Packard TT, Williams PJLeB (1981) Rates of respiratory oxygen consumption and electron transport in surface seawater from the Northwest Atlantic. Oceanol. Acta 4:351–358

Reinthaler T, van Aken HM, Veth C, Williams PJleB, Arístegui J, Robinson C, Lebaron P, Herndl GJ (2006) Prokaryotic respiration and production in the meso- and bathypelagic realm of the eastern and western North Atlantic basin. Limnol. Oceanogr. 51:1262-1273

Simon M, Rosenstock B, Zwisler W (2004) Coupling of epipelagic and mesopelagic heterotrophic picoplankton production to phytoplankton biomass in the Antarctic polar frontal region. Limnol. Oceanogr. 49:1035-1043

Turnewitsch R, B. M. Springer, K. Kiriakoulakis, J. C. Vilas, J. Arístegui, G. Wolff, F. Peine, S Werk, Graf G, Waniek JJ (2007) Determination of particulate organic carbon (POC) in seawater: The relative methodological importance of artificial gains and losses in two glass-fiber-filter-based techniques. Mar. Chem. 105:208-228

UNESCO (1994) Protocols for the Joint Global Ocean Flux Study (JGOFS) Core Measurement. Intergovernmental Oceanographic Commission, Manual and Guides 29:169

Varela M, van Aken HM, Sintes E, Herndl GJ (2008) Latitudinal trends of Crenarchaeota and Bacteria in the meso- and bathypelagic water masses of the Eastern North Atlantic. Environ. Microb. 10:110-124

Chapter III

Prokaryotic extracellular enzymatic activity in relation to biomass production and respiration in the meso- and bathypelagic waters of the (sub)tropical Atlantic

F. Baltar, J. Arístegui, E. Sintes, H. M. van Aken, J. M. Gasol, G. J. Herndl

Environmental Microbiology 11 (8), 1998-2014 (2009)

Abstract

Prokaryotic extracellular enzymatic activity, abundance, heterotrophic production and respiration were determined in the meso- and bathypelagic (sub)tropical North Atlantic. While prokaryotic heterotrophic production (PHP) decreased from the lower euphotic layer to the bathypelagic waters by two orders of magnitude, prokaryotic abundance and cell-specific PHP decreased only by one order of magnitude. In contrast to cell-specific PHP, cell-specific extracellular enzymatic activity (alpha- and beta-glucosidase, leucine aminopeptidase, alkaline phosphatase) increased with depth as did cell-specific respiration rates. Cell-specific alkaline phosphatase activity increased from the intermediate water masses to the deep waters up to 5-fold. Phosphate concentrations, however, varied only by a factor of two between the different water masses, indicating that phosphatase activity is not related to phosphate availability in the deep waters. Generally, cell-specific extracellular enzymatic activities were inversely related to cell-specific prokaryotic leucine incorporation. Thus, it is apparent that the utilization of deep ocean organic matter is linked to higher cell-specific extracellular enzymatic activity and respiration and lower cell-specific PHP than in surface waters.

Introduction

The main source of dissolved organic matter (DOM) in the open ocean is phytoplankton extracellular release and grazing processes either directly on phytoplankton (‘sloppy feeding’) or within the microbial food web (Nagata 2000). Also, heterotrophic prokaryotes release copious amounts of DOM via their polysaccharidic envelope surrounding the cells (Heissenberger & Herndl 1994, Heissenberger et al. 1996, Stoderegger & Herndl 1998). Most of the grazing-related DOM release is in the form of high molecular weight DOM (HMW-DOM, >1000 Da) and also senescent phytoplankton release mainly HMW-DOM (Lignell 1990). This HMW-DOM, although comprising only about 20% of the total euphotic DOM pool and even less in the meso- and bathypelagic waters (Benner et al. 1992), is preferentially utilized by heterotrophic

prokaryotes over the bulk of the low molecular weight DOM (LMW-DOM) (Amon & Benner 1996). The preferential prokaryotic utilization of HMW-DOM requires cleavage of the substrate prior to uptake since only molecules <600 Da can be transported through the prokaryotic cell wall (Weiss et al. 1991).

Thus, prokaryotic plankton synthesize extracellular enzymes to hydrolyze organic matter (Hoppe et al. 2002). Commonly, macromolecules are cleaved by a consortium of hydrolases: endohydrolases cleave off oligomers and exohydrolases cleave the terminal ends of the oligomers to produce monomers (Hoppe et al. 2002). Fluorogenic substrate analogs are used to quantify in situ extracellular enzymatic activity (Hoppe et al. 2002). While fluorogenic model substrates lack the chemical complexity of natural substrates (Arnosti et al. 2005), they have been commonly applied to determine potential extracellular enzymatic activities over a broad range of aquatic environments (Chrost 1989, Rath et al. 1993, Misic et al. 2006, Misic & Fabiano 2006, Williams & Jochem 2006) since they were introduced to microbial ecology (Hoppe 1983). Most of the previous studies on extracellular prokaryotic activity focused on the euphotic zone and, in the marine environment, on coastal areas (e.g., Sala et al. 2001, Zaccone et al. 2003, Misic & Fabiano 2006, Williams & Jochem 2006). Only very few vertical profiles of extracellular enzymatic activities are available for the open oceans extending from the euphotic zone to the meso- or bathypelagic realm (Hoppe et al. 1993, Koike & Nagata 1997, Hoppe & Ullrich 1999, Fukuda et al. 2000).

Generally, heterotrophic prokaryotes in the meso- and bathypelagic layers of the ocean are limited by the availability of easily metabolizable organic matter. A specific sequence in the utilization of DOM has been shown for the oceanic water column as indicated by the increasing C:N:P ratio of the DOM in different depth layers (Hopkinson & Smith 2005). From the changes in the elemental composition of the DOM with depth, it appears that the phosphorus compounds are preferentially utilized, followed by nitrogenous compounds (Benner 2002, Hopkinson & Smith 2005). Consequently, the deep-ocean DOM is depleted in N and P but rich in C which is, however, rather refractory as indicated by its average age of about 6000 yrs (Bauer et al. 1992).

Sedimenting particulate organic matter (POM) might represent a major source of organic matter for deep-water microheterotrophic communities (Tamburini et al. 2003). As in DOM, POM also exhibits an increase in the C:N ratio with depth (Schneider et al. 2003, **Chapter II**). In contrast to organic N and P in the deep ocean, inorganic N and P

are readily accessible to heterotrophic prokaryotes (Kirchman 2000). Hence, it is reasonable to assume that deep-water prokaryotic extracellular enzymatic activity is primarily expressed to target the carbon moieties in the available deep-water organic matter. Hoppe & Ullrich (1999) detected high phosphatase activity in the mesopelagic waters of the Arabian Sea coinciding with high concentrations of phosphate. These authors suggested that heterotrophic prokaryotes might use phosphatase activity to mitigate C limitation.

In this study, we addressed the question whether deep-water prokaryotes maintain the same relation between extracellular enzymatic and metabolic activity as at the base of the euphotic waters (≈100 m depth). Therefore, we determined the extracellular enzymatic activity of two glycolytic enzymes (α-glucosidase and β-glucosidase), one proteolytic enzyme (leucine aminopeptidase) and alkaline phosphatase, along with prokaryotic abundance, production and potential respiration via ETS (electron transport system) measurements at ≈ 100 m depth, in the mesopelagic (200-1000 m depth) and bathypelagic (1000-4500 m depth) waters of the (sub)tropical Atlantic. We hypothesized that with increasing depth, heterotrophic prokaryotic communities express higher cell-specific extracellular enzymatic activity while cell-specific biomass production decreases in response to the increasingly recalcitrant organic matter.

Material and Methods

Study site and sampling

During the ARCHIMEDES-2 cruise with RV *Pelagia* (November to December 2006), extracellular enzymatic activity was measured on 16 out of 20 stations occupied in total in the eastern (sub)tropical Atlantic along an eastern and a western N-S transect (Fig. 1). Meso- and bathypelagic samples were taken from the main water masses down to 4500 m depth and additionally, one subsurface sample per station was taken at the base of the euphotic layer at around 100 m depth. Sampling was performed with 22 10-L NOEX bottles mounted in a frame also holding sensors for conductivity, temperature, depth, chlorophyll fluorescence, and optical backscattering. Subsurface waters consisted

of the more saline subtropical and the less saline tropical subsurface waters. The waters below the thermocline (250 to 500 m depth) were formed by either North Atlantic Central Water (NACW; only found at the northernmost station – St. 50) or South Atlantic Central Water (SACW; rest of the stations). Underneath these central waters, the high salinity Mediterranean Sea Outflow Water (MSOW) at the northern stations, and the low salinity Antarctic Intermediate Water (AAIW) at the southern stations (from St. 13 – 37) were found between 700 to 900 m depth. Deeper in the water column, Upper Northeast Atlantic Deep Water (UNEADW), Middle Northeast Atlantic Deep Water (MNEADW) and Lower Northeast Atlantic Deep Water (LNEADW) is flowing southwards with cores at around 1800 m, 2500-2750 m and 4000 m depth, respectively. The specific water masses were identified based on their distinct temperature-salinity characteristics, oxygen content and inorganic nutrient signatures (Table 1).

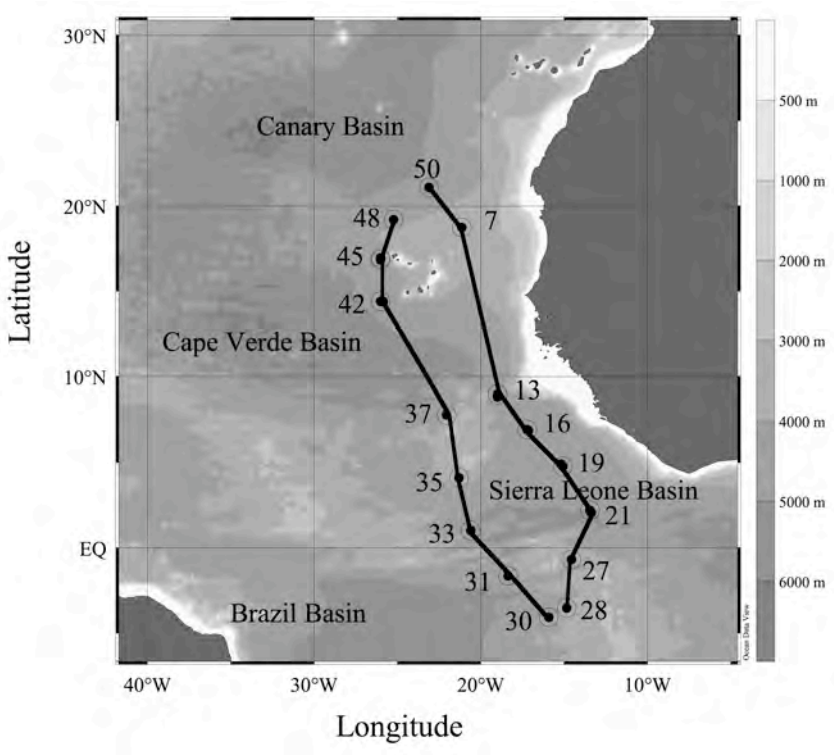


Fig. 1. (A) Stations occupied during ARCHIMEDES-II. Both transects (eastern and western) began at Station 50, but the eastern transect finished at St. 28 and the western at St 30

Water from the NOEX bottles was collected in acid-rinsed (0.1 N HCl) polycarbonate flasks rinsed three times with sample water prior to collecting samples for prokaryotic abundance, prokaryotic heterotrophic production, potential respiration

and extracellular enzymatic activities. The samples were either immediately processed for specific measurements aboard the ship or stored for subsequent analyses in the home lab as described below.

Table 1. Mean (±SE) values of physical, chemical and biological properties, characterizing the different water masses sampled during ARCHIMEDES-II. PA: Prokaryotic abundance. PHP: Prokaryotic heterotrophic production. ETS: Electron transport system. Number of measurements given in parenthesis. Subsurface: ≈100 m depth, SACW: South Atlantic Central Water, AAIW: Antarctic Intermediate Water, MSOW: Mediterranean Sea Outflow Water, UNEADW: Upper Northeast Atlantic Deep Water, MNEADW: Middle Northeast Atlantic Deep Water, LNEADW: Lower Northeast Atlantic Deep Water

Water mass	*Pressure Range* *(dbar)*	*Temperature* *(°C)*	*Salinity*	*Oxygen* *(µmol.kg^{-1})*	*NO_3* *(µmol.kg^{-1})*	*PO_4* *(µmol.kg^{-1})*	*PA* *(in 10^5 cells.mL^{-1})*	*PHP* *(µmolC.m^{-3}.d^{-1})*	*ETS* *(µmolC.m^{-3}.d^{-1})*
Subsurface	96-102	16.78 ± 1.73	35.894 ± 0.417	97.4 ± 31.1	19.4 ± 7.3	1.3 ± 0.43	3.58 ± 0.84	8.6 ± 4.8	204 ± 148
	(21)	(21)	(21)	(21)	(21)	(21)	(20)	(18)	(20)
SACW	246-503	10.52 ± 2.35	35.120 ± 0.347	78.5 ± 23.2	27.8± 7.6	1.8 ± 0.09	1.92 ± 0.70	1.66 ± 1.40	88.7 ± 52.0
	(42)	(42)	(42)	(42)	(42)	(42)	(42)	(39)	(39)
AAIW	748-901	4.97 ± 0.48	34.570 ± 0.059	122.2 ± 14.0	32.0 ± 5.0	2.1 ± 0.4	0.90 ± 0.30	0.19 ± 0.09	67 ± 34
	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(12)	(11)
MSOW	851-904	6.39 ± 0.45	34.889 ± 0.094	97.7 ± 6.9	28.8 ± 8.1	1.8 ± 0.6	0.76 ± 0.23	0.18 ± 0.02	66.5 ± 45
	(9)	(9)	(9)	(9)	(9)	(9)	(9)	(7)	(9)
UNEADW	1498-2002	3.74 ± 0.18	34.981 ± 0.020	208.0 ± 10.2	30.2 ± 6.4	1.9 ± 0.4	0.52 ± 0.14	0.045 ± 0.02	32.1 ± 18
	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(13)	(14)
MNEADW	2100-2752	2.76 ± 0.14	34.939 ± 0.015	215.2 ± 3.2	22.2 ± 0.3	1.5 ± 0.03	0.29 ± 0.72	0.030± 0.019	30.3 ± 35
	(19)	(119)	(19)	(19)	(19)	(19)	(19)	(16)	(17)
LNEADW	3499-4503	1.93 ± 0.21	34.883 ± 0.019	216.3 ± 3.0	23.5 ± 0.6	1.6 ± 0.05	0.23 ± 0.63	0.034 ± 0.04	39.1 ± 21
	(8)	(8)	(8)	(8)	(8)	(8)	(7)	(8)	(8)

Prokaryotic abundance (PA) determined by flow cytometry

Prokaryotic plankton collected from the different depth layers of the water column were enumerated using flow cytometry. Samples (2 ml) were fixed with 1% paraformaldehyde (final concentration), shock-frozen in liquid nitrogen for 5 min and stored at -80°C (Kamiya et al., 2007). Picoplankton cells were stained with SYBR-Green I and enumerated with a FACSCalibur flow cytometer (Becton Dickinson) within 2 months. Immediately before analysis, the thawed picoplankton samples were stained with SYBR-Green I at room temperature in the dark for 15 min. Fluorescent microspheres (Molecular Probes Inc.) with a diameter of 1 µm were added to all samples as an internal standard. Counts were performed with an argon laser at 488 nm wavelength. Prokaryotic cells were enumerated according to their right angle scatter and green fluorescence. The counting window of the flow cytometer was set to exclude eukaryotic picoplankton.

Prokaryotic heterotrophic production (PHP) by [^{3}H] leucine incorporation

Bulk PHP was measured by incubating triplicate 10-40 mL samples and formaldehyde-killed blanks (2% final concentration) with 10 nM [^{3}H]-leucine (final concentration, specific activity 160 Ci mmol^{-1}; Amersham) in temperature-controlled incubators in the dark at *in situ* temperature for 4-10 h (Kirchman et al. 1985). Incubations were terminated by adding formaldehyde (2% final concentration) before filtering the samples and the blanks through 0.2-µm polycarbonate filters (25 mm filter diameter; Millipore). Subsequently, the filters were rinsed three times with 5% ice-cold trichloroacetic acid, dried, and placed in scintillation vials. Scintillation cocktail (8 ml Canberra-Packard Filter Count) was added, and after 18 h, counted in a liquid scintillation counter (LKB Wallac model 1212). The mean disintegrations per minute (DPM) of the formaldehyde-fixed blanks were subtracted from the mean DPM of the respective samples, and the resulting DPM converted into leucine incorporation rates. Prokaryotic carbon biomass production was estimated using a conservative theoretical conversion factor of 1.55 kg C mol^{-1} Leu assuming no internal isotope dilution (Kirchman & Ducklow 1993).

Activity of the ETS

ETS activity was measured following the modifications of the tetrazolium

reduction technique as described earlier (Arístegui & Montero 1995). Some minor modifications of the method were made to increase its sensitivity. Briefly, about 10 l of sample was filtered through a Whatman GF/F filter (47-mm diameter). Filters were folded into cryovials and immediately stored in liquid nitrogen until analysis in the laboratory. Back in the laboratory, the filters with the collected material were homogenized in 2.5 ml phosphate buffer with a Teflon-glass tissue grinder at 0–4ºC for 1.5 min. A 0.9 ml aliquot of the crude homogenate was incubated in duplicate with 0.5 ml of substrate solution (NADH, NADPH) and 0.35 ml of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) at 18ºC for 20 min. The reaction was quenched by adding 0.25 ml of a mixture of formalin and phosphoric acid. The quenched reaction mixture was centrifuged at 4000*g* at 4ºC for 20 min and the absorbance of the particle-free solution measured in a Beckman DU-650 spectrophotometer at 490 and 750 nm wavelength after adjusting the sample to room temperature. Readings at 750 nm, to correct for turbidity, were always negligible. In addition to the samples, duplicate controls were run by replacing the crude extract with a clean Whatman GF/F filter homogenized in phosphate buffer. ETS activity was calculated using the equation given in Packard & Williams (1981):

$$ETS_{ASSAY}\ (\text{mmol } O_2\ m^{-3}\ h^{-1}) = H \times S \times (OD_{corr}) / (1.42 \times V \times f \times t/60) \times 22.4$$

where H is the volume of the homogenate (in ml), S is the volume of the quenched reaction mixture (in ml), OD_{corr} is the absorbance of the sample measured at 490 nm wavelength and corrected for blank absorbance, V is the volume (in l) of the seawater filtered through the Whatman GF/F filter, f is the volume of the homogenate used in the assay (in ml), t is the incubation time (in min), 60 converts minutes to hours, the factor 1.42 converts the INT-formazan formed to oxygen units (in µl) and 22.4 converts the µl O_2 to µmol O_2. ETS activity was corrected to in situ temperature using the following equation:

$$ETS_{IN\ SITU} = ETS_{ASSAY} \times e^{(Ea/R \times (1/Tass - 1/Tis))}$$

where Ea is the Arrhenius activation energy (in kcal mol^{-1}), R is the gas constant, and Tass and Tis are the temperatures (in degrees Kelvin) in the assay and *in situ*, respectively. A calculated activation energy of 16 kcal mol^{-1} was used (Arístegui &

Montero 1995). The final oxygen consumption rates were converted to carbon units using a respiratory quotient of 1. We are aware of the fact that the respiratory quotient might vary from about 0.6 to 1.3 depending on the substrate used, however, the general conclusions of this paper are not affected by the choice of the respiratory quotient.

Measurements of prokaryotic extracellular enzymatic activity

The hydrolysis of the fluorogenic substrate analogs 4-methylcoumarinyl-7-amide (MCA)-*L*-leucine-7-amido-4-methylcoumarin, 4-methylumbelliferyl (MUF)-α-*D*-glucoside, 4-MUF-β-*D*-glucoside and MUF-phosphate was measured to estimate potential activity rates of α-, β-glucosidase, aminopeptidase and alkaline phosphatase (Hoppe 1983). All the chemicals were obtained from Sigma and appropriate stocks prepared in methyl-cellosolve. The activity of the enzymes is linearly related to the fluorescence resulting from the cleavage of MUF or MCA and was detected spectrofluorometrically using a Fluorolog-3 fluorometer with a MicroMax 384 microwell plate reader (Horiba) at an excitation and emission wavelength of 365 nm and 445 nm, respectively. The samples (145 µl) were incubated in microwell plates, in the dark and at *in situ* temperature for 24 h. The linearity of the increase in fluorescence upon addition of fluorogenic substrate over time was checked by incubating samples up to 48 h. The increase in the relative fluorescence over time was transformed to cleavage activity using a standard curve established with different concentrations of the fluorochromes MUF and MCA added to 0.2-µm filtered sample water. In order to determine enzyme kinetics, model substrate concentrations should cover a sufficiently wide range. In this study, 12 different concentrations were used for the different substrates ranging from 0.6 µM to 1200 µM. For routine measurements of α- and β-glucosidase activities, a final concentration of 10 µM was applied, previously determined as saturating concentration. As a consequence of adding saturating concentrations, the rates measured with this technique are potential rates.

Profiles of enzyme kinetics for alkaline phosphatase and leucine-aminopeptidase were determined at each station. The saturation curves were transformed into a Lineweaver-Burk or Hanes-Woolf plot to reveal maximum enzyme activity (V_{max}, nmol l^{-1} h^{-1}) and substrate affinity (Michaelis-Menten half saturation constant; K_m, µM). Cell-specific ectoenzyme activity (amol $cell^{-1}$ h^{-1}) was calculated by dividing V_{max} by prokaryotic abundance. The ratio cell-specific V_{max}/K_m, which is the slope of the Michaelis–Menten equation at low substrate concentrations, was also calculated

(Healey 1980). This ratio is an indicator of the ability of prokaryotes to attain a high hydrolysis rate at low substrate concentrations since organisms might compensate for a high K_m by a high V_{max}.

Statistical analysis

Data were log transformed to attain normality before the *t*-student test was applied. To check the normality of the individual data sets, the Kolmogorov–Smirnoff test was used. For calculating uncertainties on ratios, error propagation was taking into account; standard deviations were calculated using the formula for the propagation of error (Bevington & Robinson 2003) as follows:

$$\Delta z / z = \sqrt{[((\Delta x)^2 / x) + ((\Delta y)^2 / y)]}$$

where z is the percent abundance or the percent assimilating and is equal to x divided by y, as described above. Δx and Δy are the standard deviations associated with x and y, respectively. Δz is the standard deviation calculated for z. Standard deviations were then converted to standard errors for each calculation.

Results

Prokaryotic abundance (PA) and prokaryotic heterotrophic production (PHP)

Total PA (Fig. 2A) decreased exponentially with depth by one order of magnitude in both the eastern and western section of the transect in the North Atlantic (R^2=0.73, n=65 and R^2=0.89, n=60, respectively). In all the water masses, PA was higher in the western than the eastern section (*t*-test; p = 0.03, n = 60) with the largest difference between the two sections detected in the Antarctic Intermediate Water (AAIW).

As PA, also PHP (Fig. 2B) decreased exponentially with depth (R^2=0.31, n=59 and R^2=0.52, n=49 for the eastern and western transect, respectively), however, by two orders of magnitude from the subsurface layer (≈100 m depth) to the Lower Northeast

Atlantic Deep Water (LNEADW) (Table 1). No significant difference in the mean PHP was detectable between the individual water masses of the eastern and western transect (*t*-test; p = 0.53, n = 49). In the subsurface layer, PHP was about two times higher in the eastern than in the western section, probably because of the proximity to the upwelling waters at the continental slope (Fig. 2B). Cell-specific PHP decreased also exponentially with depth (R^2=0.42, n=59 and R^2=0.57, n=49 for the eastern and western transect, respectively), albeit only by one order of magnitude (Fig. 2C). Mean cell-specific PHP was not significantly different between both transects (*t*-test; p = 0.30, n = 49).

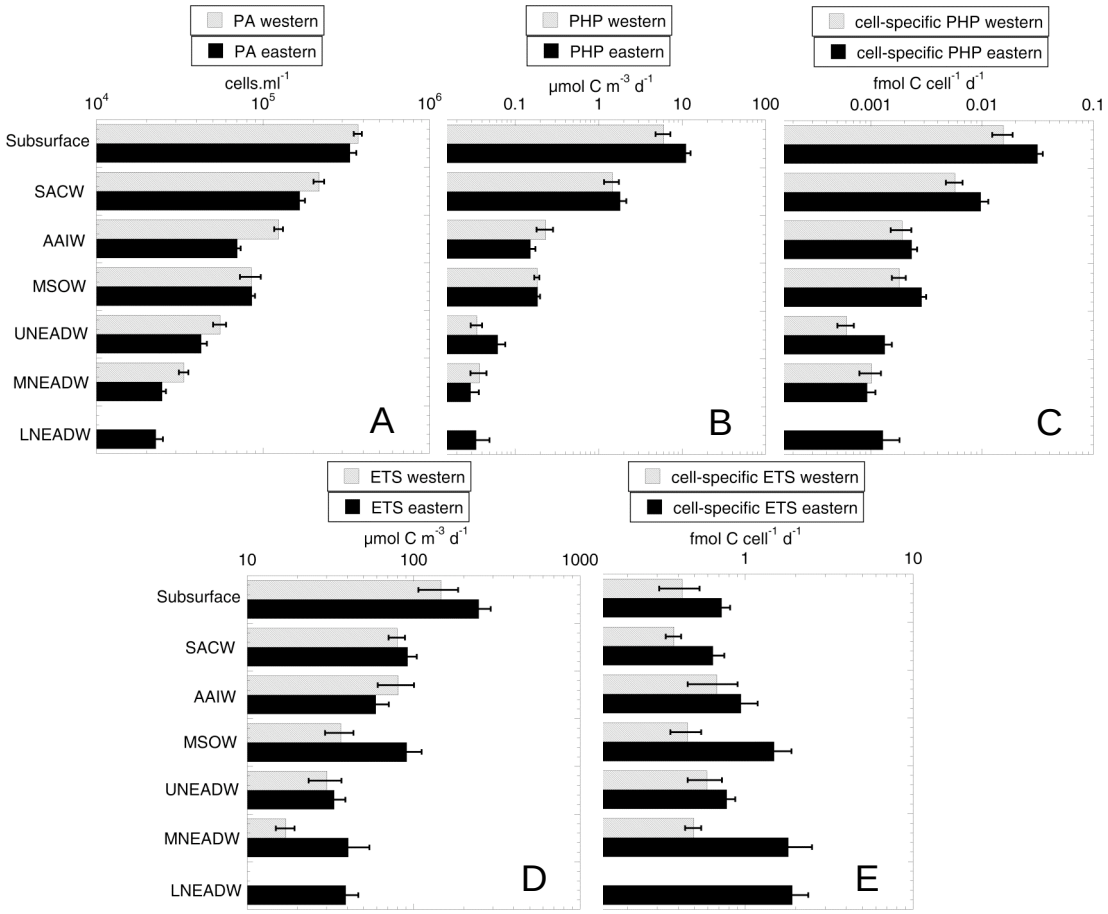


Fig. 2. Distribution of basic prokaryotic parameters in the different water masses through the eastern and western transect (see Table 1 for water depths of each water mass) (A) prokaryotic abundance (cell^{-1} ml^{-1}) (B) prokaryotic heterotrophic production (µmol C m^{-3} d^{-1}), (C) Cell-specific heterotrophic prokaryotic production (fmol C cell^{-1} d^{-1}), (D) ETS (µmol C m^{-3} d^{-1}), (E) cell-specific ETS (fmol C cell^{-1} d^{-1}). Error bars represent SE of 7 - 42 measurements

Potential respiration estimated via ETS measurements

Potential respiration rates as determined by ETS measurements decreased over the sampled depth-range by one order of magnitude (204 – 30.3 µmol C m^{-3} d^{-1}; Table 1,

Fig. 2D). Although ETS activity was higher in the eastern than in the western transect in all the water masses, except in the AAIW, overall, ETS activity was not significantly different between both transects (*t*-test; p = 0.30, n = 60). Individual water masses, however, exhibited pronounced differences in ETS activity such as the Middle Northeast Atlantic Deep water (MNEADW) and the Mediterranean Sea Outflow Water (MSOW) with 2 and 2.5 times higher ETS activity in the eastern than in the western transect, respectively. Although both prokaryotic abundance (Fig. 2A) and ETS measurements (Fig. 2D) decreased with depth, cell-specific ETS showed no systematic trend with depth, with the averages of the different water masses ranging from 0.42-1.8 fmol C $cell^{-1}$ d^{-1} (Fig. 2E). Cell-specific ETS was significantly higher (*t*-test; p = 0.00001, n = 53) in all the water masses of the eastern than in the western transect (including the AAIW, in contrast to bulk ETS activity). In the MNEADW and MSOW, cell-specific ETS activity was more than three times higher in the eastern than in the western transect (Fig. 2E).

Extracellular enzymatic activity (EEA)

In contrast to prokaryotic heterotrophic production, EEA decreased only slightly with depth at the two transects (Fig. 3). α-glucosidase (AGase; Fig. 3A, B) and β-glucosidase (BGase; Fig. 3C, D) activities exhibited higher lateral than vertical variability with maxima and minima of both ectoenzymes at similar locations (compare Fig. 3A & C; Fig. 3B & D). Leucine aminopeptidase (LAPase; Fig. 3E, F) and alkaline-phosphatase (APase; Fig. 3G, H) activities were generally higher than AGase and BGase, particularly at the base of the euphotic layer. LAPase and APase, however, decreased more with depth than AGase and BGase.

In contrast to cell-specific biomass production, cell-specific EEA generally increased with depth at both transects (Fig. 4). On average, cell-specific AGase increased from 0.14 amol $cell^{-1}$ h^{-1} at the base of the euphotic layer to 2.21 amol $cell^{-1}$ h^{-1} in the LNEADW, cell-specific BGase from 0.22 to 1.02 amol $cell^{-1}$ h^{-1}, cell-specific LAPase from 25.5 to 129.2 amol $cell^{-1}$ h^{-1}, and APase from 3.5 to 28.2 amol $cell^{-1}$ h^{-1}. No significant differences between the eastern and the western transect for any of the cell-specific EEA were detectable (*t*-test; p = 0.74, n = 26 for AGase; *t*-test; p = 0.89, n = 32 for BGase; and *t*-test; p = 0.24, n = 49 for LAPase) except for APase which was significantly higher in the eastern than in the western transect (*t*-test; p = 0.0028, n = 48).

Dynamics in kinetics of APase and LAPase

The maximum rates of hydrolysis (V_{max}) of APase and LAPase decreased with depth (Fig. 5A, B). V_{max} of APase was about one order of magnitude lower than the V_{max} of LAPase. No significant difference was discernable between the eastern and western transects for the V_{max} of APase (*t*-test; p = 0.53, n = 50) and for LAPase V_{max} (*t*-test; p = 0.54, n = 46). In the AAIW, however, the decreasing trend with depth was not observed, as we measured a lower APase V_{max} and higher LAPase V_{max} in this water mass than in its adjacent water masses (Fig. 5A, B).

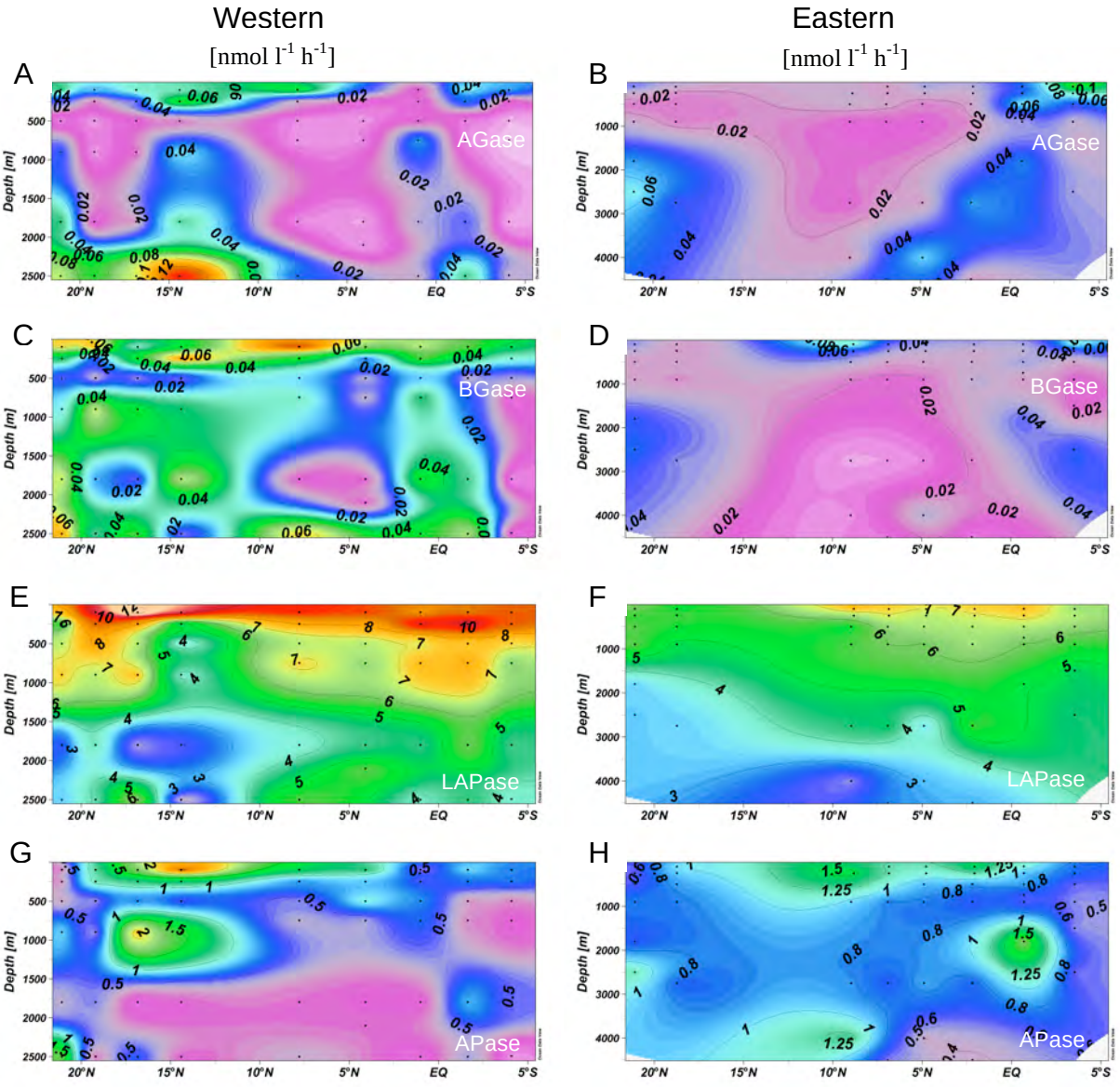


Fig. 3. Distribution of AGase (alpha-glucosidase) (A, B), BGase (beta-glucosidase) (C, D), LAPase (leucine aminopeptidase) (E, F) and APase (alkaline phosphatase) (G, H) activity throughout the water column along the western (left panels) and eastern (right panels) transect in the North Atlantic; values are given in nmol l^{-1} h^{-1}. Note the different depth range

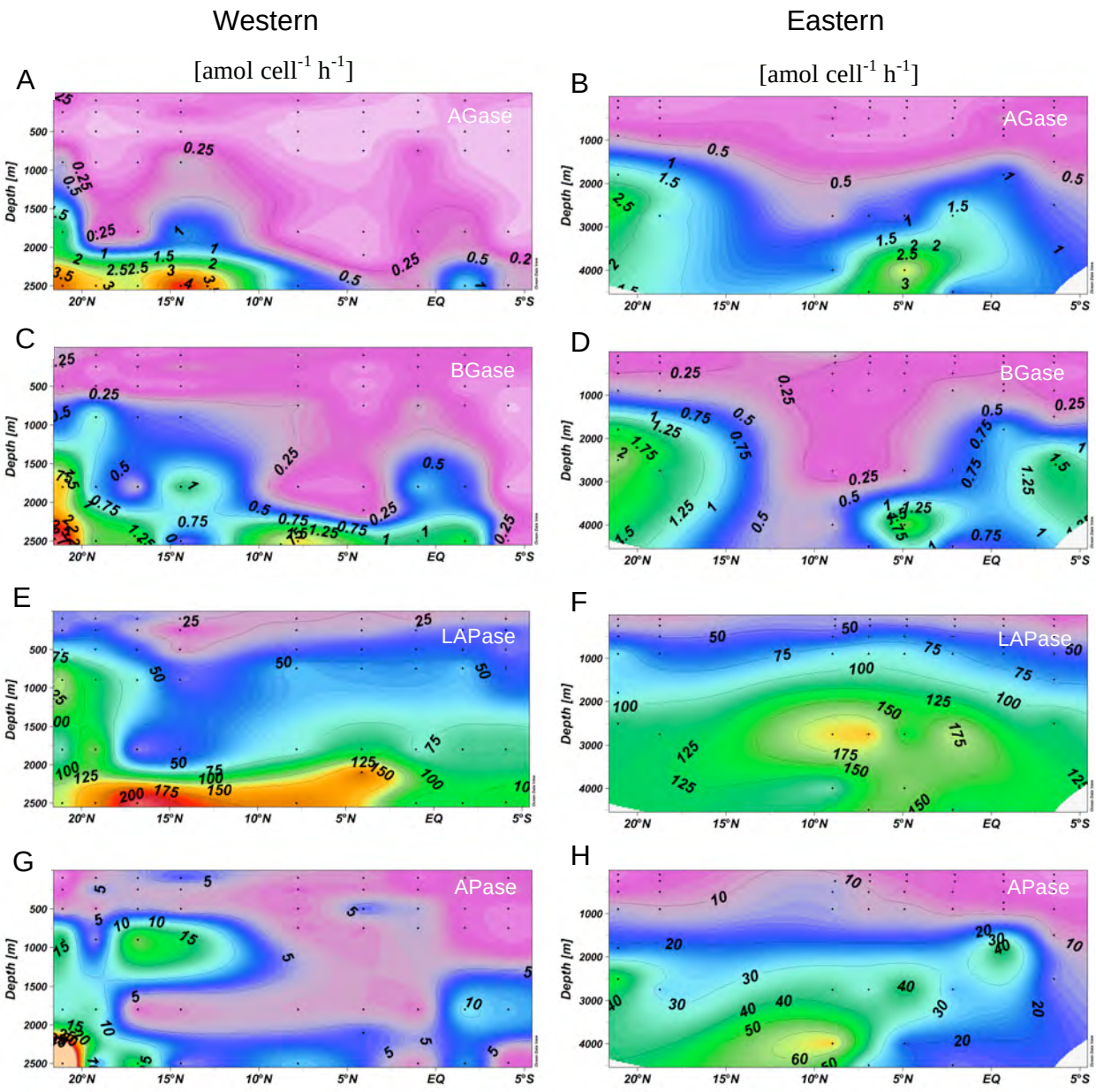


Fig. 4. Distribution of cell-specific AGase (A, B), BGase (C, D), LAPase (E, F) and APase (G, H) activity throughout the water column along the western (left panels) and eastern (right panels) transect in the North Atlantic; values are given in amol cell^{-1} h^{-1}. Note the different depth range in both transects

The half-saturation constant (K_m) of APase was higher in subsurface waters only in the upwelling regions, showing a relative minimum in intermediate waters (especially in the MSOW for APase and LAPase) and then increasing with depth (Fig. 5C, D). There were no significant differences discernable between the eastern and western transect in the K_m of APase and LAPase (*t*-test; p = 0.24, n = 49 for APase K_m; and *t*-test; p = 0.89, n = 47 for LAPase K_m).

Cell-specific potential hydrolytic activity (V_{max} $cell^{-1}$) increased with depth for both APase and LAPase (Fig. 6A, B), and was significantly higher (cell-specific APase V_{max}: *t*-test; p = 0.00001, n = 62; cell-specific LAPase V_{max}: *t*-test; p = 0.00001, n = 64) in the deep-water masses (UNEADW, MNEADW and LNEADW) than in the subsurface to intermediate water masses (subsurface, SACW, AAIW and MSOW). Significantly higher cell-specific potential hydrolytic activity was found at the eastern transect (*t*-test; p = 0.0015, n = 49 for APase V_{max}) but not for cell-specific LAPase V_{max} (*t*-test; p = 0.11, n = 46). The ratio of cell-specific V_{max} : K_m was significantly higher for both enzymes at the eastern than the western transect (*t*-test; p = 0.001, n = 49 for APase and p = 0.038, n = 46 for LAPase). This ratio increased with depth for both enzymes, ranging from 0.018-4.25 $cell^{-1}$ h^{-1} for APase (Fig. 6C) and 0.024-0.75 $cell^{-1}$ h^{-1} for LAPase (Fig. 6D), and was significantly higher (cell-specific APase V_{max} : K_m: *t*-test; p = 0.0004, n = 56; cell-specific LAPase V_{max} : K_m: *t*-test; p = 0.0002, n = 64) in the deep water masses (UNEADW, MNEADW and LNEADW) than in the subsurface to intermediate water masses (subsurface, SACW, AAIW and MSOW).

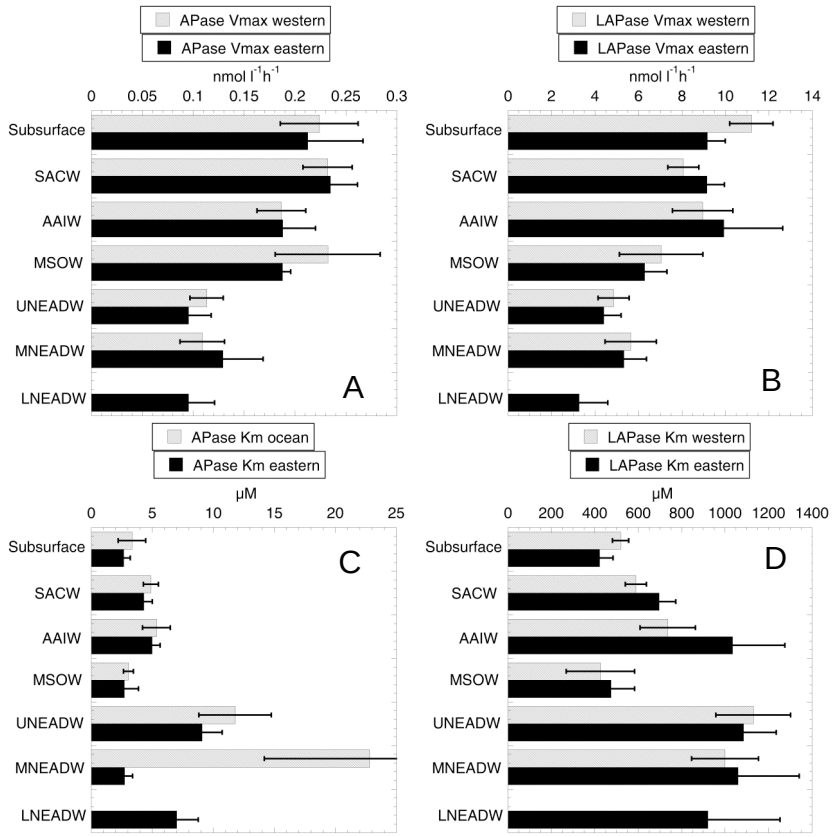


Fig. 5. Ectoenzyme kinetics of APase (A, C) and LAPase (B, D) throughout the water column along the eastern and western transect in the North Atlantic; (A, B) V_{max} (nmol h^{-1}), (C, D) K_m (µM). Error bars represent SE of 6 - 40 measurements

Thus, bulk maximum rates of hydrolysis decreased with depth while the half-saturation constant, cell-specific hydrolytic activity and cell-specific V_{max} : K_m ratio increased.

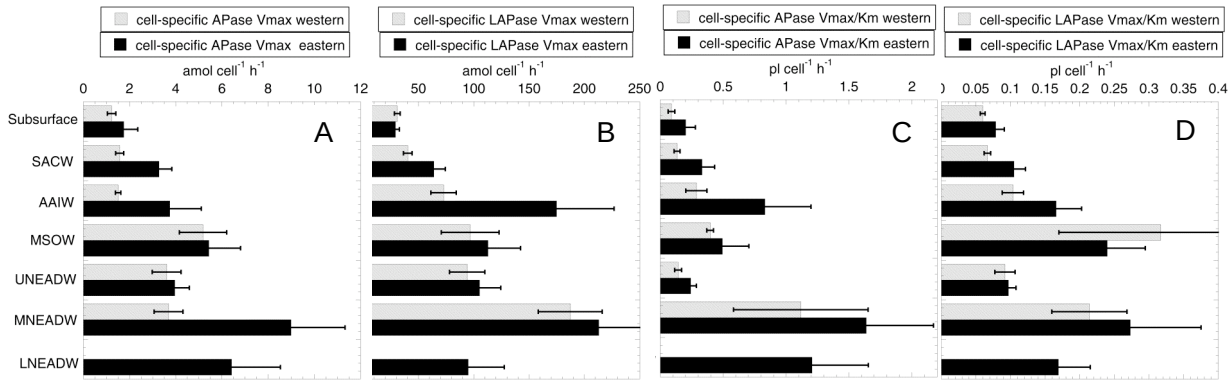


Fig. 6. Cell-specific (A) APase V_{max} (amol cell^{-1} h^{-1}) (B) LAPase V_{max} (amol cell^{-1} h^{-1}) and (C) APase V_{max}/K_m (pl cell^{-1} h^{-1}) (D) LAPase V_{max}/K_m (pl cell^{-1} h^{-1}) in the different water masses of the eastern and western transect in the North Atlantic. Error bars represent SE of 6-40 measurements

Discussion

Uncertainties in the estimation of the prokaryotic activity rates in the deep ocean

All the methods applied here to determine the different prokaryotic activity parameters have their own inherent limitations. Prokaryotic biomass production was assessed by the incorporation of leucine. While most of the bacterial groups are incorporating leucine (Cottrell & Kirchman 2003), at least some Archaea, reported to be similarly important in terms of abundance in the deep North Atlantic (Teira et al. 2006), might be chemoautotrophic and hence, not take up leucine. However, it has been shown for the North Atlantic and the Arctic Ocean that the percentage of Archaea taking up leucine is roughly similar to that of Bacteria (Herndl et al. 2005, Kirchman et al. 2007). Moreover, a large variation in the conversion factor of incorporated leucine to produced carbon biomass has been reported for surface Atlantic waters (Alonso-Saéz et al. 2007). Also, the determination of respiration via ETS relies on a conversion factor to convert the measured absorption of the assay into oxygen consumption or carbon dioxide

production (Packard et al. 1996).

The use of fluorogenic substrate analogs to estimate extracellular enzymatic activity (EEA) bears at least three problems. First, it targets only the exohydrolase activity which cleaves the terminal end of oligomers while the rate-limiting step in the cleavage of macromolecular DOM is likely the endohydrolase activity cleaving polymers into oligomers (Hoppe et al. 2002). The second problem associated with the fluorogenic substrate analogs is that they are model compounds and do not reflect the enormous diversity of potential extracellular enzyme substrates in nature. Third, with the enzyme assay applied here, we did not discriminate among potentially present different types of each extracellular enzyme. Using a zymography approach and capillary electrophoresis, up to 8 different beta-glucosidases were detectable at specific times in the coastal North Sea, all of them with specific enzyme kinetics (Arrieta & Herndl 2001, 2002).

We have normalized leucine incorporation, respiration and EEA to total prokaryotic abundance to compare the cell-specific activity of prokaryotes of sub-surface waters with that in deep-waters. This assumes that all the prokaryotic cells have similar activity levels, which is certainly not the case. A part of the enumerated cells might be dormant or dead (Heissenberger et al. 1996) and different prokaryotic groups might express different activity levels (Martínez et al. 1996, Cottrell & Kirchman 2003) and even intraspecific differences in activity levels exist (Sintes & Herndl 2006). For the study area in the North Atlantic, Varela et al. (2008) report a contribution of Bacteria (determined by FISH using the Eub338I-III oligonucleotide probe) to total picoplankton abundance ranging from about 40 to 65% with no pronounced trend with depth while the contribution of Archaea was generally below 10% of total picoplankton abundance. Hence, despite all the uncertainties concerning the inter- and intraspecific variability of the different activity levels measured here, there are no major differences in the contribution of Bacteria and Archaea to total prokaryotic abundance from about 100 m depth to the deep-waters in the study area. Taken together, the contrasting depth-related patterns in cell-specific leucine incorporation *versus* cell-specific EEA and respiration indicate that these differences are real and not methodological artifacts. The observed contrasting pattern in cell-specific leucine incorporation *versus* cell-specific EEA and respiration is most likely also not caused by measuring these parameters under surface pressure rather than under in situ pressure conditions. While the absolute values of the individual parameters measured at surface pressure conditions might deviated

from those in situ, there is no reason to assume that prokaryotic biomass production and respiration would show opposite trends under in situ than under surface pressure conditions. Otherwise, the measured growth efficiencies for bathypelagic prokaryotic communities of around 2% (Reinthaler et al. 2006) would be unrealistically deviating from this value which seems reasonable considering the comparably low growth efficiencies reported for prokaryotic communities of oligotrophic surface waters (del Giorgio et al. 1997, del Giorgio & Cole 1998). Also, until now, there is no consistent trend reported in the literature on the effect of pressure on bathypelagic prokaryotic activity including extracellular enzymatic activity. There is evidence that deep water prokaryotic activity is overestimated if measured under decompressed conditions (Jannasch & Wirsen 1982). However, other authors report inhibition of prokaryotic activity because of decompression (Tamburini et al. 2003). It is well known that prokaryotic enzymes might be adapted to a specific hydrostatic pressure (Jannasch & Taylor 1984, Somero 1992). If this holds true on a community level our bulk extracellular enzymatic activity would be underestimated under decompressed conditions. In fact, Tamburini et al. (2002) showed that LAPase and APase rates measured in samples maintained under in situ pressure conditions were around 2.3 times higher than those measured in their decompressed counterparts. Thus, our reported increase in cell-specific extracellular enzymatic activity with depth might be even higher under in situ conditions. Hence, we are confident that the general trend of increasing cell-specific extracellular enzymatic activity with depth is real and if there is a bias due to decompressing the samples, the reported rates are likely underestimations of the actual rates under in situ pressure conditions.

Alkaline phosphatase utilization in the deep: the paradox of high APase at high endproduct concentrations

It is well documented that the expression of many prokaryotic ectoenzymes is regulated by the concentration of its endproduct (Chrost 1991). Chrost & Overbeck (1987) observed in lake Plußsee, that APase significantly decreased when the ambient phosphate concentrations were higher than 0.5 µM. Ammerman & Azam (1991) reported that APase activity was usually low and sometimes undetectable in P-rich waters, and Nausch et al. (1998) observed a significant decrease in APase at ambient phosphate concentrations higher than 1 µM in the Baltic Sea. Also Zaccone et al. (2003) found an inverse relation between APase and phosphate concentrations from the surface

to deep waters in the Mediterranean Sea.

We found distinct water mass- and depth-related patterns of cell-specific APase in relation to PO_4 concentrations (Fig. 7). While phosphate concentrations varied only 2-fold over all the water masses, cell-specific APase activity varied more than 7-fold with lowest average cell-specific APase in the upper and intermediate waters increasing with depth (Fig. 7). This specific pattern indicates that the expression of APase is not regulated by the concentration of its endproduct in deep Atlantic waters, a pattern also identified by Hoppe & Ullrich (1999) in the mesopelagic waters of the Arabian Sea. It indicates that shifts in the functioning of APase might take place from the upper mesopelagic waters with their relatively higher contribution of reactive organic matter to the deeper water masses characterized by old, refractory organic matter. As hypothesized by Hoppe & Ullrich (1999), C-limited prokaryotes in the deep strata of the water column might use APase to access organic C moieties from organic matter, rather than phosphate directly.

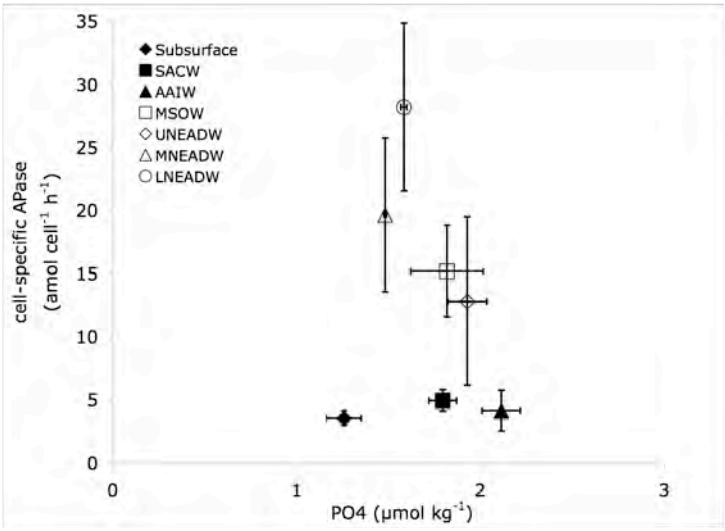


Fig. 7. Cell-specific APase (amol $cell^{-1}$ h^{-1}) versus phosphate concentration (µmol.kg^{-1}) in the different water masses. Error bars represent SE of 6-40 measurements

Relation between EEA and prokaryotic activity parameters in the deep oceanic water column

There are only few reports available on the EEA in the meso- and bathypelagic realm of the open–ocean while considerable information is available for open surface waters. The bulk APase activity reported in this study (0.04-3.7 nmol l^{-1} h^{-1}) is in the same range as reported by Hoppe & Ullrich (1999) for the deep Indian Ocean (2-4.5 nmol l^{-1} h^{-1} at 800 m), but is higher than that the range of APase activity (0.03-0.3 nmol

l^{-1} h^{-1} at 1000-4000 m) reported by Koike & Nagata (1997) for the deep central Pacific Ocean. The difference between the APase activity reported by Koike & Nagata (1997) and our data might be due to the fact that Koike & Nagata (1997) measured APase activity associated with particles (>0.2 μm) only and thus, excluding dissolved APase activity. Cell-specific EEA in the meso- and bathypelagic zones have been only reported in a few studies (Table 2). Our ranges of EEA are similar to the data from the northwestern Mediterranean Sea (Tamburini et al. 2002, Misic & Fabiano 2006), the Oman coast and the Arabian Sea (Hoppe & Ullrich 1999), and the central North Atlantic (**Chapter IV**). In all the studies (except in Misic & Fabiano 2006), an increase of cell-specific EEA was observed with depth. This increase in cell-specific EEA with depth corresponds to the increase in cell-specific respiration with depth reported for different regions (Table 3). In contrast to that, cell-specific PHP exhibits a larger variability depending on the sampling conditions and location (Table 4). Generally, cell-specific PHP decreases with depth to the bathypelagic layers (Table 4).

Table 2. Cell-specific extracellular enzymatic activity (amol $cell^{-1}$ h^{-1}) in different marine systems. LAPase: leucine aminopeptidase, APase: alkaline phosphatase, AGase: alpha-glucosidase, BGase: beta-glucosidase

Environment	Conditions	LAPase	APase	AGase	BGase	Reference
Baltic Sea	Tank incubations		2.0-14			Nausch 1998
Baltic Sea	Summer	0.3-5		0.1-3.3	0.2-3.7	Nausch et al. 1998
	Autumn	20-237		0.1-1	0.2-2	
Gulf Aqaba	Euphotic zone	<0.05-49.2			<0.1-43.8	Grossart & Simon 2002
Coastal lagoon	Hypertrophic	188-625			6.9-25	Cunha et al. 2000
San Francisco Bay	Cells < 1 um	7.2-12			0.16-0.57	Murrell et al. 1999
	All cells	16-31			0.47-1.60	
Uranouchi Inlet, Japan	Surface water	23.2-1017				Patel et al. 2000
	Bottom water	21.1-270				
Florida Bay	Estuarine system	52.2-1571.7	44.5-1029.4	1.3-74.6	0.7-23.6	Williams & Jochem 2006
Caribbean Sea	Eutrophic	31.6		1.69	0.18	Rath et al. 1993
	Oligotrophic	75.6		0.4	0.06	
Santa Monica Basin	Oligotrophic	78-618				Rosso & Azam 1987
Adriatic Sea	Marine Snow	432-4996		7-40	6-140	Karner & Herndl 92
Selected aggregates	Experimental	av. 242 ± 493				Smith et al. 1992
	Seawater	av. 52.5 ± 15				
Unaltered	Aggregates	220 ± 98		4.2±0.9	3.0±2.4	Azúa et al. 2003

Unaltered seawater microcosm	Aggregates	220 ± 98		4.2±0.9	3.0±2.4	Azúa et al. 2003
	Ambient water	560 ±89		5.0±1.0	6.8±3.4	
Freshly produced phytoplanktonic material microcosm	Aggregates	840±231		12.5±5.6	13.0±3.6	
	Ambient water	170±17		2.0±0.6	4.4±0.9	
California Bight	44 isolates from marine sources	4-3810	0.7-410	0-8	0-35	Martínez et al. 1996
Gulf of Genoa (NW Mediterranean)	Euphotic (0-200 m)	av. 25.3-54.9			av. 1.1	Misic & Fabiano 2006
	Mesopelagic (200-1000 m)	av 25.3-38.0			av. 0.7	
DYFAMED station (Mediterranean Sea)	Surface layer (10-200 m)	av. 1.9	av. 1.9			Tamburini et al. 2002
	Deep layer (200-2000 m)	av. 3.5	av. 7.7			
Oman coast, upwelling	Euphotic zone	12.6-46.9	1.2-8.3		0.02-1.2	Hoppe & Ullrich 1999
	Deep water	455-1817	10.8-86.2		7.7-52.5	
Arabian Sea	Euphotic zone	6.6-23.2	0.4-3.6		0.16-0.22	
	Deep water	33-118	5.6-23.4		0.27-1.18	
Central Atlantic	Subsurface Layer (100)	5.6-31.4	0.7-2.9	0.1-0.66	<0.1-0.5	**Chapter IV**
	Mesopelagic (200-1000 m)	9.6-39.7	1.3-5.2	<0.1-1.9	0.17-2.2	
	Bathypelagic (1000-7000 m)	5.3-147.2	4.4-20.7	<0.1-5.20	0.24-6.82	
Sub(tropical) North Atlantic	Subsurface Layer (100)	11.1-43.0	0.4-7.8	<0.1-0.52	<0.1-1.1	**This chapter**
	Mesopelagic (200-1000 m)	4.83-145.5	0.87-27.3	<0.1-1.58	<0.1-1.1	
	Bathypelagic					

Table 3. Cell-specific respiration (CSR) (fmol C $cell^{-1}$ d^{-1}) in different marine systems. Av. Indicates average

Environment	**Conditions**	**CSR**	**Reference**
Gulf of Mexico	<0.8 um	0.39	Jørgensen et al. 1999
Santa Rosa Sound estuary	<0.8 um	0.17	
Aberystwyth (Cardigan Bay, Wales, UK)	Coastal water	4.28 ± 1.12	Mukhanov et al. 2003

Sevastopol Bay (SB; Black Sea, Ukraine)	Coastal water	6.63 ± 4.51	
North Wales	<0.8 um	0.4–6.8	Blight et al. 1995
Louisiana (USA) shelf waters	<1 um	2.4–8.7	Biddanda et al. 1994
Southern North Sea	Seasonal Cycle	0.3-3.6	Reinthaler et al. 2005
Elbe Estuary, Germany	Aggregate on sampling day	22.0	Ploug et al. 2002
	Aggregate on day 6 of incubation	2.6	
Kerguelen Plateau (Southern Ocean)	Mean value upper 100m of <0.8 um	0.5-1.9	Obernosterer et al. 2008
	Bathypelagic zone (1000-4500 m)	0.23-6.90	
Temperate North Atlantic	Subsurface Layer (100-135 m)	av. 0.85	Reinthaler et al. 2006
	Oxygen minimum (402-725 m)	av. 1.67	
	Bathypelagic zone (1800-3000 m)	av. 2.74	
Subtropical North Atlantic	Subsurface Layer (100)	0.04-3.82	**Chapter II**
	Mesopelagic zone (200-1000 m)	0.12-5.23	
	Bathypelagic zone (1000-5000 m)	0.43-7.66	
Sub(tropical) North Atlantic	Subsurface Layer (100)	0.17-1.18	**This chapter**
	Mesopelagic zone (200-1000 m)	0.14-2.88	
	Bathypelagic zone (1800-4500 m)	0.23-6.90	

Table 4. Cell-specific prokaryotic heterotrophic production (PHP) (fmol C $cell^{-1}$ d^{-1}) in different marine systems

Environment	**Conditions**	**Cell-specific PHP**	**Reference**
Rhone river plume	Euphotic zone (0-100 m)	0.34-0.40	Kirchman et al. 1989
	Mesopelagic zone (150-700 m)	0.14-0.34	
Natural diatom aggregates in Øresund (Denmark)	Natural diatom aggregates	18.0-51.8	Grossart et al. 2003
	Surrounding waters	4.3-16.3	
Dona Paula bay (west coast of India)	13-month field observations from surface waters (1 m)	0.26-3.9	Bhaskar & Bhosle 2008
Coastal transition zone North Atlantic	Surface layer (5-82 m)	0.57-4.68	**Chapter I**
	Mesopelagic zone (200-760 m)	0.016-0.50	
	2000 m	0.020-0.33	
SE Mediterranean Sea	Euphotic zone (0-100 m)	0.03-0.09	Robarts et al. 1996
	Mesopelagic zone (100-1000 m)	0.03-0.20	
Gulf of Mexico	0-300 m	0.01-1.33	Skoog et al. 1999

Tasman Sea	Euphotic zone (0-100 m)	0.01-0.086	Moriarty & O'Donohue 1995
	Mesopelagic zone (100-1200 m)	0.002-0.091	
East Sea, Korea	Euphotic zone (0-200 m)	0.028-1.75	Cho et al.2000
	Mesopelagic zone (200-1000 m)	0.014-0.23	
Southern North Sea	Seasonal Cycle	0.41-3.0	Reinthaler et al. 2005
Global data set	Epipelagic (0-200)	av. 0.199 ± 0.018	Arístegui et al. 2009
	Mesopelagic zone (200-1000 m)	av. 0.112 ± 0.017	
	Bathypelagic zone (1000-4000 m)	av. 0.076 ± 0.010	
Subarctic Pacific	Euphotic zone (0-100 m)	0.0016-0.402	Nagata et al. 2000b
	Mesopelagic zone (100-1000 m)	0.0005-0.0050	
	Bathypelagic zone (1000-5834 m)	0.0003-0.0011	
Indian Ocean	Euphotic zone (0-200 m)	0.007-0.308	Ducklow 1993
	Mesopelagic zone (200-1000 m)	0.002-0.007	
Southern Ocean	Euphotic zone (0-200 m)	0.005-0.21	Simon et al. 2004
	Mesopelagic zone (200-1000 m)	0.003-0.56	
Temperate North Atlantic	Subsurface Layer (100-135 m)	0.004-0.12	Reinthaler et al. 2006
	Oxygen minimum (402-725 m)	0.002-0.14	
	Bathypelagic zone (1800-3000 m)	0.0005-0.26	
Subtropical North Atlantic	Subsurface Layer (100)	0.01-0.07	**Chapter II**
	Mesopelagic zone (200-1000 m)	0.001-0.05	
	Bathypelagic zone (1000-5000 m)	0.0004-0.01	
Central Atlantic	Subsurface Layer (100)	0.26-1.61	**Chapter IV**
	Mesopelagic zone (200-1000 m)	0.012-1.33	
	Bathypelagic zone (1000-7000 m)	0.0017-0.046	
Sub(tropical) North Atlantic	Subsurface Layer (100)	0.01-0.05	**This chapter**
	Mesopelagic zone (200-1000 m)	0.0005-0.03	
	Bathypelagic zone (1800-4500 m)	0.0001-0.004	

Cell-specific LAPase activity decreases exponentially with increasing cell-specific leucine incorporation (Fig. 8). Thus, biomass production decreases with increasing expression of extracellular enzymes.

Generally, the oceanic DOM pool is characterized by increasing C:N:P ratios with depth (Hopkinson & Smith 2005). Also, it has been observed that the amino acid : carbohydrate ratio of sinking POM decreases with depth (Haake et al. 1993), indicating that proteinaceous components of sinking POM are more rapidly degraded than the polysaccharide fraction (Skoog & Benner 1997). Experiments have consistently shown that bacteria degrade proteins faster than polysaccharides during decomposition of phytoplankton-derived detritus (Skopintsev 1981) and, thus, that the LAPase : BGase ratios (which can be interpreted as the degradation of protein relative to that of

polysaccharides) decrease during phytoplankton decay (Middelboe et al. 1995). These general trends suggest that the relative concentration of polysaccharides in sinking POM increases with depth, resulting in an intensive supply of polysaccharide-rich material to deeper waters and a decrease in the LAPase : BGase ratio with depth as observed in the present study (Fig. 9), where the LAPase : BGase ratio was significantly higher (*t*-test; $p < 0.001$, $n = 44$) in the deep water masses (UNEADW, MNEADW and LNEADW) than in the subsurface to intermediate water masses (subsurface, SACW, AAIW and MSOW).

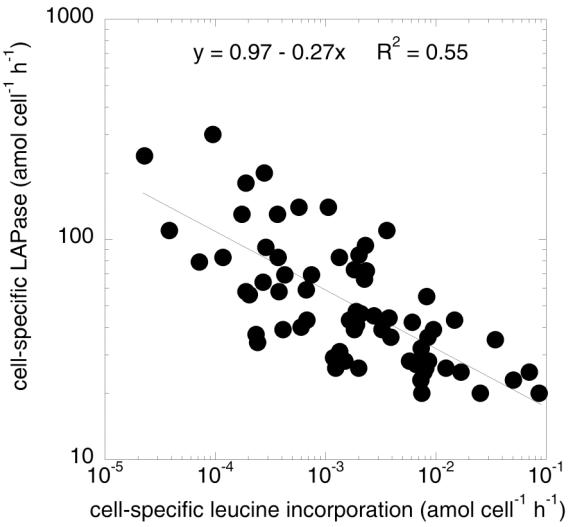


Fig. 8. Relation between cell-specific leucine incorporation and cell-specific leucine aminopeptidase (LAPase) activity. Data are derived from Fig. 2C (for cell-specific leucine incorporation) and Fig. 4E, F (for cell-specific leucine aminopeptidase)

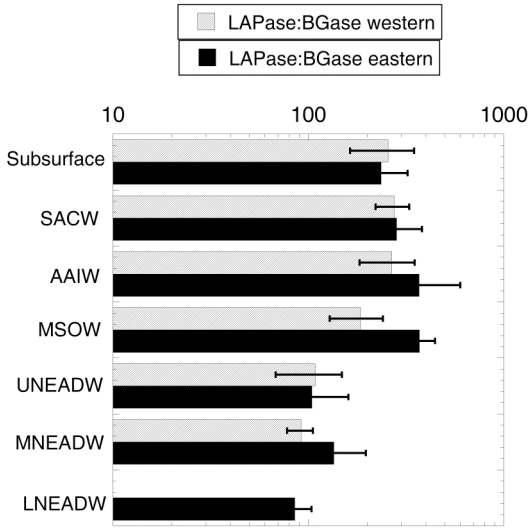


Fig. 9. Ratio of LAPase:BGase in the different water masses of the eastern and western transect in the North Atlantic. Error bars represent SE of 3-10 measurements

Davey et al. (2001), in the only other study where extracellular enzyme kinetics have been measured below the euphotic zone, report an increase in the K_m with depth in distinct profiles of LAPase to 200 m depth in the North Atlantic, while the V_{max} decreased. This trend is in agreement with our study for the full depth range (Fig. 5).

Azúa et al. (2003) studied the influence of the quality of the organic matter on the hydrolysis of polymers by marine prokaryotes in microcosms. They found that the cell-specific V_{max}/K_m ratio, which describes the ability of enzymes to compete at low substrate concentration (Healey 1980), was higher in the unamended seawater microcosm than in seawater enriched in easily utilizable DOM. These experimentally obtained results agree with our findings from the meso- and bathypelagic waters of the Atlantic characterized by major differences in the DOM reactivity between surface and deep waters. Deep-water prokaryotes require apparently a high K_m and a high cell-specific V_{max}/K_m ratio because of the refractory nature of the organic matter present in the deep ocean.

In summary, the decrease in the availability of labile organic matter (dissolved and particulate) with increasing depth leads to an increase in cell-specific extracellular enzymatic expression and concomitantly, to a reduction in the prokaryotic growth yield. The increase in the K_m and the V_{max}/K_m ratio of LAPase and APase with depth further indicates an adaptation of the extracellular prokaryotic enzymes to the refractory nature of the deep-water organic matter.

Acknowledgements

We thank the captain and crew of R/V *Pelagia* for their help during work at sea. This research was supported by a predoctoral Fellowship of the Spanish Ministry of Education and Science (AP2005-3932) to F.B., a grant of the Spanish Ministry of Education and Science to J.A. (Remolinos Oceánicos y Deposiciones Atmosféricas (RODA) project; CTM 2004-06842-C03/MAR), and a grant of the Earth and Life Science Division of the Dutch Science Foundation (ALW-NWO; ARCHIMEDES project, 835.20.023) to G.J.H. The work was carried out within the frame of the 'Networks of Excellence' MarBef and EurOceans supported by the 6th Framework Program of the European Union.

References

Alonso-Saéz L, Gasol JM, Arístegui J, Vilas JC, Vaqué D, Duarte CM, Agusti S (2007) Large-scale variability in surface bacterial carbon demand and growth efficiency in the subtropical northeast Atlantic Ocean. Limnol. Oceanogr. 52:533-546

Ammerman JW, Azam F (1991) Bacterial 5´-nucleotidase activity in estuarine and coastal marine waters: characterization of enzyme activity. Limnol. Oceanogr. 36:1427-1436

Amon RMW, Benner R (1996) Bacterial utilization of different size classes of dissolved organic matter. Limnol. Oceanogr. 41:41-51

Arnosti C, Durkin S, Jeffrey WH (2005) Patterns of extracellular enzyme activities among pelagic marine microbial communities: implications for cycling of dissolved organic carbon. Aquat. Microb. Ecol. 38:135-145

Arrieta JM, Herndl GJ (2001) Assessing the diversity of marine bacterial beta-glucosidase by capillary electrophoresis zymography. Appl. Environ. Microbiol. 67:4896-4900

Arrieta JM, Herndl GJ (2002) Changes in bacterial beta-glucosidase diversity during a coastal phytoplankton bloom. Limnol. Oceanogr. 47:594-599

Arístegui J, Montero MF (1995) The relationship between community respiration and ETS activity in the ocean. J. Plankton Res. 17:1563-1571

Arístegui J, Gasol JM, Duarte CM, Herndl GJ (2009) Microbial oceanography of the dark ocean's pelagic realm. Limnol. Oceanogr. 54(5):1501-1529

Azúa I, Uanue M, Ayo B, Arrtolozaga I, Arrieta JM, Iriberri J (2003) Influence of organic matter quality in the cleavage of polymers by marine bacterial communities. J. Plankton Res. 25:1451-1460

Bauer JE, Williams PM, Druffel ERM (1992) ^{14}C activity of dissolved organic carbon fractions in the north-central Pacific and Sargasso Sea. Nature 357:667-670

Benner R (2002) Chemical composition and reactivity. In: Hansell DA, Carlson CA (eds) Biogeochemistry of marine dissolved organic matter. Elsevier Science, New York, p 59-90

Benner R, Pakulski JD, M.McCarthy, Hedges JI, Hatcher PG (1992) Bulk chemical characteristics of dissolved organic matter in the ocean. Science 255:1561-1564

Chrost JR (1991) Microbial Enzymes in Aquatic Enironments, Vol, New York, 1991

Chrost RJ, Overbeck J (1987) Kinetics of alkaline phosphatase activity and phosphorus availability for phytoplankton and bacterioplankton in Lake Plußsee (North German eutrophic lake). Microb. Ecol. 13:229-248

Cottrell MT, Kirchman DL (2003) Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. Limnol. Oceanogr. 48:168-178

Davey KE, Kirby RR, Turley CM, Weightman AJ, Fry JC (2001) Depth variation of bacterial extracellular enzyme activity and population diversity in the northeastern North Atlantic Ocean. Deep Sea Res. II 48:1003-1017

del Giorgio PA, Cole JJ (1998) Bacterial growth yield efficiency in natural aquatic systems. Annu. Rev. Ecol. Syst. 29:503-541

del Giorgio PA, Cole JJ, Cimberis A (1997) Respiration rates of bacteria exceed phytoplankton in unproductive aquatic systems. Nature 385:148-151

Fukuda R, Sohrin Y, Saotome N, Fukuda H, Nagata T, Koike I (2000) East-west gradient in ectoenzyme activities in the subarctic Pacific: Possible regulation by zinc. Limnol. Oceanogr. 45:930-939

Haake B, Ittekot V, Honjo S, Manganini S (1993) Amino acid, hexosamine and carbohydrate fluxes to the deep Subarctic Pacific (Station P). Deep Sea Res. 40:547-560

Healey FP (1980) Slope of the Monod equation as an indicator of advantage in nutrient competition. Microb. Ecol. 5:281–286

Heissenberger A, Herndl GJ (1994) Formation of high molecular weight material by free-living marine bacteria. Mar. Ecol. Prog. Ser. 111:129-135

Heissenberger A, Leppard GG, Herndl GJ (1996) Relationship between the intracellular integrity and the morphology of the capsular envelope in attached and free-living marine bacteria. Appl. Environ. Microbiol. 62:4521-4528

Herndl GJ, Reinthaler T, Teira E, Aken Hv, Veth C, Pernthaler A, Pernthaler J (2005) Contribution of *Archaea* to total prokaryotic production in the deep Atlantic Ocean. Appl. Environ. Microbiol. 71:2303-2309

Hopkinson J, C.S., Smith EM (2005) Estuarine Respiration: An Overview of Benthic, Pelagic, and Whole System Respiration. In: Williams PJLeB (ed) Respiration in aquatic ecosystems. Academic Press, New York, p 122-146

Hoppe H-G (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Mar. Ecol. Prog. Ser. 11:299-308

Hoppe H-G, Ullrich S (1999) Profiles of ectoenzymes in the Indian Ocean: phenomena of phosphatase activity in the mesopelagic zone. Aquat. Microb. Ecol. 19:139-148

Hoppe H-G, Arnosti C, Herndl GJ (2002) Ecological significance of bacterial enzymes in the marine environment. In: Burns RG, Dick RP (eds) Enzymes in the environment: activity, ecology, and applications. Marcel Dekker, Inc., New York, p 73-108

Jannasch HW, Wirsen CO (1982) Microbial activities in undecompressed and decompressed deep-seawater samples. Appl. Environ. Microbiol. 43:1116-1124

Jannasch HW, Taylor CD (1984) Deep-sea microbiology. Annu. Rev. Microbiol. 38:487-514

Kirchman DL (2000) Uptake and regeneration of inorganic nutrients by marine heterotrophic bacteria. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, p 261-288

Kirchman DL, Ducklow HW (1993) Estimating conversion factors for thymidine and leucine methods for measuring bacterial production. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, p 513-517

Kirchman D, K'Ness E, Hodson R (1985) Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. 49:599-607

Kirchman DL, Elifantz H, Dittel AI, Malmstrom RR, Cottrell MT (2007) Standing stock and activity of Archaea and Bacteria in the western Arctic Ocean. Limnol. Oceanogr. 52:495-507

Koike I, Nagata T (1997) High potential activity of extracellular alkaline phosphatase in deep waters of the central Pacific. Deep-Sea Res. 44:2283-2294

Lignell R (1990) Excretion of organic carbon by phytoplankton: its relation to algal biomass, primary productivity and bacterial secondary productivity in the Baltic Sea. Mar. Ecol. Prog. Ser. 68:85-99

Martínez J, Smith DC, Sterward GF, Azam F (1996) Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. Aquat. Microb. Ecol. 10:223-230

Middelboe M, Søndergaard M, Letarte Y, Borch NH (1995) Attached and free-living bacteria: production and polymer hydrolysis during a diatom bloom. Microb. Ecol. 29:231-248

Misic C, Fabiano M (2006) Ectoenzymatic activity and its relationship to chlorophyll-a and bacteria in the Gulf of Genoa (Ligurian Sea, NW Mediterranean). J. Mar. Syst. 60:193-206

Misic C, Castellano M, Fabiano M, Ruggierini N, Saggiomo V, Povero P (2006) Ectoenzymatic activity in surface waters: A transect from the Mediterranean Sea across the Indian Ocean to Australia. Deep-Sea Res., Part 1 53:1517-1532

Nagata T (2000) Production mechanisms of dissolved organic matter. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, p 121-152

Nausch M, Pollehne F, Kerstan E (1998) Extracellular enzyme activities in relation to hydrodynamics in Pomerian Bight (Southern Baltic Sea). Microb. Ecol. 36:251-258

Packard TT, Williams PJLB (1981) Rates of respiratory oxygen consumption and electron transport in surface seawater from the Northwest Atlantic. Oceanol. Acta 4:351–358

Packard T, Berdalet E, Blasco D, Roy SO, St-Amand L, Lagace B, Lee K, Gagne J-P (1996) CO_2 production predicted from isocitrate dehydrogenase activity and

bisubstrate enzyme kinetics in the marine bacterium *Pseudomonas nautica*. Aquat. Microb. Ecol. 11:11-19

Schneider B, Schlitzer R, Fischer G, Nöthing EM (2003) Depth-dependent elemental compositions of particulate organic matter (POM) in the ocean. Glob. Biogeochem. Cy. 17:1032, doi:10.1029/2002GB001871

Sintes E, Herndl GJ (2006) Quantifying substrate uptake of individual cells of marine bacterioplankton populations by catalyzed reporter deposition fluorescence in situ hybridization combined with microautoradiography. Appl. Environ. Microbiol. 72:7022-7028

Skoog A, Benner R (1997) Aldoses in various size fractions of marine organic matter: implications for carbon cycling. Limnol. Oceanogr. 42:1803-1813

Skopintsev BA (1981) Decomposition of organic matter of plankton, humification and hydrolysis. In: Duursma K, Dawson R (eds) Marine organic chemistry; evolution, composition, interactions and chemistry of organic matter in seawater. Elsevier, p 125-177

Somero GN (1992) Adaptation to high hydrostatic pressure. Annu. Rev. Physiol. 54:557-577

Stoderegger K, Herndl GJ (1998) Production and release of bacterial capsular material and its subsequent utilization by marine bacterioplankton. Limnol. Oceanogr. 43:877-884

Tamburini C, Garcin J, Bianchi A (2003) Role of deep-sea bacteria in organic matter mineralization and adaptation to hydrostatic pressure conditions in the NW Mediterranean Sea. Aquat. Microb. Ecol. 32:209-218

Teira E, Lebaron P, Aken Hv, Herndl GJ (2006) Distribution and activity of Bacteria and Archaea in the deep water masses of the North Atlantic. Limnol. Oceanogr. 51:2131-2144

Weiss M, Abele U, Weckesser J, Welte W, Schiltz E, Schulz GE (1991) Molecular architecture and electrostatic properties of bacterial porin. Science 254:1627-1630

Williams C, Jochem FJ (2006) Ectoenzyme kinetics in Florida Bay: Implications for bacterial carbon source and nutrient status. Hydrobiologia 569:113-127

Zaccone R, Monticelli LS, Seritti A, Santinelli C, Azzaro M, Boldrin A, LaFerla R, D'Alcala MR (2003) Bacterial processes in the intermediate and deep layers of

the Ionian Sea in winter 1999: vertical profiles and their relationship to the different water masses. J. Geophys. Res. 108:8117

Chapter IV

High dissolved extracellular enzymatic activity in the deep Central Atlantic Ocean

F. Baltar, J. Arístegui, J. M. Gasol, E. Sintes, H. M. van Aken, G. J. Herndl

Aquatic Microbial Ecology (in press)

Abstract

The distribution of prokaryotic abundance (PA), prokaryotic heterotrophic production (PHP), and suspended particulate organic material (POM), as well as total and dissolved (operationally defined as passing through 0.2 μm pore size filters) potential extracellular enzymatic activities (EEA) (alpha- and beta-glucosidase [AGase and BGase], leucine aminopeptidase [LAPase], and alkaline phosphatase [APase]) were determined in the meso- and bathypelagic waters of the (sub)tropical Atlantic along an eastern zonal transatlantic transect and a western N-S transect. Significant differences between both transects were found for POM concentration but not for PA, PHP (except in the subsurface and oxygen minimum layer), and dissolved and total EEA. PHP decreased by three orders of magnitude from the lower euphotic zone to the bathypelagic waters, while PA and cell-specific PHP only by one and 2 orders of magnitude respectively. The proportion of dissolved to total extracellular enzymatic activity was high in the dark ocean for all the enzymes, ranging from 54-100%, 56-100%, 65-100% and 57-97% for AGase, BGase, LAPase and APase, respectively. The kinetic parameters (V_{max} and K_m) of both the dissolved and total fraction of LAPase and APase were very similar throughout the water column, suggesting a similar origin of the dissolved and particulate EEA. Significant correlations between both, dissolved and total EEA were found with prokaryotic metabolism and the POM pool. Based on the previous notion that the fraction of dissolved EEA is higher in particle-attached than in free-living microbes, our results suggest, in agreement with recent genomic evidence, that microbial activity in the dark ocean occurs mainly on colloidal and particulate material, which is prone to disruption during the sampling process. Hence, more selective sampling techniques are needed to specifically collect these deep-water aggregates representing probably hotspots of microbial activity in the deep ocean.

Introduction

Prokaryotes play a major role as biomass producers and transformers of dissolved organic matter (DOM) in the sea (Azam & Cho 1987). Heterotrophic

prokaryotes express extracellular enzymes (EE) to hydrolyze high molecular weight DOM into lower molecular weight (MW) compounds of < 600 MW, which is the MW threshold of DOM that can be taken up by prokaryotes (Weiss et al. 1991).

Since Hoppe (1983) introduced the fluorescent substrates analogues in aquatic microbial ecology to quantify potential extracellular enzymatic activity (EEA), this technique has been applied to a wide range of aquatic environments (Chrost 1989, Rath et al. 1993, Misic et al. 2006). However, most of these studies were restricted to the euphotic zone and coastal seas (Sala et al. 2001, Zaccone et al. 2003, Williams & Jochem 2006). Only a few profiles of EEA are available for the whole water column of the open ocean (Koike & Nagata 1997,Hoppe & Ullrich 1999, **Chapter III**).

The activity of EE in the dark ocean is likely related to the composition of sinking particulate organic matter (POM). Previous studies suggest that a substantial fraction of sinking POM is solubilized to DOM, fueling prokaryotic production in the meso- and bathypelagic zones (Cho & Azam 1988). The observation that particulate organic carbon (POC) decreases faster with depth than prokaryotic carbon demand, led to the formulation of the "particle decomposition paradox" by Karl et al. (1988). Cho & Azam (1988) proposed that the underlying reason for this is an over-expression of EE by the particle-associated prokaryotic community, which releases the EE into the particles. This over-expression of dissolved EE results in a loose hydrolysis – uptake coupling of cleavage products and consequently, to the potential release of these products into the surrounding water, eventually fueling free-living prokaryotes (Karner & Herndl 1992, Smith et al. 1992, Azam & Long 2001). Prokaryotic EE can be released into the environment by a suite of different processes, either actively by the prokaryotic cell or via grazing activity (Chrost 1991, Bochdansky et al. 1995, Karner & Rassoulzadegan 1995), and remain active for an extended period of time (Wetzel 1991).

The importance of dissolved EE in material cycling in soils is widely recognized (McLaren & Skujins 1967, Paul 1989, Dick & Tabatabai 1992); however, less consensus exists about the relevance of dissolved enzymes in marine communities. Most EEA in the oceans is found associated with prokaryotes (Hoppe 1983, Hoppe et al. 2002). Consequently, it has been assumed that only cell-associated EE are of ecological significance (Someville & Billen 1983, Rego et al. 1985, Chrost & Rai 1993). Nevertheless, significant dissolved EEA in the oceanic water column has been detected on macroscopic particles such as marine snow (Karner & Herndl 1992, Smith et al. 1992, Müller-Niklas et al. 1994).

Based on the notion that particle-attached prokaryotes are releasing EEs into their environment and hence, exhibit a loose hydrolysis-uptake coupling, we hypothesized that the contribution of dissolved to total EEA should be higher in the dark open ocean if deep-water prokaryotes are more dependent on POM as substrate than surface water prokaryotic communities. Surface water heterotrophic microbial communities have access to bioreactive DOM while in deep-waters, essentially all of the bulk DOM is refractory (Benner et al. 1992, Aluwihare et al. 2005).

In this study, we investigated the dynamics of both total and dissolved EEA across the (sub)tropical Atlantic from the lower euphotic zone to the bathy- and abyssopelagic layers (to 7000 m depth). We determined the EEA of two glycolytic enzymes (α-glucosidase and β-glucosidase), one proteolytic enzyme (leucine aminopeptidase) and alkaline phosphatase, and related them to prokaryotic abundance (PA) and heterotrophic production (PHP), as well as to the distribution of POC and particulate organic nitrogen (PON). We provide evidence that prokaryotic metabolism is related to the concentration of the POM pool and to the dissolved and total EEA, suggesting that colloidal and particulate organic matter might be more important for deep-water prokaryotes than hitherto assumed.

Materials and Methods

Study site and sampling

EEA, heterotrophic prokaryotic biomass production and the concentration of POM were determined in several waters masses of the (sub)tropical Atlantic Ocean. The cruise track covered more than 4500 km along an eastern zonal transatlantic ("TA") transect (from Brazil through the Romanche Fracture Zone [RFZ], a major canyon in the Mid-Atlantic Ridge at the equator) and a western N-S transect (roughly parallel to the African continental slope, "African") (Fig. 1). Samples from several depths, targeting the core of the main water masses, were taken with RV *Pelagia* at 20 out of 30 stations occupied during the ARCHIMEDES-III cruise (December 2007 to January 2008). Along the TA transect, samples were obtained from the base of the mixed layer

("subsurface", 100 m layer), the oxygen minimum layer (OML; consisting of South Atlantic Central Water), the Antarctic Intermediate Water (AAIW), the Upper North Atlantic Deep Water (UNADW), the Middle North Atlantic Deep Water (MNADW), the Lower Northeast Atlantic Deep Water (LNEADW), and the Antarctic Bottom Water (AABW). In the RFZ, the NADW and AABW mix forming transitional waters (Trans), since the RFZ is sufficiently deep to allow significant eastward flow of AABW. The AABW slowly transforms to Lower Deep Water (LDW) by mixing with NEADW (Ferron et al. 1998). Along the N-S African transect, samples were taken from the "subsurface", the OML (consisting of North and South Atlantic Central Water), the Sub-Arctic Intermediate Water (SAIW), the Upper Northeast Atlantic Deep Water (UNEADW), NEADW, and the LDW. The specific water masses were identified based on their distinct temperature-salinity characteristics and oxygen content (Table 1). Samples from the distinct water masses were collected with 12 L Niskin bottles mounted on a CTD (conductivity, temperature, depth) frame to determine total prokaryotic abundance and heterotrophic production, POM, and EEA as described below.

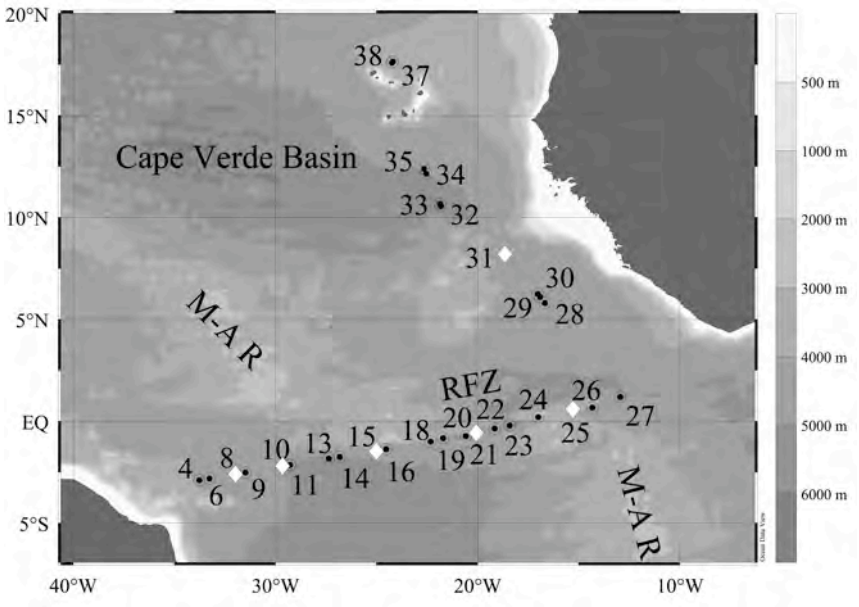


Fig. 1. Stations occupied during cruise ARCHIMEDES-III. Sampled stations are indicated by numbered dots. Kinetic profiles were performed at stations labeled by open diamonds. The Transatlantic transect (TA) ranged from Sta. 4 to Sta. 27; the African transect (African) from Sta. 28 to Sta. 38. M-A R: Mid-Atlantic Ridge, RFZ: Romanche Fracture Zone

Table 1. Characteristics of the main water masses sampled during the ARCHIMEDES-III cruise based on CTD profiles at individual stations. Ranges are given for each water mass where samples were collected. TA: Transatlantic, OML: oxygen minimum layer, AAIW: Antarctic Intermediate Water, UNADW: Upper North Atlantic Deep Water, MNADW: Middle North Atlantic Deep Water, LNADW: Lower North Atlantic Deep Water, Trans: Transitional water, AABW: Antarctic Bottom Water, SAIW: Sub-Arctic Intermediate Water, UNEADW: Upper Northeast Atlantic Deep Water, NEADW: Northeast Atlantic Deep Water, LDW: Lower Deep Water

Transect	Water mass	Depth (m)	Temperature (ºC)	Salinity	Oxygen (μmol kg^{-1})
TA	subsurface	100	14.1-23.7	35.4-37	89.2-199.1
	OML	250-750	4.6-13.7	34.4-35.4	74-152.6
	AAIW	750-1200	4.2-5.0	34.4-34.6	125.7-176.4
	UNADW	1750	3.5-3.8	34.9	206.8-247.6
	MNADW	2750-3750	2.1-2.6	34.9	209.9-222
	LNADW	3750-4500	1.7-2.1	34.8-34.9	211.3-225.2
	Trans	2750-4350	1.2-2.6	34.8-34.9	204.5-212-9
	AABW	4500-7150	0.6-0.9	34.7	190.1-199.9
African	subsurface	100	13.3-19.1	35.3-36.7	66.8-125.3
	OML	250-470	9.9-13.6	35.1-35.4	33.9-69.5
	SAIW	750	6.0-7.3	34.7-35.0	69.4-83
	UNEADW	1750	1.8-3.8	34.9	190.7-201-1
	NEADW	2750	1.8-2.7	34.9	207-209
	Trans	3750	1.8-2.1	34.9	205.7-208
	LDW	3400-5200	1.6-2.3	34.8-34.9	199.2-207.2

Prokaryotic abundance (PA) determined by flow cytometry

Prokaryotic plankton collected from the different depth layers of the water column were enumerated using flow cytometry. Samples (2 mL) were fixed with 1% paraformaldehyde (final concentration), shock-frozen in liquid nitrogen for 5 min and stored at -80°C. Picoplankton cells were stained with SYBR-Green I and enumerated with a FACSCalibur flow cytometer (Becton Dickinson) on board within 2 d. Immediately before analysis, the thawed picoplankton samples were stained with SYBR-Green I (using a final concentration of 1X as recommended by the manufacturer)

at room temperature in the dark for 15 min. Fluorescent microspheres (Molecular Probes Inc.) with a diameter of 1 μm were added to all samples as an internal standard. Counts were performed with an argon laser set at 488 nm wavelength. Prokaryotic cells were enumerated according to their right angle scatter and green fluorescence. The counting window of the flow cytometer was set to exclude eukaryotic plankton.

Prokaryotic heterotrophic production (PHP) measured by [^{3}H] leucine incorporation

Bulk PHP was measured by incubating triplicate 10-40 mL samples and triplicate formaldehyde-killed blanks (2% final concentration) with 5 nmol L^{-1} [^{3}H]-leucine (final concentration, specific activity 160 Ci $mmol^{-1}$; Amersham) in temperature-controlled incubators in the dark at *in situ* temperature for 4-10 h. Incubations were terminated by adding formaldehyde (2% final concentration) 10 min prior to filtering the samples and the blanks through 0.2-μm polycarbonate filters (25 mm filter diameter; Millipore) supported by HAWP filters (Millipore, 0.45 μm pore size). Subsequently, the filters were rinsed three times with 5% ice-cold trichloroacetic acid, dried, and placed in scintillation vials. Scintillation cocktail (8 mL Canberra-Packard Filter Count) was added, and after 18 h, counted in a liquid scintillation counter (LKB Wallac model 1212) on board. The mean disintegrations per minute (DPM) of the formaldehyde-fixed blanks were subtracted from the mean DPM of the respective samples and the resulting DPM converted into leucine incorporation rates. Prokaryotic carbon biomass production was estimated using a conservative theoretical conversion factor of 1.55 kg C mol^{-1} Leu assuming no internal isotope dilution (Kirchman & Ducklow 1993). Average cell-specific leucine incorporation rates (fmol C $cell^{-1}$ d^{-1}) were calculated by dividing bulk leucine incorporation by prokaryotic abundance.

Particulate organic carbon (POC) and organic nitrogen (PON)

Samples (4-6 L) for POC and PON were filtered through combusted (450ºC for 12 h) 25-mm Whatman GF/F filters. The filters were wrapped in combusted aluminum foil and kept frozen at -20ºC until processed. In the laboratory, the filters were thawed and dried overnight at 65ºC in a desiccator under HCl fumes to remove carbonates and finally, dried overnight in a desiccator with silica gel. Prior to analysis, samples were packed into ultraclean tin caps. The carbon analyses were performed on a Perkin-Elmer 2400 CHN (carbon, hydrogen, nitrogen) elemental analyzer according to the JGOFS

(Joint Global Ocean Flux Study) protocol (UNESCO 1994). Concentrations of POM originating from low-volume filtrations may be biased due to the possible sorption of DOM on the filter material (Moran et al. 1999). Hence, sorption blanks were determined for each station by placing two Whatman GF/F filters on top of each other in the filter holder while filtering the water sample. The adsorbed organic C and N collected by the lower filter was measured as described above and subtracted from the POC- and PON-content of the corresponding particulate sample.

Measurements of prokaryotic extracellular enzymatic activity

The hydrolysis of the fluorogenic substrate analogs 4-methylcoumarinyl-7-amide (MCA)-L-leucine-7-amido-4-methylcoumarin, 4-methylumbelliferyl (MUF)-α-D-glucoside, 4-MUF-β-D-glucoside and MUF-phosphate was measured to estimate potential activity rates of leucine-aminopeptidase (LAPase), α-, β-glucosidase (AGase, BGase), and alkaline phosphatase (APase), respectively (Hoppe 1983). All the chemicals were obtained from Sigma and appropriate stocks prepared in methyl-cellosolve. The activity of the enzymes is linearly related to the fluorescence resulting from the hydrolytic cleavage of the monomer from MUF or MCA and was detected spectrofluorometrically using a Fluorolog-3 fluorometer with a MicroMax 384 microwell plate reader (Horiba) at an excitation and emission wavelength of 365 nm and 445 nm. Samples (300 µL) were incubated in the dark at in situ temperature for 24 h. The linearity of the increase in fluorescence over time was checked on sets of samples incubated between 24-48 h resulting in the same hydrolytic rates h^{-1}. Subsamples without substrate were used as blanks to determine the background fluorescence of the samples. Previous experiments showed that abiotic hydrolysis of the substrates was not significant (data not shown). The fluorescence obtained at the beginning and at the end of the incubation was corrected for the corresponding blank. This increase in fluorescence over time was transformed to hydrolysis activity using a standard curve established with different concentrations of the fluorochromes MUF and MCA added to 0.2-µm filtered sample water. To determine enzyme kinetics, model substrate concentrations should cover a sufficiently wide range. In this study, 12 different concentrations were used for the different substrates ranging from 0.39 µmol L^{-1} to 800 µmol L^{-1} for APase and from 0.98 µmol L^{-1} to 2000 µmol L^{-1} for LAPase. For routine measurements, a final concentration of 10 µmol L^{-1} was applied to measure α- and β-glucosidase activity, 100 µmol L^{-1} for APase and 500 µmol L^{-1} for APase.

These concentrations have been previously determined as saturating concentrations. Consequently, this approach yields potential hydrolysis rates.

Profiles of enzyme kinetics for APase and LAPase were determined at each station. The saturation curves were transformed into a Lineweaver-Burk or Hanes-Woolf plot to reveal maximum enzyme activity (V_{max}, nmol L^{-1} h^{-1}) and substrate affinity (Michaelis-Menten half saturation constant; K_m, μmol L^{-1}).

We distinguished between total EEA of the sample and the dissolved fraction of the EEA and their respective enzyme kinetics. For total EEA, raw seawater was used while for dissolved EEA, samples were filtered through a low protein binding 0.2 μm Acrodisc® Syringe filter (Pall Corporation), following the protocol of Kim et al. (2007). As suggested by these authors, gentle pressure was applied and care was taken to avoid exposing cells to the air at the end of the filtration (Nagata & Kirchman 1990). As indicated by Obayashi & Suzuki (2008), depending on the filter used for size fractionation, the adsorption of extracellular enzymes can be substantial. The latter authors found that around half of the LAPase activity could be adsorbed onto mixed cellulose ester membrane filters as compared to low protein binding filters (and more than 90% for trypsin and chymotrypsin). This adsorption of enzymes on filters would lead to an overestimation of the particulate EEA fraction. Also, extracellular enzymes may be washed off from the cell surface even during gentle filtration and thus contribute to the pool of dissolved enzymes. However, due to the low abundance of prokaryotes typically found in the deep ocean, the potential release of ectoenzymes most likely would not contribute much to the pool of dissolved EEA. In our study, dissolved EEA was considered as the EEA obtained in the filtrate. Total and dissolved EEA were determined on six replicate samples. For enzyme kinetics, three replicate samples were used.

Statistical analysis

Data were log transformed to attain normality prior to applying the *t*-student test and Spearman's rank correlation coefficients were calculated. To check the normality of the individual data sets, the Kolmogorov–Smirnoff test was used. For calculating uncertainties on ratios, error propagation was taking into account; standard deviations were calculated using a formula for the propagation of error (Bevington & Robinson 2003) as follows:

$$\Delta z / z = \sqrt{[((\Delta x)^2 / x) + ((\Delta y)^2 / y)]}$$

where z is the percent abundance or the percent assimilation, and is equal to x divided by y, as described above. Δx and Δy are the standard deviations associated with x and y, respectively. Δz is the standard deviation calculated for z. Standard deviations were then converted to standard errors for each calculation.

Results

Prokaryotic abundance (PA) and prokaryotic heterotrophic production (PHP)

As expected, PA decreased with depth by one order of magnitude (Fig. 2A) over the 100-7000 m depth range. Despite the fact that PA was higher in all the water masses of the African transect than along the TA transect, no significant differences were found between the average values of the two transects (*t*-test; $p = 0.24$, $n = 139$). PHP also decreased with depth, although by three orders of magnitude (Fig. 2B), being only slightly (but significantly) higher in the subsurface and OML of the TA than in the African transect. Average cell-specific PHP decreased with depth by two order of magnitude (Fig. 2C). Similarly to PHP and in contrast to PA, average cell-specific PHP was only slightly higher in the subsurface and OML layers of the TA than in the African transect. Like for PHP, no significant differences were found for average cell-specific PHP (*t*-test; $p > 0.05$) in any of the water masses between the two transects.

Distribution of particulate organic matter

POC (Fig. 3A) was significantly higher in all the water masses of the TA than in those of the African transect (*t*-test; $p < 0.001$, $n = 106$). In the TA, POC increased by a factor of 2 from the 100 m depth horizon to the AAIW, and decreased thereafter to concentrations similar to those of the subsurface waters (Fig. 3A). In the African transect, however, POC concentrations decreased with depth from the subsurface waters to the LNADW to about half of the subsurface POC concentration, and increased in the

deepest water mass sampled (LDW) to POC concentrations similar to those of subsurface waters (Fig. 3A).

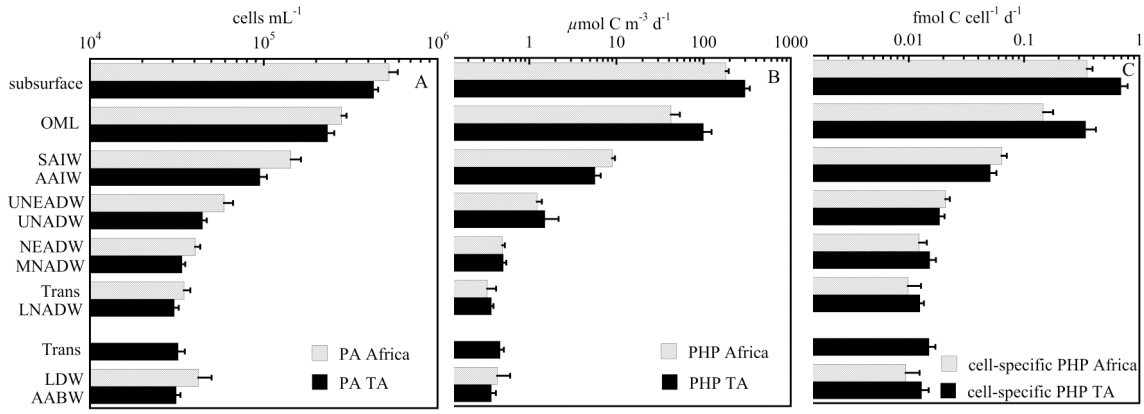


Fig. 2. Distribution of basic prokaryotic parameters in the different water masses in the Transatlantic transect (TA) and the African transect (African) (*see* Table 1 for depth ranges of each water mass). (A) Prokaryotic abundance (cells mL^{-1}), (B) Prokaryotic heterotrophic production (μmol C m^{-3} d^{-1}), (C) Average cell-specific prokaryotic heterotrophic production (fmol C $cell^{-1}$ d^{-1}). Bars represent SE of the mean

PON concentrations were generally one order of magnitude lower than POC (Fig. 3B), decreasing with depth in both transects. In contrast to POC, significantly higher (*t*-test; $p < 0.05$, $n = 105$) PON concentrations were found along the African transect than at the TA transect (compare Fig. 3A and B), albeit PON concentrations were similar in the deepest water masses (Trans, NADW, LDW and AABW) of the two transects (*t*-test; $p > 0.05$). Throughout the entire water column, the mean C:N ratio of POM (Fig. 3C) was significantly higher along the TA transect than the African transect (*t*-test; $p < 0.0001$, $n = 105$), due to both the higher POC and lower PON concentrations in the TA than in the African transect.

Total and dissolved extracellular enzymatic activity (EEA)

In contrast to prokaryotic heterotrophic production, total and dissolved EEA decreased only slightly with depth (Fig. 4). The different total EEA were significantly correlated with each other (Spearman's *rho*, $p < 0.0001$). A particularly strong correlation was found between α-glucosidase (AGase; Fig. 4A) and β-glucosidase (BGase; Fig. 4B) activity (Spearman's *rho* = 0.91, $p < 0.0001$, $n = 106$) (compare Fig. 4A and B). Leucine aminopeptidase (LAPase; Fig. 4C) and alkaline-phosphatase (APase; Fig. 4D) activities were generally higher than AGase and BGase, particularly at

the base of the euphotic layer (subsurface layer). APase, decreased less with depth than AGase, BGase and LAPase.

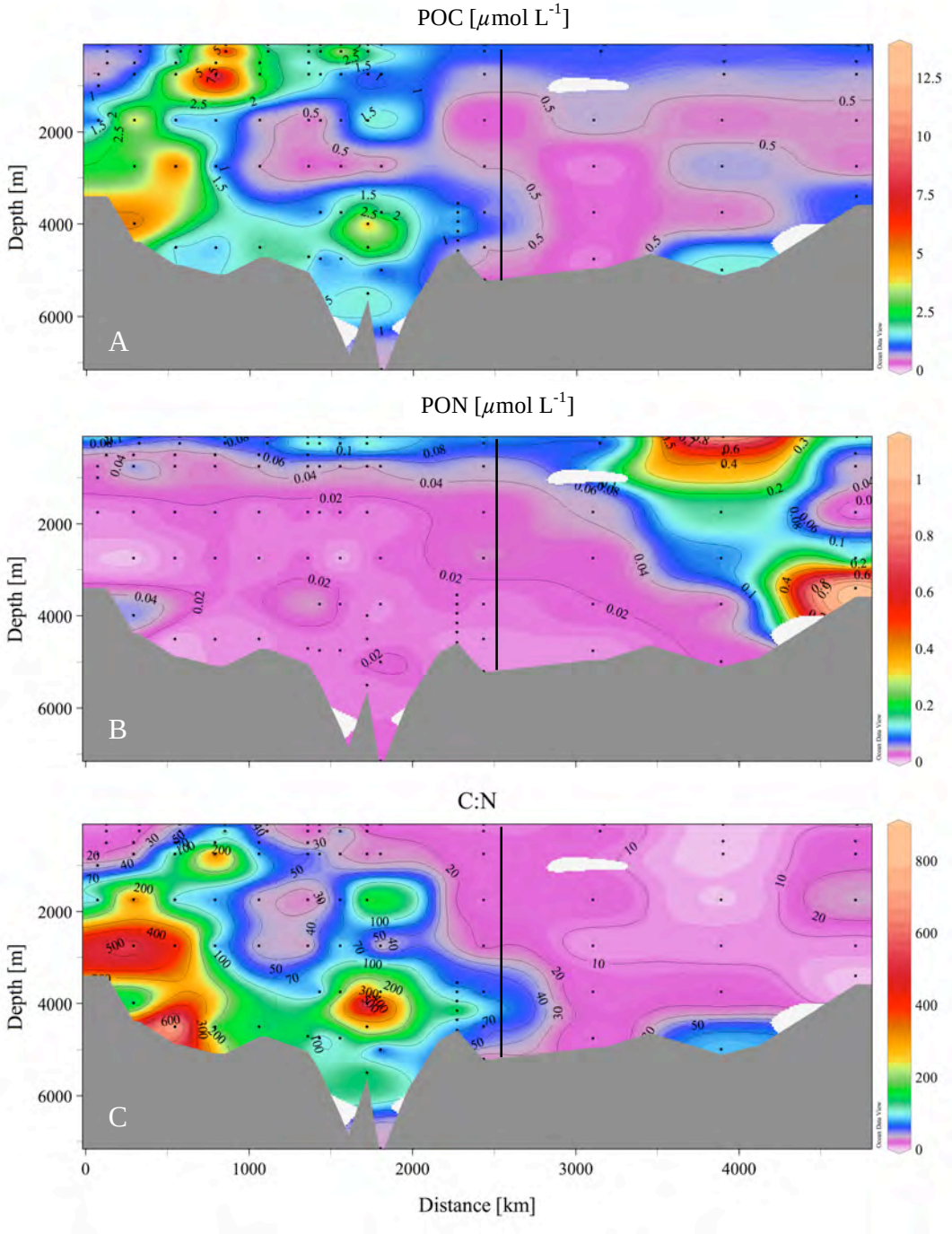


Fig. 3. Distribution of (A) POC (μmol L^{-1}), (B) PON (μmol L^{-1}), (C) POC:PON molar ratio throughout the water column along the entire ARCHIMEDES-III cruise track (from Sta. 4 to Sta. Sta. 38) The vertical full line indicates where the cruise track turned from the westward (Transatlantic) to the northward (African) transect

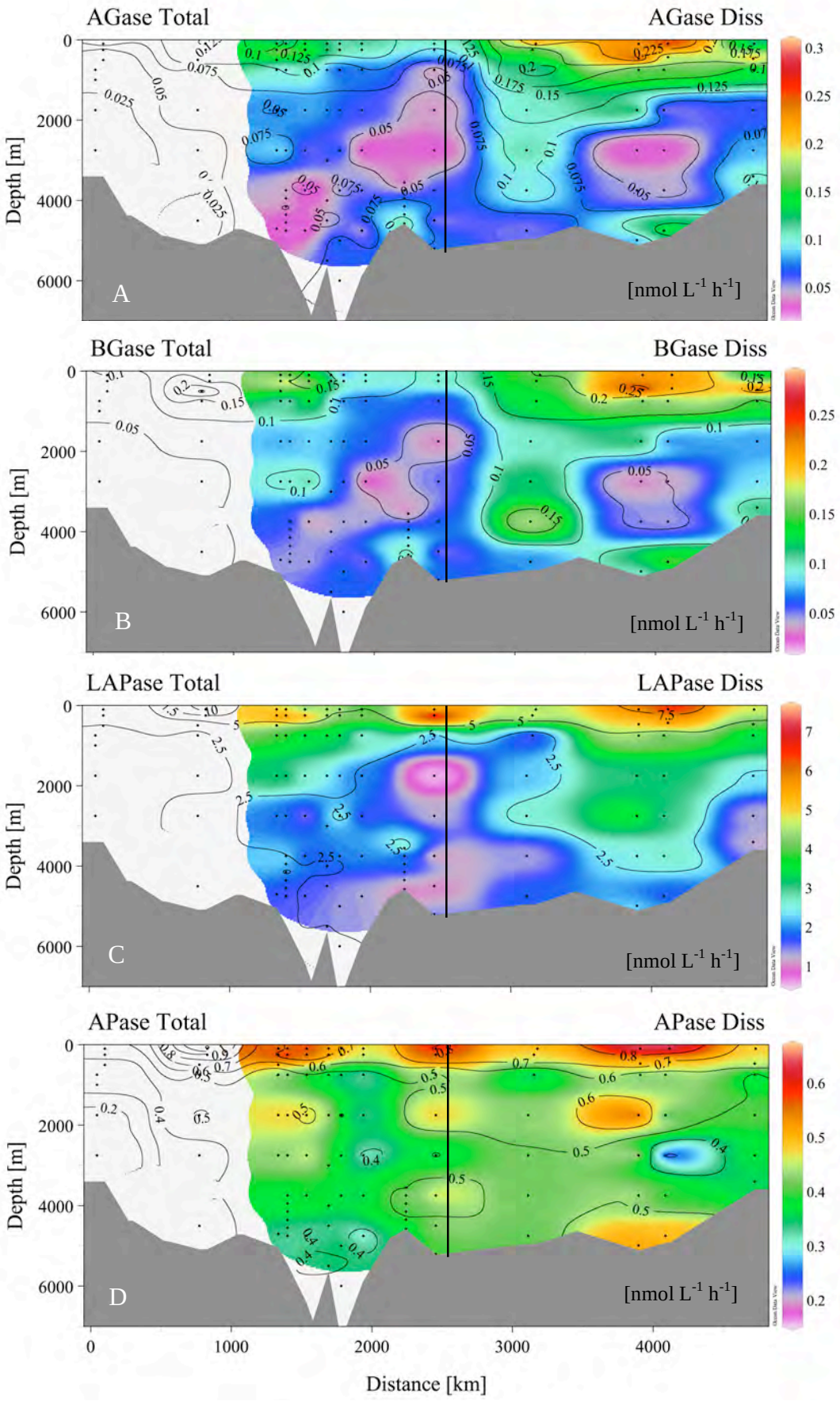


Fig. 4. Distribution of (A) AGase (alpha-glucosidase), (B) BGase (beta-glucosidase), (C) LAPase (leucine aminopeptidase) and (D) APase (alkaline phosphatase) activities throughout the water column along the entire ARCHIMEDES-III cruise track (from Sta.4 to Sta. 38); values are given in nmol L^{-1} h^{-1}. Total EEA are plotted as black isolines over the dissolved EEA (color gradient). Where color contours are missing, only total EEA was measured. Vertical full line indicates boundary between Transatlantic and African transect

Similar to the total EEA, also the different dissolved EEA were significantly correlated with each other (Spearman's *rho*, $p < 0.0001$). Again, the strongest correlation was found between the dissolved AGase and BGase (Spearman's *rho* = 0.90, $p < 0.0001$, n = 66). The distribution patterns of the dissolved and total EEA for all the enzymes (AGase, BGase, LAPase and APase) were very similar along the cruise track and throughout the water column (compare black isolines with color background in Fig. 4A-D). Hence, the different total and dissolved EEA were correlated with each other (Spearman's *rho*, $p < 0.005$). The log-log slope of the dissolved versus total EEA was very close to 1 for AGase (slope = 0.98, n = 66, R^2 =0.96), BGase (slope = 0.97, n = 66, R^2 =0.95) and LAPase (slope = 0.93, n=66, R^2 =0.98), however, lower for APase (slope = 0.75, n=66, R^2 =0.85). No significant differences for any of the EEA were detectable (*t*-test; $p < 0.05$) between the African and TA transects. Average cell-specific EEA increased with depth for all the enzymes (data not shown).

The contribution of dissolved to total EEA (Fig. 5A-D) was high in the aphotic layers for all enzymes, particularly for LAPase and APase (Fig. 5C, D), whereas the contribution of dissolved AGase and BGase to the respective total EEA was more patchy (Fig. 5A, B). Overall, no significant differences were found between the percentages of dissolved to total EEA for any of the enzymes between the two transects (*t*-test; $p < 0.05$).

Dynamics in kinetics of total and dissolved LAPase and APase

At six stations (labeled as diamonds in Fig. 1), profiles of kinetic parameters for total and dissolved LAPase and APase were obtained. Generally, the maximum hydrolysis rates (V_{max}) of total and dissolved LAPase and APase decreased with depth (Fig. 6A, B). Significant differences were found between the subsurface and OML and the deeper water masses in the V_{max} of total and dissolved LAPase (*t*-test; $p < 0.001$, n = 29) and APase (*t*-test; $p < 0.001$, n = 30). Significant differences between dissolved and total V_{max} were only found in the subsurface waters for LAPase (*t*-test; $p = 0.013$, n = 10) and in the OML (*t*-test; $p = 0.048$, n = 10) and AAIW (*t*-test; $p = 0.038$, n = 10) for APase. In these upper water masses, the differences between the V_{max} of dissolved and total APase (Fig. 6B) were higher than for LAPase (Fig. 6A). The detected difference in the V_{max} between dissolved and total APase coincided with the lower contribution of dissolved APase to the total APase pool as compared to LAPase (Fig. 4A-D).

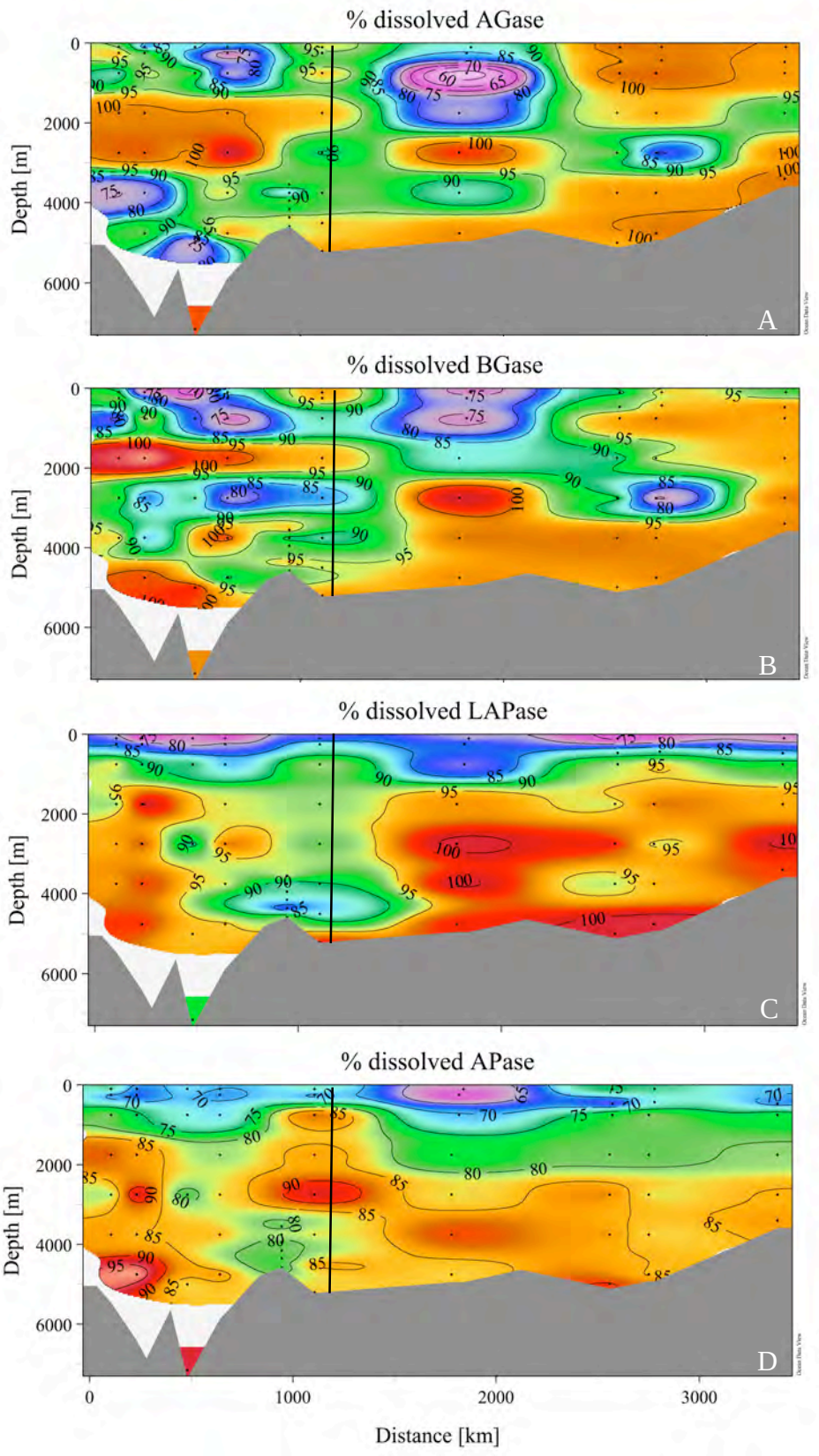


Fig. 5. Distribution of the percentage of dissolved relative to total (A) AGase (alpha-glucosidase), (B) BGase (beta-glucosidase), (C) LAPase (leucine aminopeptidase) and (D) APase (alkaline phosphatase) activity throughout the water column along the entire ARCHIMEDES-III cruise track (from Sta.18 to Sta. 38). Note that panels start at Sta. 18 because there were no measurements of dissolved EEA performed between Sta. 4 and Sta. 18 (see Fig. 4). Vertical full line indicates boundary between Transatlantic and African transect. SE were around 9, 10, 2 and 3% for AGase, BGase, LAPase and APase, respectively

In contrast to V_{max}, the half-saturation constant (K_m) of LAPase (Fig. 6C) of the total and the dissolved fractions increased with depth. The subsurface and OML exhibited a significantly lower K_m than the deeper water masses for the total (*t*-test; p = 0.00013, n = 29) and dissolved (*t*-test; p = 0.0005, n = 29) LAPase, and for total APase (*t*-test; p = 0.0004, n = 30, Fig. 6D). Similarly to the V_{max}, the difference between and dissolved K_m was higher for APase (Fig. 6D) than for LAPase (Fig. 6C), particularly in the two upper water masses. Significant differences between dissolved and total K_m were only found for APase in the OML (*t*-test; p < 0.05, n = 10).

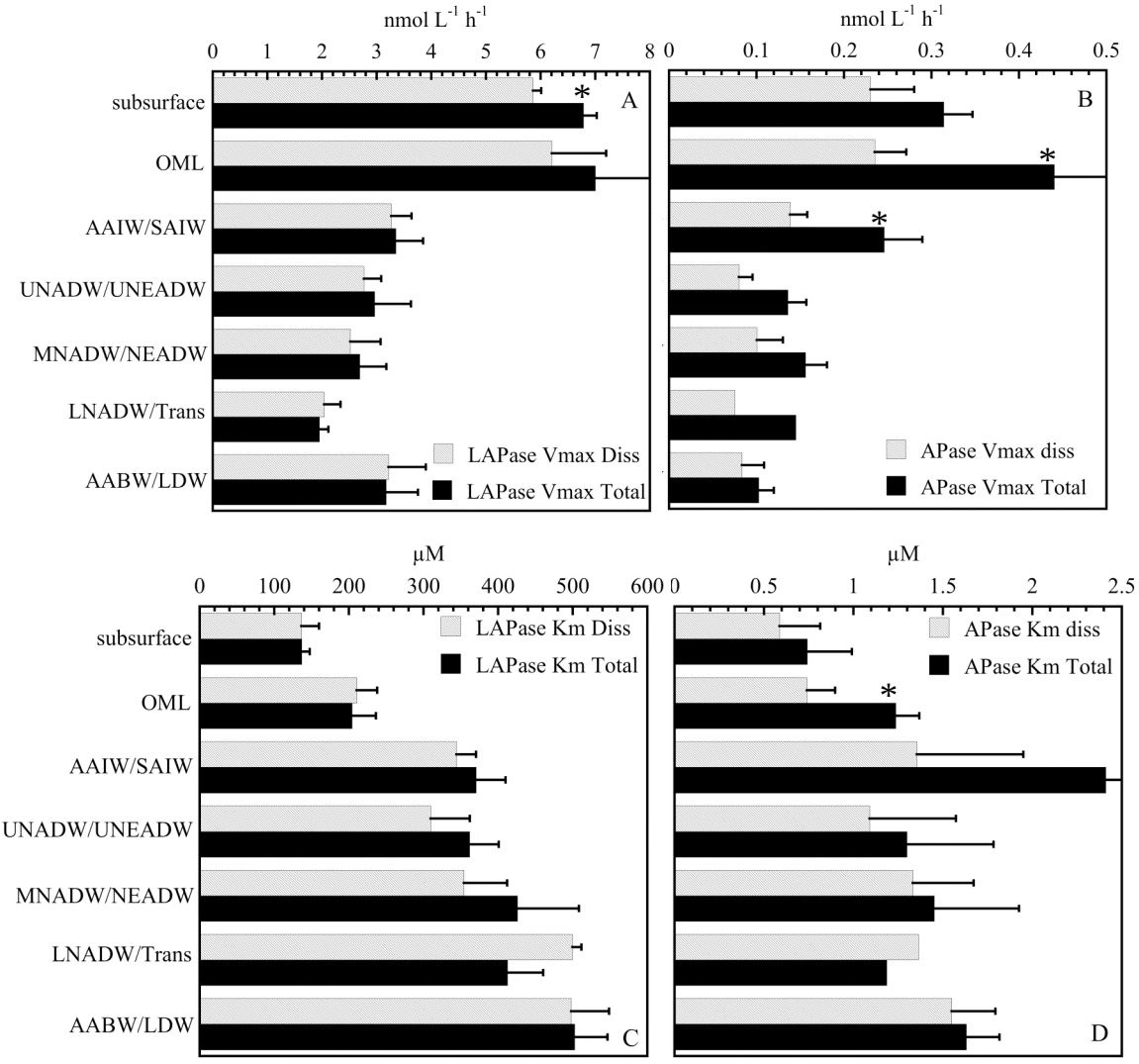


Fig. 6. Extracellular enzymatic kinetics of LAPase (A, C) and APase (B, D) at the stations identified in Fig. 1; (A, B) V_{max} (nmol h^{-1}), (C, D) K_m (μmol L^{-1}). Bars represent SE of the mean. Asterisks denote water masses where significant differences in enzyme kinetics between dissolved and total EEA were measured

Discussion

Only a few reports are available on EEA in deep oceanic water column. Our range of LAPase (0.2-13.8 nmol L^{-1} h^{-1}) activities is similar to that reported in **Chapter III** for the water column (100 to 4500 m) of the (sub)tropical North Atlantic (0.6-9.2 nmol L^{-1} h^{-1}), by Hoppe & Ullrich (1999) for the deep Indian Ocean (6-15 nmol L^{-1} h^{-1}) and by Tamburini et al. (2009) for the Tyrrhenian Sea down to 3500 m depth (0.51-8.6 nmol L^{-1} h^{-1}). Our LAPase rates, however, are higher than reported in other studies such as the Mediterranean Sea down to 2000 m depth (0.034-2.77 and 0.23-1.78 nmol L^{-1} h^{-1} by Zaccone et al. (2003) and Tamburini et al. (2002), respectively). The APase activities reported in this study (0.14-1.3 nmol L^{-1} h^{-1}) are also in the same range as the APase rates found in **Chapter III** for the (sub)tropical Atlantic water column (0.04-3.7 nmol L^{-1} h^{-1}), by Tamburini et al. (2002) for the DYFAMED station (0.01-1.8 nmol L^{-1} h^{-1}) and by Tamburini et al. (2009) for the Tyrrhenian Sea (0.21-9.9 nmol L^{-1} h^{-1}), but lower than the rates obtained by Hoppe & Ullrich (1999) for the Indian Ocean deep-waters (2-4.5 nmol L^{-1} h^{-1}), and higher than the APase activities reported by Zaccone et al. (2003) for the Ionian Sea (0.08-0.4 nmol L^{-1} h^{-1}) and the rates obtained by Koike & Nagata (1997) for the Central Pacific deep-waters (0.031-0.35 nmol L^{-1} h^{-1}). However, the latter study only measured the particulate fraction of APase (> 0.2 μm), which might explain the low values reported by these authors.

Although it has been assumed that only cell-associated EEs are of ecological significance (Someville & Billen 1983, Rego et al. 1985, Chrost & Rai 1993), the contribution of dissolved EEA to total EEA (AGase, BGase, LAPase and APase) measured in this study was high in the dark open ocean reaching almost 100% (Fig. 5A-D). Several studies have addressed the importance of dissolved EEA to total hydrolytic activity. Someville & Billen (1983) reported that most of the exoprotease activity in the eutrophic Belgian coastal waters was in the dissolved fraction. In Tokyo Bay, dissolved carboxypeptidase activity contributed between 10% and 50% of total EEA, both in freshwater and seawater communities (Hashimoto et al. 1985). In the Adriatic Sea, over a time-course of 22 h, dissolved alpha- and beta–glucosidase activity reached 73% and 65% of the activity in unfiltered water, respectively (Bochdansky et al. 1995). Over a diel cycle at a coastal site in the western Mediterranean Sea, dissolved alpha- and beta-glucosidase activity ranged between 0 and 100% of total activity (Karner & Rassoulzadegan 1995). Free laminarinase activity contributed up to 100% of total

laminarin and 48-69% of total xylan hydrolysis in a mesocosm experiment during a phytoplankton bloom (Keith & Arnosti 2001). In the Elbe estuary, Karrasch et al. (2003) reported that the dissolved EEA reaches 20-80% of the total water column activity. Ziervogel & Arnosti (2008) studying the EEA of surface waters in the northeastern Gulf of Mexico concluded that dissolved EEA plays a significant role in the hydrolysis of high-molecular weight substrates. All the above studies highlight the major contribution of dissolved EE to total hydrolytic activity, despite its variability in time and space. Nevertheless, none of these studies addressed the importance of dissolved EEA with respect to total EEA throughout the entire water column of the open ocean, and in particular the deep sea. In the only profile available comparing dissolved and total EEA in the ocean, Davey et al. (2001) found that total potential LAPase rates significantly decreased with depth (0-200 m depth) while dissolved LAPase did not. Thus, the contribution of dissolved LAPase increased towards the deeper waters. Our results are in agreement with the EEA profile reported by Davey et al. (2001), however, extending it to the bathy- and abyssopelagic realm.

This rather high contribution of dissolved to total EEA is surprising, as it is commonly assumed that free-living prokaryotes exhibit a tight hydrolysis-uptake coupling with the ectoenzymes attached to the cell wall or in the periplasmic space, where hydrolysis of macromolecular substrate occurs in intimate vicinity to the sites of hydrolysis product uptake (Hoppe et al. 2002). In contrast, particle-associated prokaryotes have been suggested to exhibit a loose hydrolysis-uptake coupling with the extracellular enzymes released into the particle. Hence, enzymatic cleavage of substrate can thus occur at distance from the cell (Smith et al. 1992). Vetter & Deming (1999) reported that released bacterial EE can produce sufficient hydrolysate from POC to support growth in the absence of any other significant source of DOC and without direct contact between the cell and particulate substrate. These empirical results obtained by Vetter & Deming (1999) support the model-based predictions formulated in an earlier paper (Vetter et al. 1998), suggesting that dissolved EE are advantageous when bacteria are attached to particles and when the substrate is within a well-defined distance to the enzyme source.

The factors determining the contribution of dissolved to total EEA are not well understood. Investigations on pure cultures of bacteria have demonstrated that EE can be released from cells in the presence of the corresponding substrate (Alderkamp et al. 2007), as a function of growth phase (Antranikian 1992), in response to bacterial

starvation (Albertson et al. 1990), viral lysis (Karner & Rassoulzadegan 1995), protozoan grazing (Bochdansky et al. 1995), or changes in microbial cell permeability (Chrost 1991). Once EE are released, they will remain active for a certain period of time. However, the lifetime of dissolved EE in marine waters is largely unknown, and different enzymes seem to have different hydrolytic lifetimes. In an enzyme degradation experiment, Bochdansky et al. (1995) found that about 70% of the initial dissolved AGase and BGase activity remained after 22 h. Ziervogel & Arnosti (2008) showed that free laminarinase was highly active only in the initial phase of a 144 h incubation, whereas xylanase, pullulanase and chondroitin hydrolase were active throughout the entire incubation period.

There are two possible reasons for the high dissolved EEA in the deep ocean. Either the dissolved EEs are a consequence of the substrate history of the water masses, or they are actively released by prokaryotes. The first option would depend on passive advection of the dissolved EEA by horizontal and vertical transport. No autochthonous release of EEs would then be required, as the dissolved EEs would be generated elsewhere. However, this would require that the dissolved EEs remain active for a prolonged period of time. The lifetime of free enzymes has been shown to be extended if associated with particles (Gianfreda & Scarfi 1991, Ziervogel et al. 2007). Koike & Nagata (1997), for the deep central Pacific, and Kim et al. (2007), for the benthic nepheloid layer of a mesotrophic lake, suggested that the high APase activity coinciding with high phosphate concentrations might be due to the transport of these enzymes attached to rapidly sinking particles from the euphotic zone and their subsequent fragmentation and dissolution. It is known that surface associations may offer dissolved EEs an improved resistance to physico-chemical degradation (Lähdesmäki & Piispanen 1992), and protection from remineralization (Lozzi et al. 2001). There is also evidence that bacterial EEs can be embedded in an exopolymeric matrix (Decho 1990). Dissolved EEs may become attached to this matrix forming a complex, similar to the enzyme-humic complexes in soils (Chrost 1990). Another potential mechanism of the incorporation of EEs into detrital particle complexes would be the trapping of digestive enzymes within partially degraded bacterial membranes which act as micelles (liposomes) (Nagata & Kirchman 1992). A large fraction of the dissolved EEs may be trapped by particles including colloids or liposomes. Furthermore, the additional stabilizing effects of low temperatures, characteristic for the deep ocean, could also facilitate the preservation of dissolved EE in the deep ocean as compared to shallow

waters. The discontinuities showed by EEA in the vertical profiles (Fig. 6), further suggest signals of advective water transport.

The kinetic parameters (V_{max} and K_m) for LAPase and APase determined in this study (Fig. 6A-D) are in the same range as obtained in a previous Atlantic study (**Chapter III**), the only other study where kinetic parameters have been determined throughout the whole oceanic water column. As in the present study, in **Chapter III** a decrease was found in the V_{max} of LAPase (~12-3 nmol L^{-1} h^{-1}) and APase (~0.3-0.1 nmol L^{-1} h^{-1}) and an increase in the K_m of LAPase (~ 400-1200 μM) and APase (~2-23 μM) with depth down to 4500 m. Tamburini et al. (2002) obtained lower K_m values for LAPase than in the present study, ranging between 0.4-1.1 μM in the Mediterranean deep-waters (down to 2000 m depth). However, the APase K_m values of 0.05-1.2 μM reported by Tamburini et al. (2002) are in the same range as the APase K_m we obtained. In contrast to Tamburini et al. (2002), but in agreement with **Chapter III** and to the present study, Davey et al. (2001) report increasing total LAPase K_m values with depth from around 70 to 250 μM from the surface waters to 200 m depth in the North Atlantic. In the latter study, however, dissolved LAPase K_m was between 500-3000 μM with no depth-related trend. These authors also found a significant decrease in the total LAPase V_{max} with depth but not in the dissolved V_{max}. The differences found between the LAPase K_m obtained by Tamburini et al. (2002) and the other studies including the present one could be due to the fact that Tamburini et al. (2002) added a substantially lower range of substrate concentration (0.05-5 μM) than in the other studies. The same distribution pattern with depth (decrease in V_{max} and increase in K_m) as reported in the present study was found in Davey et al. (2001) and in **Chapter III**. The increase in K_m and the cell-specific V_{max}/K_m ratio (which describes the ability of enzymes to compete at low substrate concentration (Healey 1980)) with depth is most likely due to the refractory nature of the organic matter present in deep waters. The high K_m found in the deep ocean for the two enzymes (Fig. 6C-D) might also suggest that the bulk of the deep-water prokaryotes are subjected to a way of life depending on pulses of organic matter supply. Hoppe et al. (1993) suggested that mesopelagic bacteria have the capability to respond or adapt rapidly to nutrient changes. Moreover, a substantially higher V_{max} than the actual uptake rates or turnover of the substrates has been reported before (Hoppe et al. 1993, Davey et al. 2001). This might indicate substrate limitation or an excess of EEs. The latter would allow prokaryotes to rapidly utilize pulses of substrates. As shown by the kinetic experiments (Fig. 6) and the contribution of

dissolved EEA (Fig. 5), APase seems to be more particle-associated than LAPase. This could be explained if, as suggested by Koike & Nagata (1997) and Kim et al. (2007), APase was more associated with solid sinking algal aggregates, which would be not as prone to destruction by filtration as gel-like particles. Overall, the fact that the kinetic parameters of total and dissolved EEs are similar in the meso- and bathypelagic waters (Fig. 6A-D) suggests that dissolved and total EE are of the same origin, with the dissolved EEs not being advected from other areas.

The same origin of dissolved and total EEA would support the second hypothesis explaining the observed dominance of dissolved EEA in the dark waters of Central Atlantic Ocean. Then, EEA might result from autochthonous prokaryotic activity associated with fragile colloidal and particulate material, prone to be disrupted during sampling. It is well-known that coagulation processes can lead to the formation of colloidal and ultimately, microparticulate organic material (Verdugo et al. 2004). Even low-molecular weight DOM has the potential to coagulate spontaneously to form polymeric gels (Azam 1998, Chin et al. 1998). These microgels may interact with other colloidal matter, forming distinct submicrometer particles that are ubiquitously present in seawater at concentrations of up to 10^9 mL^{-1} (Koike et al. 1990, Wells & Goldberg 1992, Wells 1998). This polysaccharide-based condensed matter harbors a higher concentration of nutrients than the surrounding water (Müller-Niklas et al. 1994). Bacteria have been reported to be enriched by up to three orders of magnitude on particles (Müller-Niklas et al. 1994). These would generate nutrient-enriched zones in the micrometer range, similar to the microzones proposed by Azam (1998). The above-cited papers, however, all dealt with colloidal and marine snow-type particles in the euphotic layer and upper mesopelagic realm. Whether this kind of particles prevails also in the deeper meso- and bathypelagic ocean is largely unknown. Recently, Verdugo et al. (2008) reported high concentrations of DOM polymers, spontaneously forming bioreactive polymer gels, in coastal and oceanic samples collected down to depths of 4000 m. These recently discovered self-assembled microgels are extensively colonized by microbes. The concentration of these particles is estimated to be 10 to 50 times larger than the total biomass of marine organisms, and thus could be among the richest pools of bioreactive carbon on our planet. Moreover, they could supply a major fraction of the microbial substrate, particularly important in the deep ocean where substrate is limited.

In the present study, the POM distribution was significantly different between the TA and the African transects (Fig. 3A-C), probably due to distinct oceanographic conditions (different water masses and circulation patterns, proximity to upwelling regions, etc). However, along the two transects, the POC concentrations were rather constant with depth while PON decreased with depth (particularly in the TA transect), leading to higher POC : PON ratio in deeper layers. The observed rather stable distribution of POC with depth is in striking contrast to the generally observed decrease in sinking POC collected with sediment traps (Antia et al. 2001), and might reflect the potential of deep-water DOC to coagulate and form POC as proposed by Verdugo et al. (2008).

Thus, there is evidence that carbon-rich colloidal and/or particulate organic matter is abundant in the deep ocean. The release of EEs by the particle-associated prokaryotes into the particle might help maintaining relatively high metabolic activity as compared to their free-living counterparts. In fact, bacterial foraging theory suggests that independent of possible assemblage differences, individual bacteria may produce more EE in diffusionally constrained space such as particle aggregates, where the return of hydrolysate is potentially high (Vetter et al. 1998) or, when confronted with polymeric organic matter (Chrost 1991, Vetter & Deming 1999). Moreover, Allison (2005) has argued that microbes releasing extracellular enzymes have a competitive advantage over other microbes in environments with low rates of enzyme diffusion, such as sediments or particles.

To further investigate whether prokaryotes are associated with suspended particulate organic material in the deep ocean, prokaryotic metabolic activity was compared with the POM pool (Table 2). Significant correlations were found between the POM pool, PHP, and the dissolved and total EEA, as well as the contribution of dissolved to total EEA for all the enzymes studied. PHP was correlated (Spearman's *rho*, $p < 0.002$) to dissolved and total AGase, BGase, LAPase and APase (Table 2). The strongest correlations with PHP were found for LAPase (PHP to total LAPase: Spearman's *rho* = 0.67, $p < 0.0001$, n = 93; PHP to dissolved LAPase: Spearman's *rho* = 0.71, $p < 0.0001$, n = 53; PHP to percentage of dissolved to total LAPase: Spearman's *rho* = - 0.62, $p < 0.0001$, n = 53), and with the contribution of dissolved to total APase (Spearman's *rho* = - 0.68, $p < 0.0001$, n = 53). Furthermore, POC was significantly correlated (Spearman's *rho*, $p < 0.0001$) to the four dissolved and total EEA, and to the contribution of dissolved to total LAPase and APase, but not with the percentage of

dissolved AGase and BGase (Table 2). It is noteworthy that the strongest correlations between EEA (dissolved and total) and PHP and POC are those for LAPase and APase, which are the EEA exhibiting the highest rates. In contrast, PON was only significantly correlated to total LAPase (Spearman's *rho* = 0.36, $p < 0.0001$, n = 50) and dissolved LAPase (Spearman's *rho* = 0.36, $p < 0.0001$, n = 50). In addition, the POC : PON ratio was also significantly correlated to the four dissolved and particulate EEA (Spearman's *rho*, $p < 0.0001$), probably due to the lower influence of PON on prokaryotic metabolism than POC (Table 2). The significant relation between total and dissolved EEA, and between EEA and the POM pool and prokaryotic metabolism suggests an active response to the organic matter pool by the prokaryotes.

Table 2. Correlation coefficients (Spearman's *rho*) between extracellular enzymatic activity (total, dissolved and percentage of dissolved relative to total) of AGase (alpha-glucosidase), BGase (beta-glucosidase), LAPase (leucine aminopeptidase) and APase (alkaline phosphatase), and prokaryotic heterotrophic production (PHP) and suspended particulate organic matter (POC: particulate organic carbon; PON: particulate organic nitrogen). Sample size (n) varies between 50 and 93; *p < 0.05, **p < 0.01

	PHP	POC	PON	POC : PON
Total AGase	0.45**	0.49**	0.15	-0.29*
Total BGase	0.48**	0.45**	0.14	-0.29*
Total LAPase	0.52**	0.51**	0.20	-0.30**
Total APase	0.67**	0.64**	0.36**	-0.31**
Dissolved AGase	0.53**	0.51**	0.18	-0.37**
Dissolved BGase	0.46**	0.42**	0.16	-0.29*
Dissolved LAPase	0.53**	0.41**	0.15	-0.37**
Dissolved APase	0.71**	0.69**	0.40**	-0.40*
% AGase diss.	-0.20	0.01	-0.23	-0.20
% BGase diss.	-0.32*	-0.17	-0.26	-0.05
% LAPase diss.	-0.67**	-0.48**	-0.35*	0.19
% APase diss.	-0.62**	-0.36**	-0.24	0.18

The observed pattern of the relative contribution of dissolved to total EEA is most likely not caused by measuring these parameters under surface pressure rather than

under in situ pressure conditions. Thus far, no consistent trend emerged from studies on the effect of hydrostatic pressure on bathypelagic prokaryotic activity including EEA. There is evidence that deep-water prokaryotic activity is overestimated if measured under decompressed conditions (Jannasch & Wirsen 1982), while other studies report an inhibition of prokaryotic activity under decompressed conditions (Tamburini et al. 2003). It is well known that prokaryotic enzymes might be adapted to a specific hydrostatic pressure (Jannasch & Taylor 1984, Somero 1992). In fact, Tamburini et al. (2002) showed that LAPase and APase rates measured in samples maintained under in situ pressure conditions were around 2 times higher than measured under decompressed conditions. Thus, our reported EEA might be increasingly underestimated with depth as compared to the in situ EEA and hence, the high EEA reported in the deep Atlantic might actually be even higher under in situ pressure conditions. Overall, however, there is no reason or evidence to assume that the pressure effect would act differently on the dissolved and the particulate EEA, and thus, the relation between dissolved and total EEA reported here should be unaffected by potential pressure effects.

Evidence has been accumulating recently, suggesting that a particle-associated life mode of prokaryotes in the deep ocean might be more common than hitherto assumed. Arístegui et al. (2005) and Reinthaler et al. (2006) reported an increasing nucleic acid content per cell with depth, indicative for a larger genome size, which, in turn, might point to an opportunistic life style (Lauro & Bartlett 2007). DeLong et al. (2006) found that deep-water prokaryotes are enriched in transposases, polysaccharide and antibiotic synthesis genes and high numbers of chaperone-encoding genes, all suggesting a predominately particle-attached life mode. Recently, González et al. (2008) found that the genome of a representative of a common bacterioplankton group (*Polaribacter* sp. MED 152 of the Flavobacteria) contains a substantial number of genes for attachment to surfaces or particles, gliding motility, and polymer degradation. Kirchman (2008) commenting on the findings of González et al. (2008) indicates that: “due to the fact that detrital particles are not very numerous in the oceans, the ‘‘desert’’ between particles may be studded with colloids, gels, and various forms of high molecular weight DOM, all potential sources of carbon and energy”. In **Chapter II** we report a relation between suspended POM and prokaryotic respiration in the dark ocean, suggesting that microbial life in the deep ocean is likely more dependent on slowly sinking or buoyant, laterally advected suspended particles than thus far anticipated.

The notion that microbes grow associated with (colloidal) particles in the deep ocean, where they may maintain relatively high metabolic activities, could explain also the high levels of dissolved EEA found in the dark realm of the ocean. However, the colloidal micro-environment where microbial assemblages might thrive is likely substantially altered during sample collection using standard methods, leading probably to the disruption of the size-continuum of the organic matter field. Consequently, new sampling strategies are needed to selectively collect deep ocean particles and thus, decipher the actual role of particle-attached versus free-living microbes in the deep ocean's biogeochemistry and ecology.

Acknowledgments

We thank the captain and crew of R/V *Pelagia* for their help during work at sea. M. Espino and I.J. Alonso-González performed the particulate organic matter analyses. We also thank three anonymous reviewers for their comments and suggestions to improve the manuscript. This research was supported by a predoctoral Fellowship of the Spanish Ministry of Education and Science (AP2005-3932) to F.B., a grant of the Earth and Life Science Division of the Dutch Science Foundation (ALW-NWO; ARCHIMEDES project, 835.20.023) to G.J.H., and a grant of the Spanish Ministry of Education and Science to J. A. (Remolinos Oceánicos y Deposición Atmosférica (RODA) project; CTM 2004-06842-C03/MAR). The work was carried out within the frame of the EU 'Networks of Excellence' MarBef and EurOceans.

References

Albertson NH, Nystrom T, Kjelleberg S (1990) Macromolecular-synthesis during recovery of the marine *Vibrio* sp. S14 from starvation. J. General Microb. 136:2201-2207

Alderkamp AC, Rijssel Mv, Bolhuis H (2007) Characterization of marine bacteria and the activity of their enzyme systems involved in degradation of the algal storage glucan laminarin. FEMS Microb. Ecol. 59:108-117

Allison SD (2005) Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. Ecol. Lett. 8:626-635

Aluwihare LI, Repeta DJ, Pantoja S, Johnson CG (2005) Two chemically distinct pools of organic nitrogen accumulate in the ocean. Science 308:1007-1010

Antia AN, Koeve W, Fischer G, Blanz T, Schulz-Bull D, Scholten J, Neuer S, Kremling K, Kuss J, Peinert R, Hebbeln D, Bathmann U, Conte M, Fehner U, Zeitzschel B (2001) Basin-wide particulate carbon flux in the Atlantic Ocean: Regional export patterns and potential for atmospheric CO_2 sequestration. Glob. Biogeochem. Cy. 15:845-862

Antranikian G (1992) Microbial degradation of starch. In: Wilkelman G (ed) Microbial Degradation of Natural Products. John Wiley & Sons, Weinheim, Germany, p 105-115

Arístegui J, Duarte CM, Gasol JM, Alonso-Sáez L (2005) Active mesopelagic prokaryotes support high respiration in the subtropical northeast Atlantic Ocean. Geophys. Res. Lett. 32:L03608, doi:03610.01029/02004GL021863

Azam F (1998) Microbial control of oceanic carbon flux: the plot thickens. Science 280:694-696

Azam F, Cho BC (1987) Bacterial utilization of organic matter in the sea. In: Ecology of microbial communities. Cambridge University Press, Cambridge, p 261-281

Azam F, Long RA (2001) Sea snow microcosms. Nature 414:495-498

Azúa I, Uanue M, Ayo B, Arrtolozaga I, Arrieta JM, Iriberri J (2003) Influence of organic matter quality in the cleavage of polymers by marine bacterial communities. J. Plankton Res. 25:1451-1460

Benner R, Pakulski JD, M.McCarthy, Hedges JI, Hatcher PG (1992) Bulk chemical characteristics of dissolved organic matter in the ocean. Science 255:1561-1564

Bevington PR, Robinson DK (2003) Data reduction and error analysis for the physical sciences, McGraw Hill, San Francisco, CA

Bochdansky AB, Puskaric S, Herndl GJ (1995) Influence of zooplankton grazing on free dissolved enzymes in the sea. Mar. Ecol. Prog. Ser. 121:53-63

Burns RG (1982) Enzyme activity in soil: location and a possible role in microbial ecology. In: Soil Biology and Biochemistry, Vol 14, p 423-427

Chin WC, Orellana MV, Verdugo P (1998) Spontaneous assembly of marine dissolved organic matter into polymer gels. Nature 395:568-572

Cho BC, Azam F (1988) Major role bacteria in biogeochemical fluxes in the ocean's interior. Nature 332:441-443

Chrost RJ (1989) Characterization and significance of ß-glucosidase activity in lake water. Limnol. Oceanogr. 34:660-672

Chrost RJ (1990) Microbial ectoenzymes in aquatic environments. In: Aquatic microbial ecology. Biochemical and molecular approaches. Springer, New York, p 47-78

Chrost RJ (1991) Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. In: Microbial enzymes in aquatic environments. Springer Verlag, New York, p 29-59

Chrost RJ, Rai H (1993) Ectoenzyme activity and bacterial secondary production in nutrient-improverished and nutrient-enriched mesocosms. Microb. Ecol. 25:131-150

Davey KE, Kirby RR, Turley CM, Weightman AJ, Fry JC (2001) Depth variation of bacterial extracellular enzyme activity and population diversity in the northeastern North Atlantic Ocean. Deep Sea Res. II 48:1003-1017

Decho AW (1990) Microbial exopolymers secretions in ocean environments: their role(s) in food webs and marine processes. Oceanogr. Mar. Biol. Ann. Rev. 28:73-153

DeLong EF, Preston CM, Mincer T, Rich V, Hallam SJ, Frigaard NU, Martínez A, Sullivan MB, Edwards R, Brito BR, Chisholm SW, Karl DM (2006) Community genomics among stratified microbial assemblages in the ocean's interior. Science 311:496-503

Dick WA, Tabatabai MA (1992) Significance and potential uses of soil enzymes. In: Metting FB (ed) Soil Microbial Ecology. Marcel Dekker, New York

Ferron B, Mercier H, Speer K (1998) Mixing in the Romanche Fracture Zone. J. Phys. Oceanogr. 28:1929-1945

Gianfreda L, Scarfi MR (1991) Enzyme stabilization: state of the art. Molecular and Cellular Biochemistry 100

González JM, Fernández-Gómez B, Fernández-Guerra A, Gómez-Consarnau L, Sánchez O, Coll-Lladó M, del Campo J, Escudero L, Rodríguez-Martínez R, Alonso-Sáez L, Latasa M, Paulsen I, Nedashkovskaya O, Lekumberri I, Pinhassi J, Pedrós-Alió C (2008) Genome analysis of the proteorhodopsin-containing marine bacterium *Polaribacter* sp. MED152 (Flavobacteria). Proc. Natl. Acad. Sci. USA 105:8724-8729

Hashimoto S, Fujiwara K, Fuwa K, Saino T (1985) Distribution and characteristics of carboxypeptidase activity in pond, river, and seawater in the vicinity of Tokyo. Limnol. Oceanogr. 30:631-645

Hoppe H-G (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Mar. Ecol. Prog. Ser. 11:299-308

Hoppe H-G, Ullrich S (1999) Profiles of ectoenzymes in the Indian Ocean: phenomena of phosphatase activity in the mesopelagic zone. Aquat. Microb. Ecol. 19:139-148

Hoppe H-G, Ducklow H, Karrasch B (1993) Evidence for dependency of bacterial growth on enzymatic hydrolysis of particulate organic matter in the mesopelagic ocean. Mar. Ecol. Prog. Ser. 93:277-283

Hoppe H-G, Arnosti C, Herndl GJ (2002) Ecological significance of bacterial enzymes in the marine environment. In: Burns RG, Dick RP (eds) Enzymes in the environment: activity, ecology, and applications. Marcel Dekker, Inc., New York, p 73-108

Jannasch HW, Wirsen CO (1982) Microbial activities in undecompressed and decompressed deep-seawater samples. Appl. Environ. Microbiol. 43:1116-1124

Jannasch HW, Taylor CD (1984) Deep-sea microbiology. Annu. Rev. Microbiol. 38:487-514

Karl DM, Knauer GA, Martin JH (1988) Downward flux of particulate organic matter in the ocean: a particle decomposition paradox. Nature 332:438-441

Karner M, Herndl GJ (1992) Extracellular enzymatic activity and secondary production in free-living and marine snow associated bacteria. Mar. Biol. 113:341-347

Karner M, Rassoulzadegan F (1995) Extracellular enzyme activity: indications for high short-term variability in a coastal marine ecosystem. Microb. Ecol. 30:143-156

Karrasch B, Ullrich S, Mehrens M, Zimmermann-Timm H (2003) Free and particle-associated extracellular enzyme activity and bacterial production in the lower Elbe estuary, Germany. Acta Hydroch. Hydrob. 31:297-306

Keith SC, Arnosti C (2001) Extracellular enzyme activity in a river-bay-shelf transect: variations in polysaccharide hydrolysis rates with substrate and size class. Aquat. Microb. Ecol. 24:243-253

Kim C, Nishimura Y, Nagata T (2007) High potential activity of alkaline phosphatase in the benthic nepheloid layer of a large mesotrophic lake: implications for phosphorus regeneration in oxygenated hypolimnion. Aquat. Microb. Ecol. 49:303-311

Kirchman DL (2008) New light on an important microbe in the ocean. Proc. Natl. Acad. Sci. USA 105:8487-8488

Kirchman DL, Ducklow HW (1993) Estimating conversion factors for thymidine and leucine methods for measuring bacterial production. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, p 513-517

Koike I, Nagata T (1997) High potential activity of extracellular alkaline phosphatase in deep waters of the central Pacific. Deep-Sea Res. 44:2283-2294

Koike I, Hara S, Terauchi K, Kogure K (1990) Role of sub-micrometre particles in the ocean. Nature 345:242-244

Lauro F, Bartlett DH (2007) Prokaryotic lifestyles in deep sea habitats. Extremophiles 12:15-25

Lozzi I, Calami L, Fusi P, Bosetto M, Stotzky G (2001) Interaction of horseradish peroxidase with montmorillonite homoionic to Na^+ and Ca^{2+}: effects on enzymatic activity and microbial degradation. Soil Biol. Biochem. 33:1021-1028

Lähdesmäki P, Piispanen R (1992) Soil enzymology: role of protective colloid systems in the preservation of exoenzyme activities in soil. Soil Biol. Biochem. 24:1173-1177

McLaren AD, Skujins J (1967) The environments of soil bacteria. In: Gray TRG, Parkinson D (eds) The Ecology of Soil Bacteria. Liverpool University Press, Liverpool

Misic C, Castellano M, Fabiano M, Ruggierini N, Saggiomo V, Povero P (2006) Ectoenzymatic activity in surface waters: A transect from the Mediterranean Sea across the Indian Ocean to Australia. Deep-sea res., Part 1 53:1517-1532

Moran SB, Charette MA, Pike SM, Wicklund CA (1999) Differences in seawater particulate organic carbon concentrations in samples collected using small- and large-volume methods: the importance of DOC adsorption to the filter blank. Mar. Chem. 67:33-42

Müller-Niklas G, Schuster S, Kaltenböck E, Herndl GJ (1994) Organic content and bacterial metabolism in amorphous aggregations of the northern Adriatic Sea. Limnol. Oceanogr. 39:58-68

Nagata T, Kirchman DL (1990) Filtration-induced release of dissolved free amino acids: application to cultures of marine protozoa. Mar. Ecol. Prog. Ser. 68:1-5

Nagata T, Kirchman DL (1992) Release of macromolecular organic complexes by heterotrophic marinen flagellates. Marine Ecology Progress Series 83:233-240

Obayashi Y, Suzuki S (2008) Adsorption of extracellular proteases in seawater onto filters during size fractionation. J. Oceanogr. 64:367-372

Paul EA (1989) Soil Microbiology and Biochemistry. In. Academic Press, San Diego

Rath J, Schiller C, Herndl GJ (1993) Ectoenzymatic activity and bacterial dynamics along a trophic gradient in the Caribbean Sea. Mar. Ecol. Prog. Ser. 102:89-96

Rego JV, Billen G, Fontigny A, Someville M (1985) Free and attached proteolytic activity in water environments. Mar. Ecol. Prog. Ser. 21:245-249

Reinthaler T, Aken Hv, Veth C, Williams PJl, Arístegui J, Robinson C, Lebaron P, Herndl GJ (2006) Prokaryotic respiration and production in the meso- and bathypelagic realm of the eastern and western North Atlantic basin. Limnol. Oceanogr. 51:1262-1273

Sala MM, Karner M, Arin L, Marrasé C (2001) Measurement of ectoenzyme activities as an indication of inorganic nutrient imbalance in microbial communities. Aquat. Microb. Ecol. 23:301-311

Smith DC, Simon M, Alldredge AL, Azam F (1992) Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. Nature 359:139-142

Somero GN (1992) Adaptation to high hydrostatic pressure. Annu. Rev. Physiol. 54:557-577

Someville M, Billen G (1983) A method for determining exoproteolytic activity in natural waters. Limnol. Oceanogr. 28:190-193

Tamburini C, Garcin J, Ragot M, Bianchi A (2002) Biopolymer hydrolysis and bacterial production under ambient hydrostatic pressure through a 2000 m water column in the NW Mediterranean. Deep Sea Res. II 49:2109-2123

Tamburini C, Garcin J, Bianchi A (2003) Role of deep-sea bacteria in organic matter mineralization and adaptation to hydrostatic pressure conditions in the NW Mediterranean Sea. Aquat. Microb. Ecol. 32:209-218

Tamburini C, Garel M, Ali BA, Mérigot B, Kriwy P, Charrière B, Budillon G (2009) Distribution and activity of Bacteria and Archaea in the different water masses of the Tyrrhenian Sea. Deep-Sea Research Part II 56:700-712.

UNESCO (1994) Protocols for the Joint Global Ocean Flux Study (JGOFS) Core Measurement. Intergovernmental Oceanographic Commission, Manual and Guides 29:169

Verdugo P, Alldredge AA, Azam F, Kirchman DL, Passow U, Santschi P (2004) The oceanic gel phase: a bridge in the DOM–POM continuum. Mar. Chem. 92:67-85

Verdugo P, Orellana MV, Chin WC, Petersen TW, Eng Gvd, Benner R, Hedges JI (2008) Marine biopolymer self-assembly: implications for carbon cycling in the ocean. The Royal Society of Chemistry 139:393-398

Vetter YA, Deming JW (1999) Growth rates of marine bacterial isolates on particulate organic substrates solubilized by freely released extracellular enzymes. Microb. Ecol. 37:86-94

Vetter YA, Deming JW, Jumars PA, Krieger-Brockett BB (1998) A predicitive model of bacterial foraging by means of freely released extracellular enzymes. Microb. Ecol. 36:75-92

Weiss M, Abele U, Weckesser J, Welte W, Schiltz E, Schulz GE (1991) Molecular architecture and electrostatic properties of bacterial porin. Science 254:1627-1630

Wells ML (1998) A neglected dimension. Nature 391:530-531

Wells ML, Goldberg ED (1992) Marine submicron particles. Mar. Chem. 40:5-18

Wetzel RG (1991) Extracellular enzymatic interactions: storage, redistribution, and interspecific communication. In: Chrost RJ (ed) Microbial enzymes in aquatic environments. Springer Verlag, New York, p 6-28

Williams C, Jochem FJ (2006) Ectoenzyme kinetics in Florida Bay: Implications for bacterial carbon source and nutrient status. Hydrobiologia 569:113-127

Zaccone R, Monticelli LS, Seritti A, Santinelli C, Azzaro M, Boldrin A, LaFerla R, D'Alcala MR (2003) Bacterial processes in the intermediate and deep layers of the Ionian Sea in winter 1999: vertical profiles and their relationship to the different water masses. J. Geophys. Res. 108:8117

Ziervogel K, Arnosti C (2008) Polysaccharide hydrolysis in aggregates and free enzyme activity in aggregate-free seawater from the North-Eastern Gulf of Mexico. Environ. Microb. 10:289-299

Ziervogel K, Karlsson E, Arnosti C (2007) Surface associations of enzymes and of organic matter: consequences for hydrolytic activity and organic matter remineralization in marinne systems. Mar. Chem. 104:241-252

Chapter V

Mesoscale eddies: hotspots of prokaryotic activity and differential community structure in the ocean

F. Baltar, J. Arístegui, J. M. Gasol, I. Lekunberri, G. J. Herndl

The ISME Journal (in revision)

Abstract

To investigate the effects of mesoscale eddies on prokaryotic assemblage structure and activity we sampled 2 cyclonic (CE) and 2 anticyclonic (AE) eddies in the permanent eddy field downstream the Canary Islands. The eddy stations were compared with two far-field stations (FF) located also in the Canary Current but outside the influence of the eddy field. The distribution of prokaryotic abundance (PA), bulk prokaryotic heterotrophic activity (PHA), various indicators of single-cell activity (such as nucleic acid content, proportion of live cells, and fraction of cells actively incorporating leucine), as well as bacterial and archaeal community structure were determined from the surface to 2000 m depth. In the upper epipelagic layer (0-200 m), the effect of eddies on the prokaryotic community was more apparent, as indicated by the higher PA, PHA, fraction of living cells, and percentage of active cells incorporating leucine within eddies than at FF stations. Prokaryotic community composition differed also between eddy and FF stations in the epipelagic layer. In the mesopelagic layer (200-1000 m), there were also significant differences in PA and PHA between eddy and FF stations, although in general, there were no clear differences in community composition or single-cell activity. The effects on prokaryotic activity and community structure were stronger in AE than CE, decreasing with depth in both type of eddies. Overall, both type of eddies show distinct community compositions (as compared to FF in the epipelagic), and represent oceanic "hotspots" of prokaryotic activity (in the epi- and mesopelagic realms).

Introduction

Mesoscale eddies are ubiquitous features in the ocean (Cheney & Richardson 1976, Arístegui et al. 1997, van Haren et al. 2006), with strong implications on regional biogeochemistry and productivity. Anticyclonic eddies have been seen to accumulate organic matter within their cores (e.g. Arístegui et al. 2003, Mathis et al. 2007) and to exhibit elevated microbial respiration (Arístegui & Montero 2005, Mouriño-Carballido & McGillicuddy 2006) and heterotrophic production (Ewart et al. 2008, **Chapter I**). Cyclonic eddies are known to enhance nutrient inputs to the surface ocean increasing

new production (Falkowski et al. 1991, Harris et al. 1997, Moran et al. 2001) and chlorophyll concentrations (Arístegui et al. 1997, McGillicuddy Jr. et al. 1998, Tarran et al. 2001). Current estimates suggest that up to 50% of the global new primary production may be caused by eddy-induced nutrient fluxes (Falkowski et al. 1991, McGillicuddy Jr. et al. 1998, Letelier et al. 2000). Thus, eddies exert a major control on the generation, accumulation and downward transport of biogenic production in the ocean, as well as on the associated remineralization processes mediated by prokaryotes.

Despite the recognized key role of prokaryotes within the marine biogeochemical cycles (e.g. Azam et al. 1983), only a reduced number of studies, sometimes contradictory, have been published analyzing the response of heterotrophic prokaryotes to eddy activity. Some of these studies reported increased prokaryotic abundance (PA) inside cold-core eddies (Lochte & Pfannkuche 1987, Harris et al. 1997, Thyssen et al. 2005) and in the frontal waters between cyclonic and anticyclonic eddies (Arístegui & Montero 2005). Other studies, however, did not find differences in depth-integrated prokaryotic biomass between the in- and outside of cyclonic eddies (González et al. 2001, Tarran et al. 2001). In the Canary Islands region, higher prokaryotic heterotrophic production (PHP) rates were measured within eddies compared with the surrounding waters (Bode et al. 2001, **Chapter I**). In the Sargasso Sea, Ewart et al. (2008) found an increase in PHP at the periphery of a cyclonic eddy relative to the eddy center, as well as in the core of an anticyclonic eddy. The latter authors found a tight coupling between phytoplankton and prokaryotic activity, suggesting that the variability of phytoplankton community structure plays a key role influencing PHP in these mesoscale features.

Less information is available concerning changes in prokaryotic community structure due to eddy influence. In a DMSP-producing coccolithophorid bloom in a North Atlantic cold-core eddy, Gonzalez et al. (2000) found that *Roseobacter*, SAR86, and SAR11 were the dominant groups of Bacteria associated with the bloom. However, no differences in the dominant groups were found between in- and outside the eddy. In contrast, Benitez-Nelson et al. (2007) reported mixed-layer bacterioplankton communities being similar inside and outside a cyclonic eddy, but below 50 m depth *Planctomycetes*, *Bacteroidetes*, and certain *Proteobacteria* (thought to degrade high molecular weight dissolved organic matter) were present. Zhang et al. (2009) found a greater crenarchaeaal contribution in the upper mesopelagic waters inside two cyclonic eddies (as compared to outside) that they related to a higher contribution of refractory dissolved organic matter. They also found a significantly higher bulk D-:L-Aspartic

acid uptake ratio in the core of two cyclonic eddies as compared to the outside areas, but no influence of the cyclonic eddies was found in the ratio of D-:L-Aspartic acid positive cells of *Bacteria* and *Archaea*. However, information on *Archaea* and on the activity of prokaryotes at the single-cell level comparing cyclonic and anticyclonic features is not available.

Here we report the abundance, relative nucleic acid content, viability, bulk and single-cell activities and community structure of prokaryotic assemblages in four island-induced eddies (2 cyclonic [CE] and 2 anticyclonic [AE]), compared with two unaffected (far-field [FF]) reference sites northwest of the Canary archipelago. We examined the effect of eddies in an oligotrophic region, where the impact on the prokaryotic community should be significant. Based on previous studies on the role of mesoscale eddies in oceanic biogeochemistry and productivity, we hypothesize that eddies could generate oceanic "hotspots" of activity and shifts in prokaryote assemblage composition at least in the epipelagic (0-200 m) and mesopelagic (200-1000 m) layers. We also investigated whether bathypelagic (1000-4000 m depth) prokaryotic assemblages underneath eddies respond to the presumably elevated vertical carbon fluxes.

Methods

Study site and sampling

The positions of mesoscale eddies were deduced at first instance by satellite images of sea-surface temperature during cruise RODA-I (11 August to 9 September 2006) on board the RV 'Hespérides' (Fig. 1). Once at the supposed eddy sites, their structures were characterized by means of XBT (expendable bathythermographs). Once located the eddy center, temperature, salinity and fluorescence were recorded down to 2000 m depth using a SeaBird 911 plus CTD system, mounted on a General Oceanics rosette sampler, equipped with twenty-four 12 L Niskin bottles. Eddy stations were compared to two far-field (FF) stations situated northwest of the Canary archipelago.

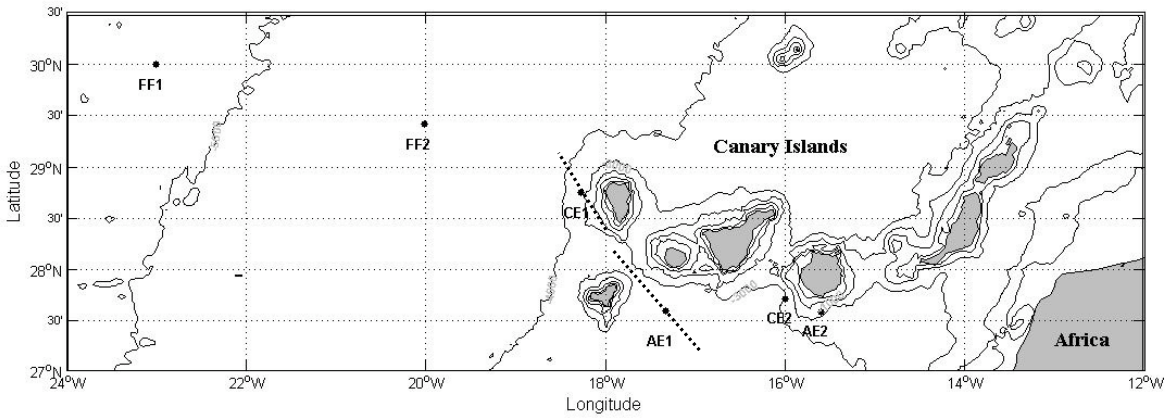


Fig. 1. Position of the sampled stations located in the far-field (FF) and in the core of cyclonic (CE) and anticyclonic eddies (AE) in the Canary Current system, during the RODA I cruise in August 2007. Sections crossing CE1 and AE1 are represented in Figure 3

These stations were placed inside the Canary Current, but outside the influence of the eddy field. Samples for prokaryotic abundance and heterotrophic activity and nucleic acid content were collected at each station from 10-13 depths ranging from 5 to 2000 m, including the deep chlorophyll maximum (DCM; 40 to 125 m), the deep scattering layer (DSL; 450-550 m) and the oxygen minimum zone (OMZ; 720 to 850 m).

Prokaryotic abundance (PA), nucleic acid content and membrane-compromised bacteria (NADS) determined by flow cytometry

Picoplankton collected from the different depth layers of the water column were enumerated using flow cytometry (FCM) with a FACSCalibur (Becton-Dickinson) with a laser emitting at 488 nm. Samples (1.5 ml) were fixed with paraformaldehyde (1% final concentration), incubated at 4°C for 15 to 30 min and then stored frozen in liquid nitrogen until analysis. Prior to counting the cells by FCM and after unfreezing, 200 µl of sample were stained with a DMSO-diluted SYTO-13 (Molecular Probes) stock (10:1) at 2.5 µM final concentration. Prokaryotes were identified by their signatures in a plot of side scatter (SSC) versus green fluorescence (FL1). High and low nucleic acid cells (HNA, LNA) were separated in the scatter plot of SSC-FL1 (Gasol et al. 1999).

HNA cells exhibited higher FL1 than LNA cells. Picocyanobacteria were discriminated in a plot of FL1 versus red fluorescence (FL3).

Viable and damaged prokaryotic cells were estimated in non-fixed samples following the nucleic acid double-staining (NADS) protocol (Gregori et al. 2001, Falcioni et al. 2008). NADS+, green cells (assumed to be active, with intact membranes) and NADS-, red cells (assumed to be inactive, with compromised cell membranes) were identified by simultaneous double staining with a membrane-permeant (SYBR Green; Molecular Probes) and impermeant (propidium iodide) probe. Immediately after collecting the samples, they were incubated in the dark with the probes for 15 min. NADS + and NADS- cells were enumerated by FCM and differentiated in a scatter plot of FL1 (green) -FL3 (red emission after blue-light excitation). Samples for prokaryotic abundance and NADS were run at a flow rate of ~60-70 µl min^{-1}, which was determined volumetrically after every 10 samples run.

Prokaryotic heterotrophic activity (PHA) estimated by [^{3}H] leucine incorporation

Prokaryotic heterotrophic activity was estimated from the incorporation of tritiated leucine using the centrifugation method (Smith and Azam 1992). ^{3}H-Leucine (Leu, Amersham, specific activity = 171 Ci $mmol^{-1}$) was added at saturating concentration (40 nmol l^{-1}) to 4 replicate 1.2 ml subsamples. Duplicate controls were established by adding 120 µl of 50% trichloroacetic acid (TCA) 10 min prior to isotope addition. The Eppendorf tubes were incubated at *in situ* temperature in temperature-controlled chambers for 2 to 7 h. Incorporation of leucine in the quadruplicate sample was stopped by adding 120 µl ice-cold 50% TCA. Subsequently, the subsamples and the controls were kept at –20°C until centrifugation (at ca. 12000 · *g*) for 20 min, followed by aspiration of the water. Finally, 1 ml of scintillation cocktail was added to the Eppendorf tubes before determining the incorporated radioactivity after 24 to 48 h on a Wallac scintillation counter with quenching correction using an external standard.

Catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH)

Immediately after collecting the samples from the Niskin bottles, 10 to 40 ml subsamples were fixed with paraformaldehyde (2% final concentration) and stored at 4°C in the dark for 12 to 18 h. The cells were collected on 0.2 µm polycarbonate filters

(Millipore, GTTP, 25 mm filter diameter) supported by cellulose nitrate filters (Millipore, HAWP, 0.45 μm), washed twice with 0.2 μm filtered Milli-Q water, dried and stored in a microfuge vial at –20°C until further processing in the laboratory. The filters were embedded in low-gelling-point agarose and incubated either with lysozyme for the *Bacteria* probes Eub338-III (mixture of probes Eub338, Eub II and Eub III; Amann et al. 1990, Daims et al. 1999), or Proteinase-K for the marine *Euryarchaeota* Group II probe Eury806 and for the marine Crenarchaeota Group I probe Cren537 (Teira et al. 2004) and GI-554 (Massana et al. 1997). To determine the coverage of the two *Crenarchaeota* probes, hybridization was performed on a set of samples with the oligonucleotide probes Cren 537 and GI-554 separately, and applied as a mix (CrenTotal). Filters were cut in sections and hybridized with horseradish peroxidase (HRP)-labeled oligonucleotide probes and tyramide-Alexa488 for signal amplification, following the protocol described in Teira et al. (2004). Cells were counter-stained with a DAPI mix: 5.5 parts Citifluor, 1 part Vectashield (Vector Laboratories) and 0.5 parts phosphate-buffered saline (PBS) with DAPI (final concentration 1 μg ml^{-1}). The slides were examined under a Zeiss Axioplan 2 microscope equipped with a 100 W Hg lamp and appropriate filter sets for DAPI and Alexa488. More than 800 DAPI-stained cells were counted per sample in a minimum of 30 fields of view. For each microscopic field, 2 different categories were enumerated: (1) total DAPI-stained cells and, (2) cells stained with the specific probe. The counting error, expressed as the percentage of the standard error between replicates, was 2% of the DAPI counts.

The use of different probes targeting *Crenarchaeota* revealed differences in detection efficiencies (CARD-FISH positive) and proportions of *Crenarchaeota* taking up leucine (MICRO-CARD-FISH positive, see below). Overall, the relative abundance of *Crenarchaeota* using GI-554 was very similar to that obtained by applying both probes simultaneously (CrenTotal) but, in deep waters, higher than the abundance obtained with the Cren537 probe. De Corte et al. (2009) also report a highly variable detection efficiency for *Crenarchaeota* using GI-554 and Cren537 in the Eastern Mediterranean Sea. However, these authors found no consistent depth-related trends in the relative abundance of both Cren537-positive and GI-554-positive cells. De Corte et al. (2009) showed that these dissimilarities were due to differences in the coverage of each probe. In this work, not only a higher crenarchaeal abundance was obtained using probe GI-554 than the Cren537, but also the proportion of active cells incorporating leucine within this group was always higher (see below).

MICRO-CARD-FISH

MICRO-CARD-FISH (CARD-FISH combined with micro-autoradiography) was performed following the protocol described by Teira et al. (2004). Briefly, samples (10-40 ml) were incubated at in situ temperature with 20 nM final concentration of ^{3}H-Leucine (Leu, Amersham, specific activity = 171 Ci mmol^{-1}). Some samples were killed with paraformaldehyde prior to adding the tritiated leucine and were used as controls. Incubation times varied according to the different depths and ranged between 2 and 24 h. After the incubation, samples were fixed overnight with paraformaldehyde (2% final conc.) at 4°C, gently filtered onto 0.2 μm polycarbonate filters (Millipore, GTTP, 25 mm diameter), and stored at -80ºC. The filters were afterwards hybridized following the CARD-FISH protocol cited above. The autoradiographic development was conducted by transferring previously hybridized filter sections onto slides coated with photographic emulsion (type NTB-2, melted at 43°C for 1 h). Subsequently, the slides were placed in a dark box with a drying agent and exposed at 4°C for 36 to 48 h. The slides were developed and fixed using Kodak specifications (Dektol developer [1:1 dilution with Milli-Q water] for 2 min, rinsed with Milli-Q water for 10 s, and fixed for 5 min, followed by a Milli-Q water rinse for 2 min). Cells were counter-stained with the same DAPI mixture used for the CARD-FISH protocol. The silver grains in the autoradiographic emulsion were detected by switching to the transmission mode of the microscope. More than 800 DAPI-stained cells were counted per sample. To enumerate the proportion of *Crenarchaeota* cells taking up leucine using the two different *Crenarchaeota* probes, the procedure was repeated using each of the probes (Cren 537 and GI-554) alone or in combination.

DNA sampling, extraction and purification, and fingerprinting of the communities

For DNA fingerprinting of prokaryotic communities, 2-5 L were filtered onto 0.2 μm polycarbonate filters (Millipore, GTTP, 47 mm filter diameter) and the filters stored in microfuge vials in liquid nitrogen for 24 h and then at –80°C until further processing in the laboratory. DNA extraction was performed using the UltraClean Soil DNA Isolation Kit MoBio kit (MoBIO laboratories, Carlsbad, CA, USA) and the protocol of the manufacturer.

Terminal-restriction fragment length polymorphism (T-RFLP) for archaeal communities. PCR conditions and chemicals were applied as described in Moeseneder et al. (2001). One µl of the DNA extract was used as a template in a 50 µl PCR mixture. For PCR, the Archaea-specific primers 21F-FAM and 958R-JOE were used (Moeseneder et al. 2001). The samples were amplified by an initial denaturation step at 94ºC (for 3 min), followed by 35 cycles of denaturation at 94ºC (1 min), annealing at 55ºC (1 min), and an extension at 72ºC (1 min). Cycling was completed by a final extension at 72ºC for 7 min. The PCR products were run on 1 % agarose gel. The gel was stained with a working solution of SYBR Gold and the obtained bands were excised, purified with the Quick gel extraction kit (Genscript, Piscataway, NJ, USA), and quantified using a Nanodrop spectrophotometer. Fluorescently labeled PCR products were digested at 37ºC overnight. Each reaction contained 30 ng of cleaned PCR product, 5 U of tetrameric restriction enzyme (*Hha*I) and the respective buffer filled up to a final volume of 50 µl with ultra-pure water (Sigma, St Louis, MO, USA). The restriction enzyme was heat inactivated and precipitated by adding 4.5 µl LPA solution and 100 µl of 100% isopropanol. The samples were kept at room temperature for 15 min followed by centrifugation at 15000 *g* for 15 min. Thereafter, the supernatant was discarded and the pellet rinsed with 100 µl 70% isopropanol and precipitated again by centrifugation (15000 *g* for 5 min). Subsequently, the supernatant was removed and the sample dried in the cycler at 94ºC for 1 min and stored at -20ºC until further analysis.

The pellet was resuspended in 2 µl of ultra-pure water and the product denatured in 7.8 µl of Hi-Di formamide at 94ºC for 3 min. Each sample contained 0.2 µl GeneTrace 1000 (ROX) marker (Applied Biosystems, Foster City, CA, USA). Fluorescently labeled fragments were separated and detected with an ABI Prism 310 capillary sequencer (Applied Biosystem) run under GeneScan mode (van der Maarel et al. 1998, Moeseneder et al. 1999). The size of the fluorescently labeled fragment was determined by comparison with the internal GeneTrace 1000 (ROX) size standard. Injection was performed electrokinetically at 15 kV and 60ºC for 15 s (adjustable). The output from the ABI Genescan software was transferred to the Fingerprinting II (Bio-Rad) software to determine peak area and for standardization using size markers. The obtained matrix was further analyzed with the Primer software (Primer-E) to determine similarities of the T-RFLP fingerprints between samples.

Automated ribosomal intergenic spacer analysis (ARISA) of the bacterial community. ARISA was used to analyze bacterial community composition with the primer 1392F and a 5'TET labeled version of the primer 23S rDNA as described by Fisher and Triplett (1999) and Hewson and Fuhrman (2004). One µl of the DNA extract was used as a template in a 50 µl PCR mixture. Thermocycling was preceded by a 3 min heating step at 94ºC, followed by 30 cycles of denaturing at 94ºC (15 s), annealing at 55ºC (30 s), and an extension at 72ºC (3 min). Cycling was completed by a final extension at 72ºC for 9 min. The PCR products were purified with the Quick purification kit (Genscript, Piscataway, NJ, USA), and quantified using a Nanodrop spectrophotometer. Purified products were then diluted to 8 ng μl^{-1} to load a standardized amount for fragment analysis and thereby preventing differences originated from different amounts of loaded DNA. Each sample of the final product was mixed with 10 µl of Hi-Di formamide at 94ºC for 3 min, 0.15 µl CST 300-1800 and 0.15 µl GeneTrace 1000 (ROX) marker (Applied Biosystems, Foster City, CA, USA). Fragments were discriminated using an ABI Prism 310 capillary sequencer (Applied Biosystem) and the resulting electropherograms were analyzed using the ABI Genescan software. The output from the ABI Genescan software was transferred to the Fingerprinting II (Bio-Rad) software to determine peak area and for standardization using size markers. Peaks contributing <0.09% of the total amplified DNA (as determined by relative fluorescence intensity) were eliminated as considered to be indistinguishable from baseline noise (Hewson and Fuhrman, 2004). The obtained matrix was further analyzed with Primer software (Primer-E) to determine similarities of the ARISA fingerprints between samples.

Results and Discussion

Oceanographic setting

Cyclonic eddies (CE), anticyclonic eddies (AE) and far-field (FF) stations showed contrasting temperature distributions, with generally lower temperatures in eddies than in FF for both surface and upper mesopelagic waters (except AE1; Fig. 2A).

The temperature-salinity (T-S) diagram (Fig. 2B) indicates that all the stations shared the same meso- and bathypelagic water mass structure (with the exception of FF2 with a slight influence of Mediterranean Sea Outflow Water). Differences in T-S properties were only found in the epipelagic layer due to the coastal-ocean salinity gradient and the mesoscale variability generated by the perturbation of the surface flow by the islands (Fig. 2A, B). The cyclonic eddies CE1 (Fig. 3A) and CE2 showed very similar temperatures throughout the water column, while the anticyclonic eddies (AE1 and AE2) exhibited temperature differences due to their different stages of development (Fig. 2A). AE1 was a typical mature anticyclonic eddy, with warm waters mixed down to 200 m (Fig. 3B). AE2, close to Gran Canaria Island, was an anticyclonic eddy at an early stage of formation, with a warm mixed layer in the upper 60 m, but a strong thermocline underneath.

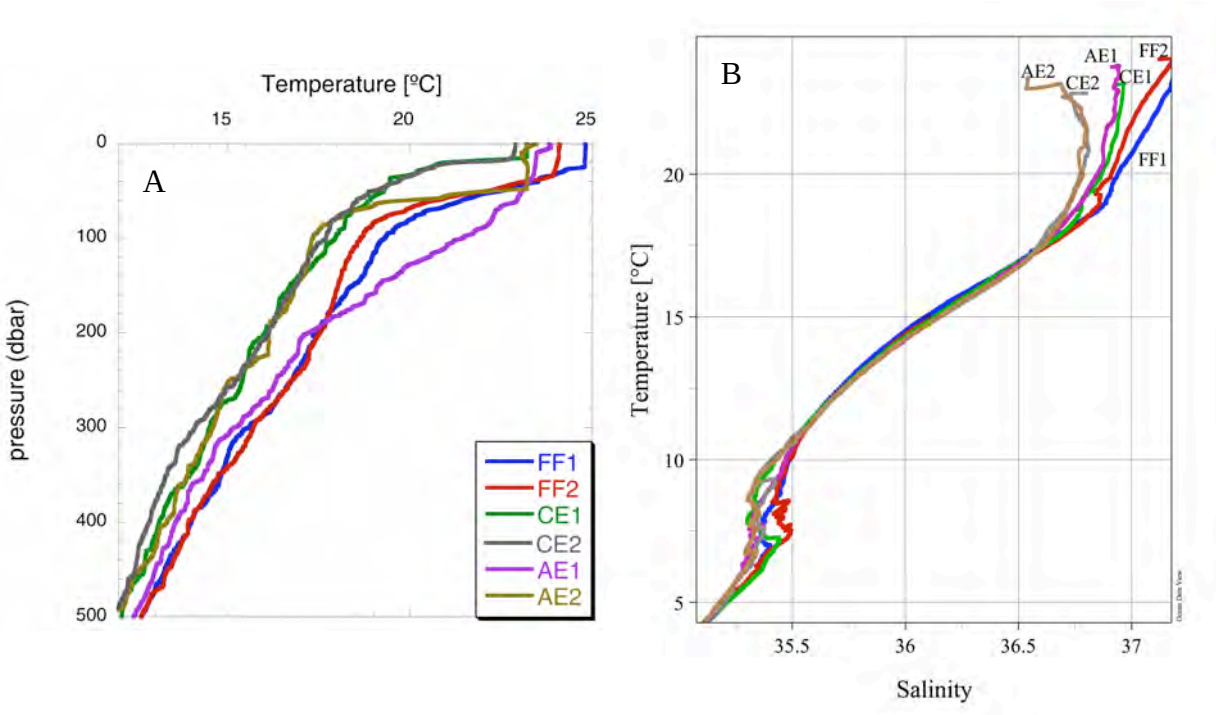


Fig. 2. Vertical profiles of temperature (ºC) (A), and temperature – salinity diagram (B) at the six stations (abbreviations as indicated in Fig. 1)

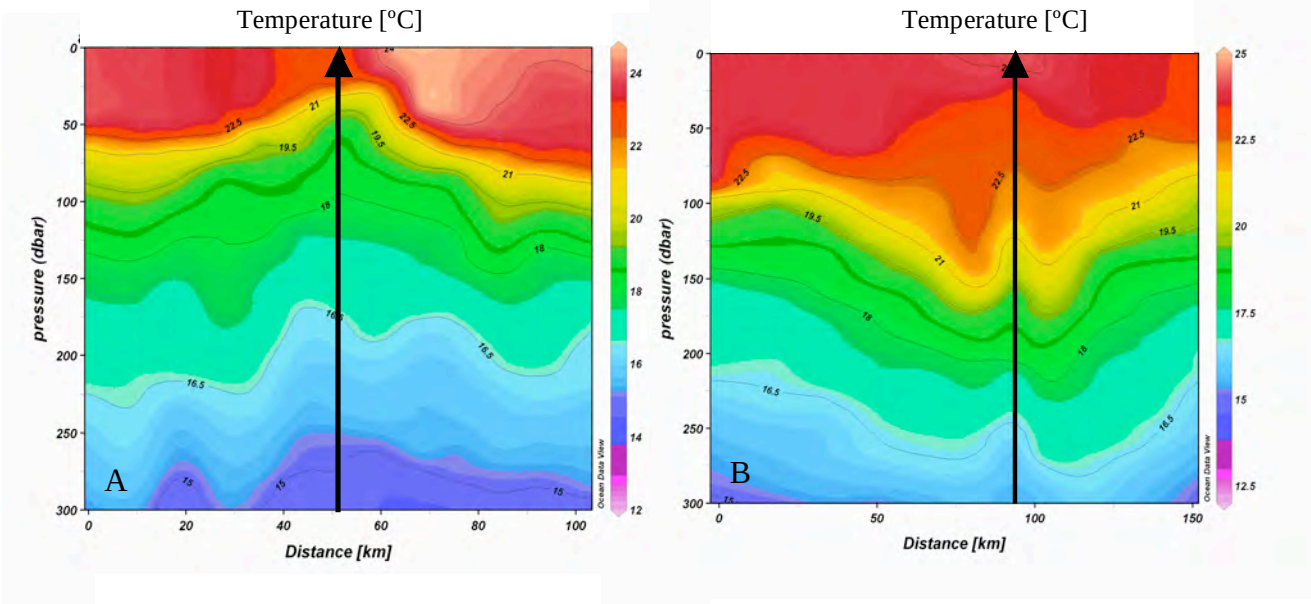


Fig. 3. Cross section of temperature (ºC) of CE1 (A) and AE1 (B) are shown as examples. Black arrows indicate the position of the CTD cast at the core of the eddies

Differences in prokaryotic structure and function in the epipelagic zone

We observed a marked effect of eddies on the prokaryotic community, generating hotspots of abundance, bulk activity, community composition and heterotrophic activity at the single-cell level (Figs. 4, 6, 7, 8). Generally, the differences between eddy and FF stations were more apparent in the epipelagic layer than in deeper waters.

The average prokaryotic abundance (PA) of AE (although not that of CE) was significantly higher than at the FF stations (ANOVA test, $p < 0.05$; Fig. 4A). Both AE and CE stations exhibited significantly higher (ANOVA test, $p < 0.05$) bulk prokaryotic heterotrophic activity (PHA) than the FF stations (Fig. 4C), despite PHA being exceptionally high in the surface layers of FF1 (Fig. 4D). In another study carried out in the same region (from the NW African upwelling to the offshore oligotrophic subtropical NE Atlantic), Alonso-Saéz et al. (2007) also found the highest leucine incorporation rates at the offshore stations. They suggested that these high leucine incorporation rates might not reflect proportionally higher PHA but rather shifts in the leucine-to-carbon conversion factor which is used to calculate PHA from leucine incorporation rates.

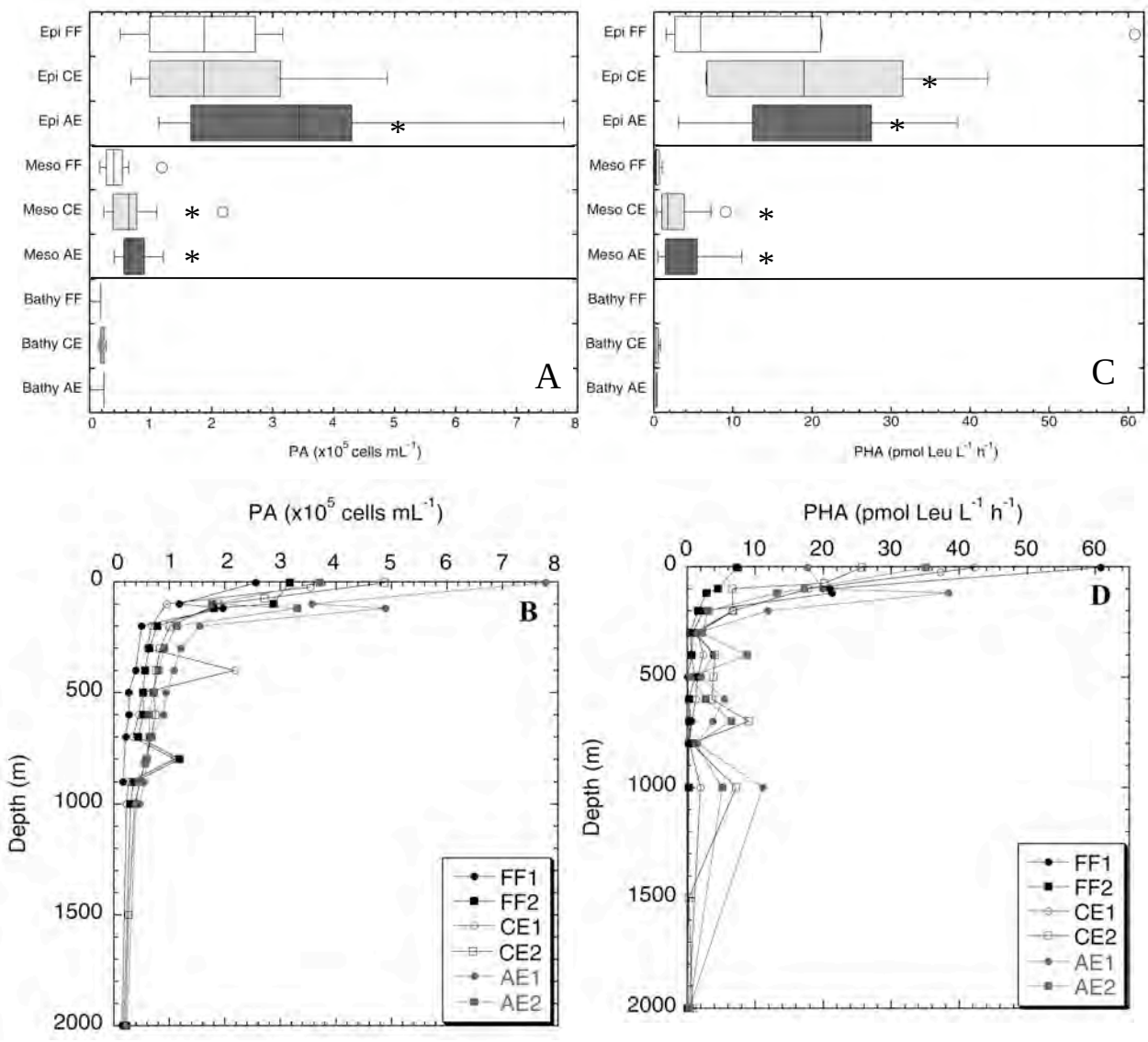


Fig. 4. Distribution of prokaryotic abundance (PA, cells ml^{-1}) and bulk prokaryotic heterotrophic activity (PHA, pmol Leu l^{-1} h^{-1}) in the epipelagic ("Epi", 0-200 m), mesopelagic ("Meso", 200-1000 m) and bathypelagic ("Bathy", 2000 m) layers grouped in far-fields (FF), anticyclonic eddies (AE) and cyclonic eddy (CE) stations (A,B), and profiles from surface to 2000 m depth at every sampled station (C, D). Outliers are indicated as open circles. Asterisks indicate variables significantly different (ANOVA test $p < 0.05$) from far-field stations

No significant differences (ANOVA test, $p > 0.05$) were detectable, however, in the relative abundance of *Bacteria*, *Crenarchaeota* and *Euryarchaeota* between eddy and FF stations (Fig. 5). The highest relative abundance of *Euryarchaeota* (14.8 % of DAPI stainable cells) was observed in the DCM of the well-developed anticyclonic eddy (AE1) (Table 1), where also the highest PA was found (Fig. 4A). Nevertheless, the relative contribution of *Bacteria* decreased with depth (Fig. 5). In contrast, the relative

abundance of *Crenarchaeota* significantly increased from the epipelagic to mesopelagic layer in all stations.

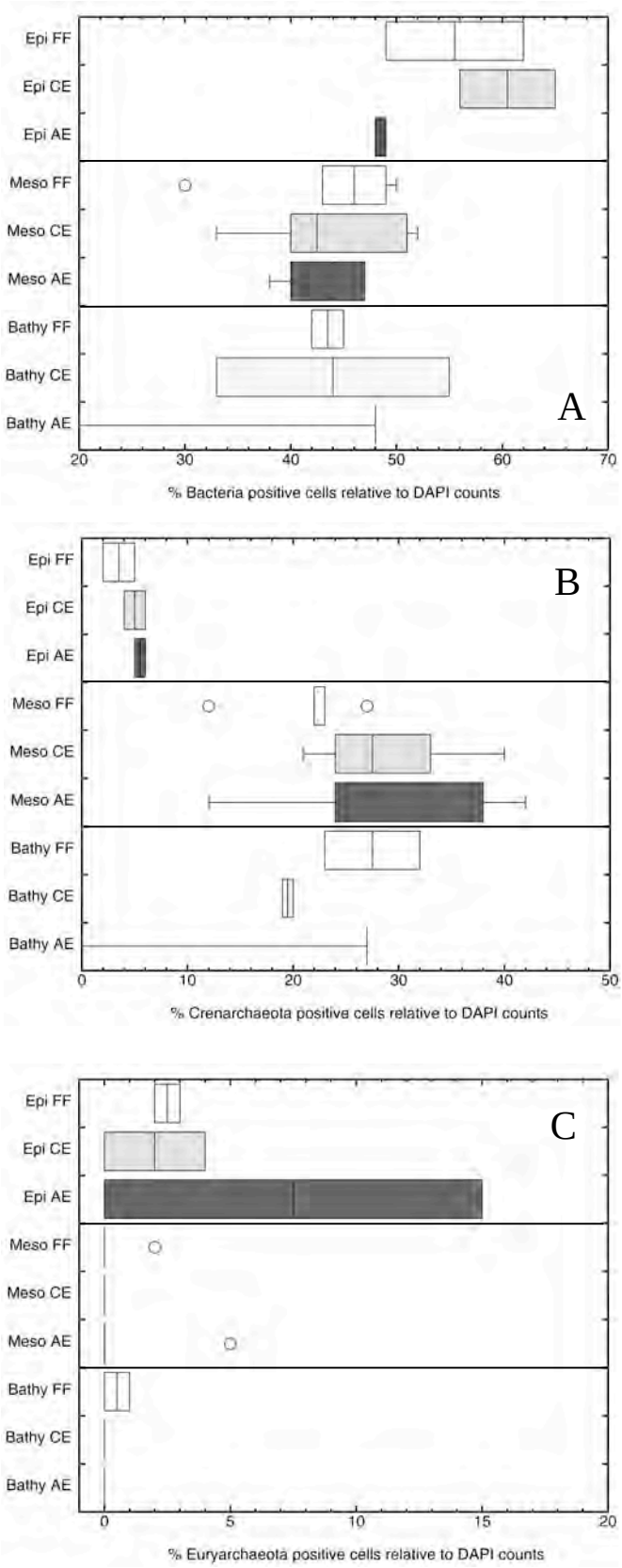


Fig. 5. Distribution of the proportion of (A) *Bacteria* (Eub (I-III)), (B) *Crenarchaeota* (hybridized simultaneously with Cren537 and GI-554), (C) *Euryarchaeota* (Eury806) as percentage of DAPI-stained cells in the epipelagic ("Epi", 0-200 m), mesopelagic ("Meso", 200-1000 m) and bathypelagic ("Bathy", 2000 m) layers grouped in far-fields (FF), anticyclonic (AE) and cyclonic eddies (CE) stations. Outliers are indicated by open circles

Table 1. Relative abundances of prokaryotic groups detected by 16S rRNA oligonucleotide probes and CARD-FISH as percentage of DAPI-stained cells at the deep chlorophyll maximum (DCM), the deep scattering layer (DSL), the oxygen minimum zone (OMZ), 1000 m and at 2000 m depth. Probes: Eub (I-III): *Bacteria*, Cren537: *Crenarchaeota* Cren537 probe positive; Cren554: *Crenarchaeota* Cren554 probe positive; Cren Total: *Crenarchaeota* positive hybridizing with Cren537and Cren554 probes together; Eury: marine *Euryarchaeota* Group II

Stations	**Depth (m)**	**Eub (I-III)**	**Cren 537**	**Cren 554**	**Cren Total**	**Eury**
FF1	120 (DCM)	62	< 1	2	2	3
	550 (DSL)	49	< 1	22	22	< 1
	700 (OMZ)	43	6	28	27	< 1
	1000	30	1	19	23	< 1
	2000	42	5	16	23	< 1
FF2	120 (DCM)	49	4	4	5	2
	800 (OMZ)	50	4	11	12	2
	1000	46	4	24	23	< 1
	2000	45	4	30	32	1
CE1	25 (DCM)	65	7	6	6	4
	500 (DSL)	44	10	35	40	< 1
	800 (OMZ)	52	13	24	25	< 1
	1000	51	6	30	30	< 1
	2000	55	1	19	20	< 1
CE2	75 (DCM)	56	4	4	4	< 1
	500 (DSL)	33	24	34	33	< 1
	700 (OMZ)	41	8	22	24	< 1
	1000	40	7	19	21	< 1
	2000	33	7	18	19	< 1
AE1	120 (DCM)	48	7	4	6	15
	500 (DSL)	44	26	41	42	< 1
	750 (OMZ)	47	11	39	38	5
	1000	47	5	10	12	< 1
AE2	120 (DCM)	49	5	5	5	< 1
	800 (OMZ)	38	12	22	25	< 1
	1000	40	11	24	24	< 1
	2000	48	13	26	27	< 1

As indicated by the fingerprinting approaches, the bacterial (Fig. 6A) and archaeal (Fig. 6B) assemblages found within eddies were clearly distinct from the assemblages found in the FF stations only in the epipelagic layer. FF showed very similar bacterial and archaeal structure in the deep chlorophyll maximum (DCM). In contrast, although CE1 and CE2 showed a very similar temperature profiles, the bacterial assemblage structures were very different. The contrary occurred at AE1 and AE2 stations that showed contrasting temperature patterns but a high similarity in

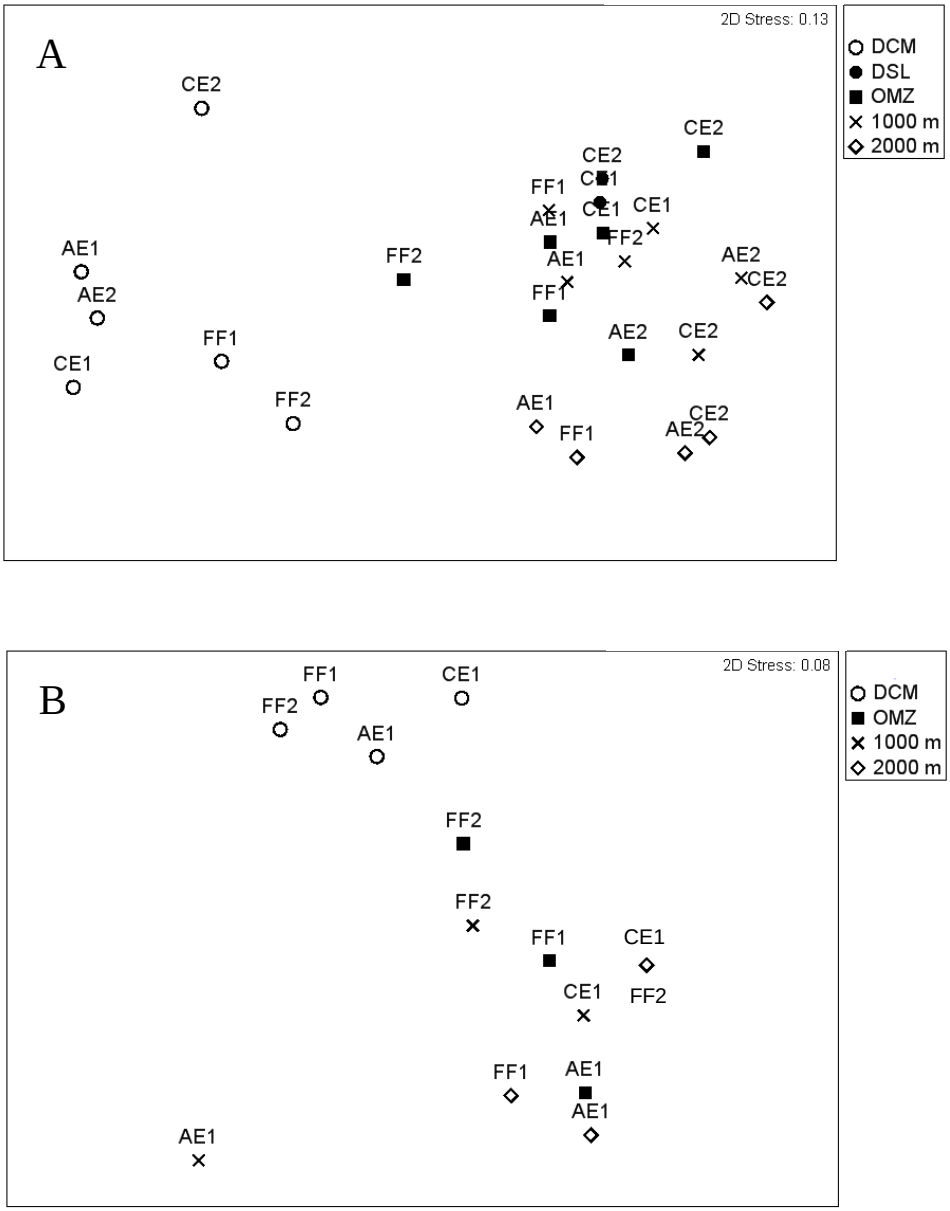


Fig. 6. Non-metric multidimensional scaling (NMDS) plot of the band pattern in (A) bacterial community composition as revealed by ARISA and (B) archaeal community composition as revealed by T-RFLP. Both, band presence alone, and band intensity were used for the statistics, yielding similar results

bacterial assemblage structure. Therefore, temperature was not the main parameter controlling prokaryotic assemblage structure but probably other processes (such as grazing, organic and inorganic matter supply, phytoplankton community structure) potentially modified by the presence of the eddies. The total number of operational taxonomic units (OTUs) detected was 104 for bacterial (ARISA) and 15 for archaeal (TRFLP) communities, respectively. The number of archaeal OTUs per sample decreased with depth (from 7 ± 2 to 3 ± 1 in the epipelagic and bathypelagic, respectively), with no significant differences between FF and eddies at any depth layer.

In contrast, the number of bacterial OTUs per sample was significantly higher in FF (16) than in eddies (6 ± 1 and 7 ± 3 in the AE and CE stations, respectively) in the epipelagic layer but not in deeper waters. In deeper waters (meso- and bathypelagic), the number of bacterial OTUs increased, ranging from 16-20, 14-22 and 16-21 for FF, CE and AE, respectively. About 50% and >30% of the OTUs were shared between both types of eddies and FF for *Archaea* and *Bacteria*, respectively. Around 20% of the archaeal and 8% of the bacterial OTUs were present at all the depths sampled, suggesting that the bacterial community was more stratified than the archaeal community.

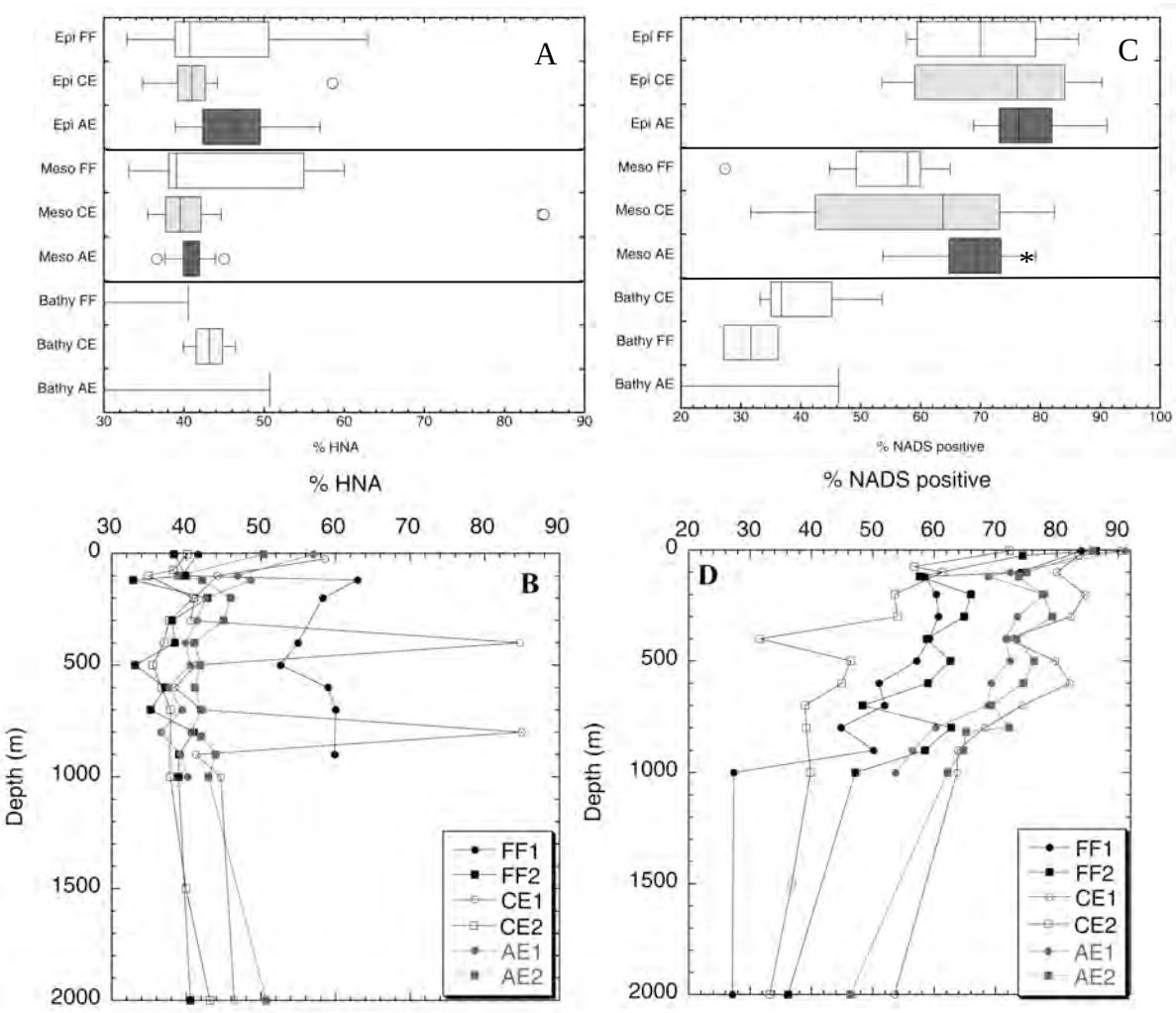


Fig. 7. Distribution of the percentage of high nucleic acid containing cells (HNA) and % NADS-determined "live" cells in the epipelagic ("Epi", 0-200 m), mesopelagic ("Meso", 200-1000 m) and bathypelagic ("Bathy", 2000 m) layers grouped in far-fields (FF), anticyclonic eddies (AE) and cyclonic eddies (CE) stations (A,B), and profiles from surface to 2000 m depth at each station (C, D). Outliers are indicated by open circles. Asterisks indicate variables significantly different (ANOVA test $p < 0.05$) from far-field stations

The percentages of high nucleic acid (HNA) (Fig. 7A, B) and of NADS+ cells (Fig. 7C, D) were not significantly different (ANOVA test, $p > 0.05$) between eddies and FF in the three depth layers. The percentage of HNA cells remained fairly constant with depth (Fig. 7A) while the percentage of NADS+ cells decreased with depth (Fig. 7B).

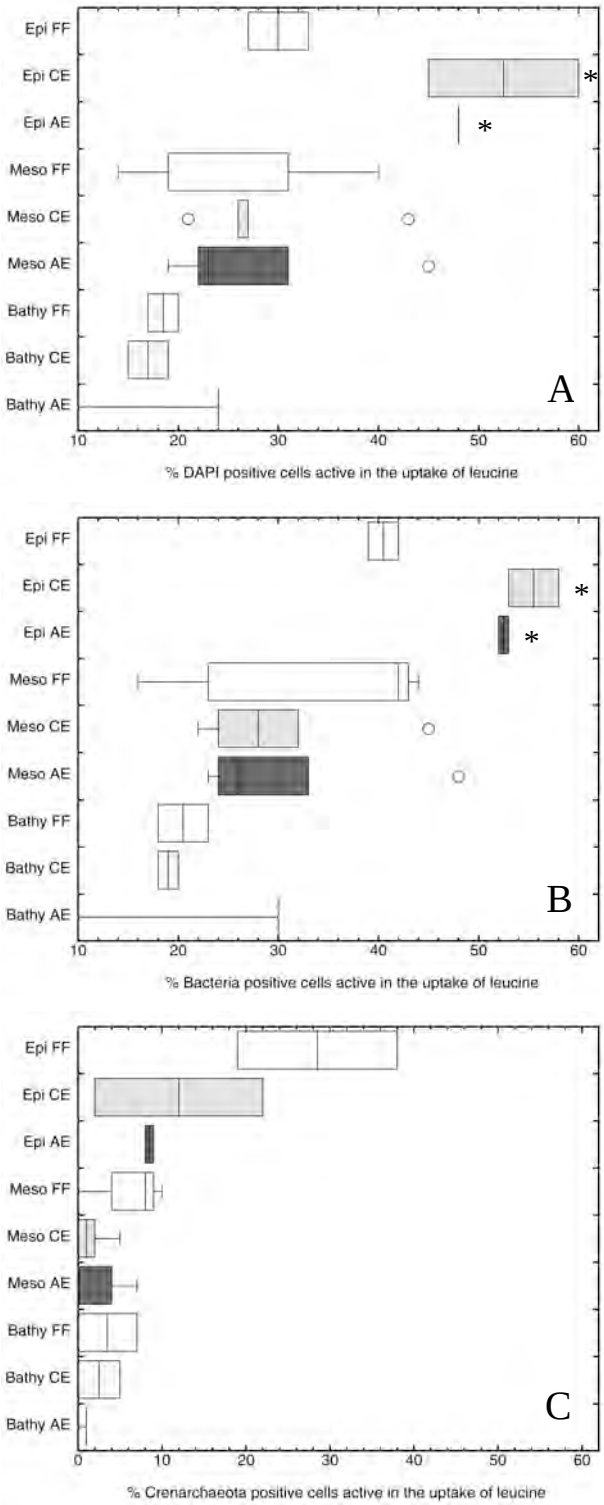


Fig. 8. Distribution of the proportion of (A) the bulk prokaryotic community (DAPI-stained cells), (B) *Bacteria* (Eub (I-III)) and (C) *Crenarchaeota* (GI-554) taking up leucine in the epipelagic ("Epi", 0-200 m), mesopelagic ("Meso", 200-1000 m) and bathypelagic ("Bathy", 2000 m) layers grouped in far-field (FF), anticyclonic (AE) and cyclonic eddy (CE) stations. Outliers are shown by open circles. Asterisks indicate variables significantly different (ANOVA test $p < 0.05$) from far-field stations

The proportion of the prokaryotic community taking up leucine (fraction of leucine positive cells) (Fig. 8A), and the fraction of leucine+ *Bacteria* (Fig. 8B), were higher (ANOVA test, $p < 0.05$) in the epipelagic zone of eddies than at the FF stations (Table 2). Conversely, the percentage of leucine+ *Crenarchaota* and *Euryarchaeota* was not significantly different (Fig. 8C, D). Although *Euryarchaeota* were not very abundant, they showed the highest proportion of leucine+ cells (ranging between 48-64 % of *Euryarchaeota*) (Table 2).

Table 2. Percentage of *Bacteria* (Eub I-III), *Crenarchaeota* Cren537 probe positive (Cren537), *Crenarchaeota* Cren554 probe positive (Cren554) and *Euryarchaeota* marine Group II (Eury) taking up leucine as detected by MICRO-CARD-FISH, at the deep chlorophyll maximum (DCM), the deep scattering layer (DSL), the oxygen minimum zone (OMZ), 1000 m and at 2000 m depth. n. d.: non-determined

Stations	**Depth (m)**	**DAPI**	**Eub (I-III)**	**Cren 537**	**Cren 554**	**Eury**
FF1	120 (DCM)	33	39	15	38	48
	550 (DSL)	31	44	4	8	n.d.
	700 (OMZ)	31	42	3	9	n.d.
	1000	19	23	< 1	< 1	n.d.
	2000	17	18	< 1	< 1	n.d.
FF2	120 (DCM)	27	42	< 1	19	64
	800 (OMZ)	40	43	8	10	49
	1000	14	16	< 1	4	n.d.
	2000	20	23	< 1	7	n.d.
CE1	25 (DCM)	60	58	15	22	57
	500 (DSL)	43	45	< 1	5	n.d.
	800 (OMZ)	27	28	< 1	< 1	n.d.
	1000	21	22	< 1	2	n.d.
	2000	19	18	< 1	5	n.d.
CE2	75 (DCM)	45	53	< 1	2	n.d.
	700 (OMZ)	27	32	< 1	1	n.d.
	1000	26	24	< 1	< 1	n.d.
	2000	15	20	< 1	< 1	n.d.
AE1	120 (DCM)	48	53	< 1	9	53
	500 (DSL)	45	48	< 1	4	n.d.
	750 (OMZ)	31	33	< 1	< 1	49
	1000	22	24	< 1	7	n.d.
AE2	120 (DCM)	48	52	< 1	8	n.d.
	800 (OMZ)	23	26	< 1	< 1	n.d.
	1000	19	23	< 1	4	n.d.
	2000	24	30	< 1	1	n.d.

The variability found in community structure and activity between eddies and FF probably reflects the accumulation of organic matter at eddy centers and boundary

zones in the eddy field region, as described in previous studies (Arístegui et al. 2003, Arístegui & Montero 2005). Frontal structures have been shown to promote the accumulation of organic matter and concomitantly of prokaryotes (Floodgate et al. 1981, Pomeroy et al. 1983, Ducklow 1988). In particular, Arístegui & Montero (2005) observed that frontal structures between eddy pairs in the Canary region favored the accumulation of bacteria. Also, Baltar et al. (2009) found dense accumulations of autotrophic and heterotrophic pico- and nanoplankton organisms at eddy-eddy and eddy-filament boundary regions. Additionally, the changes found in the prokaryotic activity and community structure may be induced by the higher availability of inorganic nutrients generated by the eddies. The growth of prokaryotic and eukaryotic autotrophs in many aquatic systems is limited by the availability of nitrogen, phosphorus, iron and silica (e.g. Elser et al. 1990, 1995). This increased supply of nutrients for surface-water prokaryotes can be produced directly by the upwards pumping of deep water (in the CE) and/or the accumulation of planktonic organism (in the center and borders of AE). Due to the accumulation of microorganisms in AE, the protistan grazing on prokaryotes (an important mechanism of nutrient regeneration in the ocean) may be high. This increased supply of inorganic nutrients may have a stronger effect on prokaryotes than on eukaryotic phytoplankton, because aquatic bacteria are better competitors for phosphorus than eukaryotic algae at low ambient nutrient concentrations (Thingstad et al. 1998). In addition, theory (Klausmeier et al. 2004) and experiments (Sommer 1994) confirm that different nutrient profiles in the water column select for phytoplankton species with different stoichiometries. This increase in the availability of nutrients may modulate phytoplankton community structure and concomitantly, modify prokaryotic assemblage structure and activity (Kelly & Chistoserdov 2001, Klausmeier et al. 2004). In that sense, Ewart et al.. (2008) found a tight relationship between enhanced PHA, phytoplankton biomass and the specific phytoplankton species, suggesting that phytoplankton community structure was an important factor influencing bacterial activity.

Propagation of effects into deeper layers

The differences in bulk PHA and PA observed in the epipelagic zone between eddy and FF stations propagated, albeit attenuating, into the mesopelagic waters where

also higher bulk PHA and PA were detected at the eddy stations (Fig. 4). Moreover, some single-cell activity proxies also indicated differences between AE and FF. A significantly higher percentage of NADS+ cells was found in the mesopelagic layer of AE (Fig. 7C). However, the proportion of heterotrophically active meso- and bathypelagic prokaryotes was similar in eddies and FF (Fig. 8).

In the mesopelagic zone, both CE and AE exhibited significantly higher PA than FF stations (ANOVA test, $p < 0.05$). In the bathypelagic zone, no significant differences in PA were detectable among the different sites. Also, like in the epipelagic zone, eddy stations (AE and CE) exhibited significantly (ANOVA test, $p < 0.05$) higher leucine uptake rates than FF stations in the mesopelagic realm (but not in the bathypelagic) (Fig. 4C).

No significant differences (ANOVA test, $p > 0.05$) were detectable in the relative abundance of *Bacteria*, *Crenarchaeota* and *Euryarchaeota* (Fig. 5), the bacterial or archaeal community structures (Fig. 6), the percentage of HNA cells (Fig. 7A, B), and the proportion of *Bacteria*, *Crenarchaota* or *Euryarchaeota* taking up leucine (Table 2) between the eddy stations and the FF reference stations in the dark ocean. However, a significantly (ANOVA test, $p < 0.05$) higher percentage of NADS+ cells (Fig. 7C, D) was found for the mesopelagic realm of AE as compared to FF (Fig. 7C, D), but not in the epi- or bathypelagic layers.

Taken together, these results suggest that only the prokaryotic communities from the epipelagic zone of the eddy stations (and not from deeper waters) are metabolically more active at the single-cell level than those of the FF stations (Fig. 6, 7, 8). The differences found in bulk PA, PHA and NADS+ cells between eddies and FF in the mesopelagic layer could be related to the increase of the organic matter flux generated by eddies. In a complementary study investigating the flux of particles collected with drifting sediment traps (I. Alonso-González, pers.com.), the same eddies investigated here were found to enhance particulate organic carbon (POC) export with respect to FF stations by a factor of 2-4. These results are in contrast to a study of Maiti et al. (2008) on a mature cyclonic eddy in the lee of Hawaii. They found that, although the eddy was highly productive at the surface, it was not efficient at exporting particulate carbon and nitrogen to deeper waters. In fact, they observed that particle production occurred in the upper 100 m and was rapidly remineralized in the upper 150 m.

Peaks of prokaryotic activity and abundance in the mesopelagic zone

Although prokaryotic abundance and metabolism generally decreased with depth, pronounced peaks were sometimes detected in the mesopelagic layer. In particular, PA peaks were observed at 400 m depth at station CE1, coinciding with the Deep Scattering Layer (DSL), and in the oxygen minimum zone (OMZ; ca. 800 m depth) of CE1 and FF2 (Fig. 4B). Also, marked peaks in PHA, HNA and NADS+ cells were found at several depths of the mesopelagic zone, at different stations (Fig. 4B, D and Fig. 7B, D). The two peaks of PA at station CE1 corresponded to large prokaryotes with a high % of HNA (compare Fig. 4B and 7B), whereas the peak of prokaryotes at the OMZ of station FF2 corresponded to cells with the same % of HNA as in surface communities (Fig. 7B). In the latter peak, a higher proportion of prokaryotes was identified as incorporating leucine (40 % leucine+ of DAPI stainable cells) compared with other depths sampled at station FF2 (14 - 27 % leucine+ of DAPI stainable cells). Also, it coincided with a pronounced peak in NADS+ cells (Fig. 7D), and a shift in bacterial (Fig. 6A) and archaeal (Fig. 6B) community composition. In fact, it is noteworthy that the only deep-water sample clustering with the epipelagic communities of both Bacteria and Archaea was that belonging to the OMZ in station FF2 (Fig. 6). This might indicate that the prokaryotic community present at this particular depth was more related to that of the DCM than to the assemblages of the corresponding depths of other stations.

Mesopelagic hotspots of microbial respiration (Arístegui et al. 2003), prokaryotic nucleic acid content (**Chapter I**), leucine incorporation and leucince/thymidine incorporation ratio (Gasol et al. 2009), dissolved organic carbon (Arístegui et al.. 2003), and zooplankton biomass, gut fluorescence and respiration (Hernández-León et al. 2001, Yebra et al. 2005) have been reported in previous studies for the eddy field region south of the Canary Islands. Hence, the observed patchiness in mesopelagic activity in these waters seems to be related to the complex hydrographic regime of the region around the Canary Islands.

Relationship between prokaryote viability and single-cell heterotrophic activity throughout the water column

The proportion of viable cells (NADS+ cells) correlated well with the fraction of leucine+ prokaryotic cells (Spearman $R = 0.71$, $p < 0.0001$, $n = 26$, Fig. 9). Furthermore, the percentage of heterotrophically active *Bacteria* was correlated to the

percentage of NADS+ cells (Spearman R = 0.67, $p < 0.0002$, n = 26), but no correlation was found for any archaeal group. These results, together with the observed lower relative abundance of both archaeal groups compared with *Bacteria*, suggest that the fraction of heterotrophically active (leucine+) and viable (NADS+) cells was dominated by *Bacteria* and not by Archaea throughout the water column. Our results agree with the higher proportion of bacterial cells active in the uptake of leucine, as compared to archaeal cells in the North Atlantic water column (Herndl et al. 2005). As shown in Fig. 9, the fraction of viable and heterotrophically active prokaryotes is more similar (i.e. closer to the 1:1 line) at low than at high values. This indicates that the NADS method likely detects cells that are intact but not necessarily very active, while MICRO-CARD-FISH identifies cells that are active (depending on the concentration of leucine used and on the exposure time), following the prokaryotic "physiological structure" model of del Giorgio & Gasol (2008).

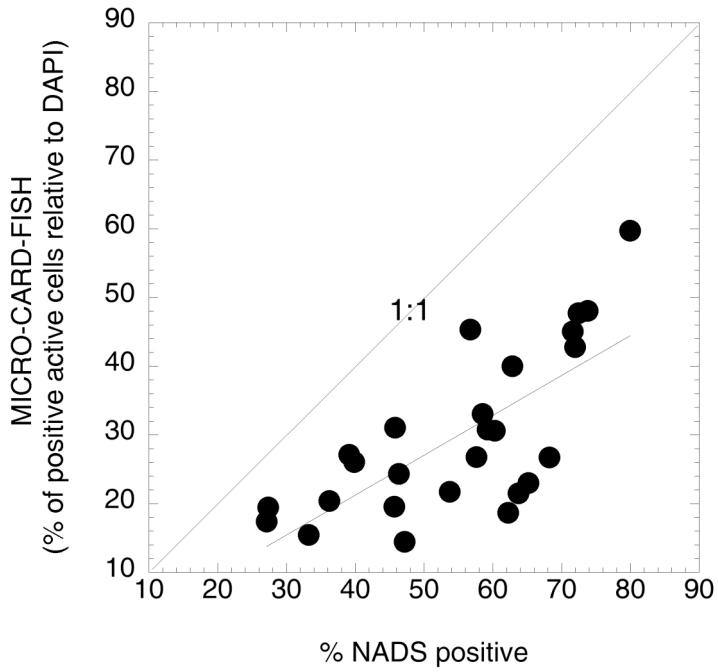


Fig. 9. Relationship between the NADS-determined "live" cells and the proportion of active prokaryotic cells taking up leucine (determined by MICRO-CARD-FISH)

Conclusions

Our results show that mesoscale eddies play a differential role in the distribution and function of prokaryotes in the ocean. The largest effects and differences were observed in the upper 1000 m, suggesting that prokaryotic communities are probably linked to the mesoscale heterogeneity, and the increase in productivity and downward flux of organic matter enhanced by eddy action. In general, the effect of anticyclonic eddies was stronger than that of cyclonic ones. Due to the recognized major role of eddies in ocean circulation, more effort should be put in the future to study the microbial processes within these mesoscale features. This would allow constraining the fate of carbon in the ocean and concomitantly building more accurate models of global biogeochemical cycles.

Acknowledgments

This research was supported by two grants of the Spanish Ministry of Education and Science to J.A. (Oceanic Eddies and Atmospheric Deposition – RODA, CTM 2004-06842-C03/MAR, and Shelf–Ocean Exchanges in the Canaries–Iberian Large Marine Ecosystem -CAIBEX, CTM 2007-66498-C02), a grant of the Earth and Life Science Division of the Dutch Science Foundation (ALW-NWO; ARCHIMEDES project, 835.20.023) to G.J.H. and a predoctoral Fellowship of the Spanish Ministry of Education and Science (AP2005-3932) to F.B. I.L and J.M.G were also supported by project MODIVUS (CTM2005-04795/MAR). The work was carried out within the frame of the EU 'Networks of Excellence' MarBef and EurOceans. We acknowledge the insightful comments of two anonymous reviewers, which helped improve the manuscript. We thank the captain and crew of RV Hespérides for their help during work at sea. We also thank A. Marrero-Díaz for providing the temperature cross-sections, and A.M. Caballero-Alfonso for her help during sampling and DNA extraction.

References

Alonso-Saéz L, Gasol JM, Arístegui J, Vilas JC, Vaqué D, Duarte CM, Agusti S (2007) Large-scale variability in surface bacterial carbon demand and growth efficiency in the subtropical northeast Atlantic Ocean. Limnol. Oceanogr. 52:533-546

Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA (1990) Combination of 16 rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microbiol. 56: 1919-1925.

Arístegui J, Montero MF (2005) Temporal and spatial changes in plankton respiration and biomass in the Canary Islands region: the effect of mesoscale variability. J. Mar. Syst. 54: 65-82.

Arístegui J, Tett P, Hernández-Guerra A, Basterretxea G, Montero MF, Wild K, Sangrá P, Hernández-León S, Cantón M, García-Braun JA, Pacheco M, Barton ED (1997) The influence of island-generated eddies on chlorophyll distribution: a study of mesoscale variation around Gran Canaria. Deep-Sea Res. 44:71-96

Arístegui J, Barton ED, Montero MF, Garcia-Muñoz M, Escánez J (2003) Organic carbon distribution and water column respiration in the NW Africa-Canaries Coastal Transition Zone. Aquat. Microb. Ecol. 33: 289-301.

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10: 257-263.

Baltar F, Arístegui J, Montero MF, Espino M, Gasol JM, Herndl GJ (2009) Mesoscale variability modulates seasonal changes in the trophic structure of nano- and picoplankton communities across the NW Africa-Canary Islands transition zone. Prog. Oceanogr. 83: 180-188.

Benitez-Nelson CR, Bidigare RR, Dickey TD, Landry MR, Leonard CL, Brown SL, Nencioli F, Rii YM, Maiti K, Becker JW, Bibby TS, Black W, Cai WJ, Carlson CA, Chen F, Kuwahara VK, Mahaffey C, McAndrew PM, Quay PD, Rappé MS, Selph KE, Simmons MP, Yang EJ (2007) Mesoscale eddies drive increased silica export in the Subtropical Pacific Ocean. Science 316:1017-1021

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

Cheney RE, Richardson PL (1976) Observed decay of a cyclonic Gulf Stream ring. Deep-Sea Res. 23: 143-155.

Daims H, Bruhl A, Amann R, Schleifer KH, Wagner M (1999) The domain-specific probe EUB338 is insufficient for the detection of all bacteria: developments and evaluation of a more comprehensive probe set. Syst. Appl. Microbiol. 22: 434-444.

De Corte D, Yokokawa T, Varela MM, Agogué H, Herndl GJ (2009) Spatial distribution of *Bacteria* and *Archaea* and *amo*A gene copy numbers throughout the water column of the Eastern Mediterranean Sea. ISME J. 3: 147-158.

Elser JJ, Marzolf ER, Goldman CR (1990) Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North Ameria: a review and critique of experimental enrichments. Can. J. Fish. Aquat. Sci. 47: 1468-1477.

Elser JJ, Stabler LB, Hassett RP (1995) Nutrient limitation of bacterial growth and rates of bacterivory in lakes and oceans: a comparative study. Aquat. Microb. Ecol. 9: 105-110.

del Giorgio PA, Gasol JM (2008) Physiological structure and single-cell activity in marine bacterioplankton. In: Kirchman DL (ed). Microbial ecology of the ocean, 2nd edn. Wiley-Liss: New York. pp 243-298.

Ducklow HW (1988) Bacterial biomass in warm-core Gulf Stream ring 82-B: mesoscale distributions, temporal changes and production. Deep-Sea Res. 33: 1789-1812.

Ewart CS, Meyers MK, Wallner ER, McGillicuddy Jr DJ, Carlson CA (2008) Microbial dynamics in cyclonic and anticyclonic mode-water eddies in the northwestern Sargasso Sea. Deep-Sea Res. II 55: 1334-1347.

Falcioni T, Papa S, Gasol JM (2008) Evaluating the flow-fytometric nucleic acid double-staining protocol in realistic rituations of planktonic bacterial death. Appl. Environ. Microb. 74: 1767-1779.

Falkowsky PG, Ziemann DA, Kolber DA, Bienfang PK (1991) Role of eddy pumping in enhancing primary production in the ocean. Nature 352: 55-58.

Fisher MM, Triplett EW (1999) Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Appl. Environ. Microbiol. 65: 4630-4636.

Floodgate GD, Fogg GE, Jones DA, Lochte K, Turley CM (1981) Microbiology and zooplankton activity at a front at Liverpool Bay. Nature 240: 133-136.

Gasol JM, Zweifel UL, Peters F, Fuhrman JA, Hagström Å (1999) Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl. Environ. Microbiol. 65: 4475-4483.

Gasol JM, Alonso-Sáez L, Vaqué D, Baltar F, Calleja ML, Duarte CM, Arístegui J (2009) Mesopelagic prokaryotic bulk and single-cell heterotrophic and community composition in the NW Africa-Canary Islands coastal-transition zone. Prog. Oceanogr. 83: 189-196.

González JM, Simó R, Massana R, Covert JS, Casamayor EO, Pedrós-Alió C, Moran MA (2000) Bacterial community structure associated with a dimethylsulfoniopropionate-producing North Atlantic algal bloom. Appl. Environ. Microbiol. 66: 4237-4246.

González N, Anadón R, Mouriño B, Fernández E, Sinha B, Escánez J, de Armas D (2001) The metabolic balance of the planktonic community in the North Atlantic subtropical gyre: the role of mesoscale instabilities. Limnol. Oceanogr. 46: 946-952.

Gregori G, Citterio S, Ghani A, Labra M, Sgorbati S, Brown S, Denis M (2001) Resolution of viable and membrane-compromised bacteria in freshwater and marine waters based on analytical flow cytometry and nucleic acid double staining. Appl. Environ. Microbiol. 67: 4662-4670.

Harris RP, Boyd P, Harbour DS, Head RN, Pingree RD, Pomroy AJ (1997) Physical, chemical and biological features of a cyclonic eddy in the region of 61° 10'N 19° 50'W in the North Atlantic. Deep Sea Res. I 11: 1815-1839.

Hernández-León S, Gómez M, Pagazaurtundua M, Portillo-Hahnefeld A, Montero I, Almeida C (2001) Vertical distribution of zooplankton in Canary Island waters: implication for export flux. Deep-Sea Res. I 48: 1071-1092.

Herndl GJ, Reinthaler T, Teira E, Aken Hv, Veth C, Pernthaler A, Pernthaler J (2005) Contribution of *Archaea* to total prokaryotic production in the deep Atlantic Ocean. Appl. Environ. Microbiol. 71: 2303-2309.

Hewson I, Fuhrman JA (2004) Richness and diversity of bacterioplankton species along an estuarine gradient in Moreton Bay, Australia. Appl. Environ. Microbiol. 70: 3425-3433.

Kelly KM, Chistoserdov AY (2001) Phylogenetic analysis of the succession of bacterial communities in the Great South Bay (Long Island). FEMS Microb. Ecol. 35: 85-95.

Kerkhof LJ, Voytek MA, Sherrell RM, Millie D, Schofield O (1999) Variability in bacterial community structure during upwelling in the coastal ocean. Hydrobiologia 401: 139-148.

Klausmeier CA, Litchman E, Daufresne T, Levin SA (2004) Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature 429: 171-174.

Letelier RM, Karl DM, Abbott MR, Flament P, Freilich M, Lukas R, Strub T (2000) Role of late winter mesoscale events in the biogeochemical variability ofthe upper water column of the North Pacific Subtropical Gyre. J. Geophys. Res. 105:28723-28739

Lochte K, Pfannkuche O (1987) Cyclonic cold-core eddy in the eastern North Atlantic. II. Nutrients, phytoplankton and bacterioplankton. Mar. Ecol. Prog. Ser. 39: 153-164.

Maiti K, Benitez-Nelson CR, Rii Y, Bidigare R (2008) The influence of a mature cyclonic eddy on particle export in the lee of Hawaii. Deep-Sea Res. II 55: 1445-1460.

Massana R, Murray AE, Preston CM, DeLong EF (1997) Vertical distribution and phylogenetic characterization of marine planktonic *Archaea* in the Santa Barbara Channel. Appl. Environ. Microbiol. 63: 50-56.

Mathis JT, Pickart RS, Hansell DA, Kadko D, Bates NR (2007) Eddy transport of organic carbon and nutrients from the Chukchi Shelf: Impact on the upper halocline of the western Arctic Ocean. J. Geophys. Res. 112: C05011, doi: 05010.01029/02006JC003899

McGillicuddy Jr. DJ, Robinson AR, Siegel DA, Jannasch HW, Johnson R, Dickey TD, McNeil J, Michaels AF, Knap AH (1998) Influence of mesoscale eddies on new production in the Sargasso Sea. Nature 394:263-266

Moeseneder MM, Arrieta JM, Muyzer G, Winter C, Herndl GJ (1999) Optimization of terminal-restriction fragment length polymorphism analysis for complex marine bacterioplankton communities and comparison with denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. 65: 3518-3525.

Moeseneder MM, Winter C, Arrieta JM, Herndl GJ (2001) Terminal restriction fragment length polymorphism (T-RFLP) screening of a marine archaeal library to determine the different phylotypes. J. Microbiol. Meth. 44: 159-172.

Morán XAG, Taupier-Letage I, Vazquez-Dominguez E, Ruiz S, Arin L, Raimbault P, Estrada M (2001) Physical-biological coupling in the Algerian Basin (SW Mediterranean): Influence of mesoscale instabilities on the biomass and production of phytoplankton and bacterioplankton. Deep Sea Res. I 48:405-437

Mouriño-Carballido B, Jr. DJ McGillicuddy (2006) Mesoscale variability in the metabolic balance of the Sargasso Sea. Limnol. Oceanogr. 51: 2675-2689.

Pomeroy LR, Atkinson LP, Blanton JO, Campbell WB, Jacobsen TR, Kerrick KH, Wood AM (1983) Microbial distribution and abundance in response to physical and biological processes on the continental shelf of southeastern U.S.A. Cont. Shelf Res. 2:1-20

Smith DC, Azam F (1992) A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. Mar. Microb. Food Webs 6: 107-114.

Sommer U (1994) The impact of light intensity and day length on silicate and nitrate competition among marine phytoplankton. Limnol. Oceanogr. 39: 1680-1688.

Tarran GA, Zubkov MV, Sleigh MA, Burkill PH, Yallop M (2001) Microbial community structure and standing stocks in the NE Atlantic in June and July of 1996. Deep Sea Res. II 48: 963-985.

Teira E, Reinthaler T, Pernthaler A, Pernthaler J, Herndl GJ (2004) Combining catalyzed reporter deposition-fluorescence in situ hybridization and microautoradiography to detect substrate utilization by Bacteria and Archaea in the deep ocean. Appl. Environ. Microbiol. 70: 44411-44414.

Thingstad TF, Zweifel UL, Rassoulzadegan F (1998) P limitation of heterotrophic bacteria and phytoplankton in the northwest Mediterranean. Limnol. Oceanogr. 43: 88-94.

Thyssen M, Lefevre D, Caniaux G, Ras J, Fernandez CI, Denis M (2005) Spatial distribution of heterotrophic bacteria in the northeast Atlantic (POMME study area) during spring 2001. J. Geophys. Res. 110: doi:10.1029/2004JC002670.

van der Maarel MJEC, Artz RRE, Haanstra R, Forney LJ (1998) Association of marine Archaea with the digestive tracts of two marine fish species. Appl. Environ. Microbiol. 64: 2894-2898.

van Haren H, Millot C, Taupier-Letage I (2006) Fast deep sinking in Mediterranean eddies. Geophys. Res. Lett.

Yebra L, Almeida C, Hernández-León S (2005) Vertical distribution of zooplankton and active flux across an anticyclonic eddy in the Canary Island waters. Deep-Sea Res. I 52: 69-83.

Zhang Y, Sintes E, Chen J, Zhang Y, Dai M, Jiao N, Herndl GJ (2009) Role of mesoscale cyclonic eddies in the distribution and activity of *Archaea* and *Bacteria* in the South China Sea. Aquat. Microb. Ecol. 56:65-79

Chapter VI

Determining prokaryotic carbon utilization in the dark ocean: growth efficiency, leucine-to-carbon conversion factors and their relation

F. Baltar, J. Arístegui, J. M. Gasol, G. J. Herndl
Aquatic Microbial Ecology (in revision)

Abstract

Experiments were conducted in the mesopelagic subtropical Northeast Atlantic Ocean to determine variation in the prokaryotic leucine-to-carbon conversion factor (CF), and prokaryotic growth efficiency (PGE). The way prokaryotic heterotrophic production (PHP) is calculated directly influences PGE (variations of PGE between 1-31% were found for a single sample). The empirically obtained deep-water CFs showed a 7-fold variability (0.13-0.85 kg C mol^{-1} Leu) and hence, were lower than the theoretical CF of 1.55 kg C mol^{-1} Leu assuming no isotope dilution. Empirically determined CFs were highly correlated to PGE, suggesting that both parameters are representations of the same basic metabolic processes. Overall, the PGEs obtained in this study suggest that mesopelagic prokaryotic assemblages can be as important in carbon processing as their epipelagic counterparts, at least in some regions.

Introduction

A key concept in the study of ecosystem ecology is the balance between catabolic and anabolic processes, usually defined as the growth efficiency. This parameter quantifies the proportion of the organic carbon incorporated into biomass (production) compared to the total organic carbon uptake (production plus respiration). In the oceanic ecosystem, prokaryotes play a mayor role in the organic carbon fluxes, being responsible for up to >90% of the community respiration (Robinson & Williams 2005). Prokaryotes are also a key component of the microbial loop, acting as a trophic link (transforming dissolved into particulate organic carbon) for higher trophic levels (Azam et al. 1983). Therefore, the organic carbon concentration that will be respired (CO_2 release) in the ocean and/or the amount of organic carbon that will flow through prokaryotes to higher trophic levels greatly depends on the prokaryotic growth efficiency (PGE) (Carlson et al. 2007).

Although estimating PGE in the ocean is essential, obtaining the metabolic rates involved in that assessment is not simple. Prokaryotic respiration (PR) is, in theory, more complicated to measure than production. Authors often use an assumed PGE to estimate PR from prokaryotic heterotrophic production (PHP). Two empirical equations

have largely been used, one estimating PGE from PHP (del Giorgio & Cole 1998) and the other from temperature (Rivkin & Legendre 2001). While these global assessments across systems provide valuable insights into overall control mechanisms of prokaryotic metabolism, PGE might vary substantially among and even within systems.

PHP is frequently determined by measuring leucine or thymidine incorporation and applying an empirically obtained conversion factor to convert the leucine or thymidine incorporation rates into carbon production (del Giorgio & Cole 1998). However, the relation between substrate incorporation and carbon produced is not constant (Ducklow 2000). This leucine (or thymidine) to carbon conversion factor (LeuCF) may vary by a factor of 10 within individual systems (e.g. Sherry et al. 2002, Alonso-Saéz et al. 2007). Thus, assuming a fixed theoretical LeuCF can yield to erroneous PHP estimates. Consequently, it is generally recommended to empirically determine LeuCFs to better constrain carbon fluxes (Bell 1990, Pulido-Villena & Reche 2003, Buesing & Marxsen 2005, Calvo-Díaz & Morán 2009).

To fully understand the biogeochemical carbon cycle in the ocean, it is imperative to take into account the entire depth range where metabolic processes are present. Dark ocean (>200 m depth) prokaryotes have been reported to account for ca. 75% of biomass, 50 % of production (Arístegui et al. 2009) and 15% of respiration (del Giorgio & Williams 2005) in the global ocean, thus playing a major role in the global carbon cycle. Although the potential role of deep-ocean prokaryotes for metabolizing organic substrates and the importance of defining the PGE for carbon flux studies are generally accepted, there are only a few studies reporting dark-ocean PGE and LeuCFs. Moreover, almost none of the available deep-ocean PGE measurements were directly estimated (but see Arístegui et al. 2005, Reinthaler et al. 2006). Recently, from a global data set of PHP and PR, Arístegui et al. (2009) suggested that PGE should decrease with depth to a value of ca. 4% in the bathypelagic realm assuming an epipelagic PGE of 15% (del Giorgio & Cole 2000). This decrease in PGE with depth has been associated with the decrease in substrate quality and quantity with depth (**Chapter III**), coinciding with higher extracellular enzymatic activities per cell than in surface waters (**Chapter III**). The few PGE values published for dark ocean prokaryotes vary considerably (1-39%, see *Results and Discussion*). Evidence is accumulating that the flux of organic carbon to the deep waters is insufficient to sustain the prokaryotic carbon demand in these layers (Reinthaler et al. 2006, Steinberg et al. 2008, **Chapter**

II). Thus, a fine-tuning of the deep ocean PGE estimates might help resolving this discrepancy between organic carbon supply and demand.

Carlucci et al. (1986) found that labeled amino acids were increasingly respired with depths where PGE is thought to be lower than in surface waters. Since leucine respiration has been linked to low LeuCFs (Alonso-Saéz et al. 2007, del Giorgio et al. pers. com.), it might be postulated that deep ocean heterotrophic prokaryotes should exhibit low LeuCFs. Despite the importance of determining empirical CF for accurate PGE estimates, there is only one report (Gasol et al. 2009) of deep ocean CFs (5 estimates from the mesopelagic realm but no PGE calculations were done). Therefore, the influence of LeuCFs on PGE is still unknown for the deep ocean, where a substantial fraction of the organic carbon processing takes place.

In the present study, we review existing information on deep ocean PGE and LeuCFs and report results from 13 experiments conducted to determine PGE in the mesopelagic zone of the subtropical Northeast Atlantic. The aim is to provide additional estimates of PGE for the dark ocean and to examine how the LeuCFs estimates affect the PGE. We hypothesized that, as shown for other organic carbon-limited systems (e.g. the oligotrophic subtropical NE Atlantic, Alonso-Saéz et al. 2007), the deep ocean CFs are lower than the theoretical CF.

Material and methods

Study site and sampling

To analyze the variability of the deep ocean leucine-to-carbon conversion factor, eight stations were occupied in the subtropical North Atlantic between the Canary and the Cape Verde archipelagos during the RODA-II cruise (February 2007) on board the RV 'Hespérides' (Fig. 1). The samples were obtained from four different sites within a cyclonic eddy (R2) generated by the presence of the islands, from near-shore waters (next to the Canary Islands), in close proximity to the Cape Blanc upwelling and from the open subtropical Atlantic Ocean. All samples were obtained from the mesopelagic realm (ranging from 350-1000 m depth) (Table 1). The samples were immediately taken to temperature-controlled chambers set at in situ temperature (± 0.2ºC).

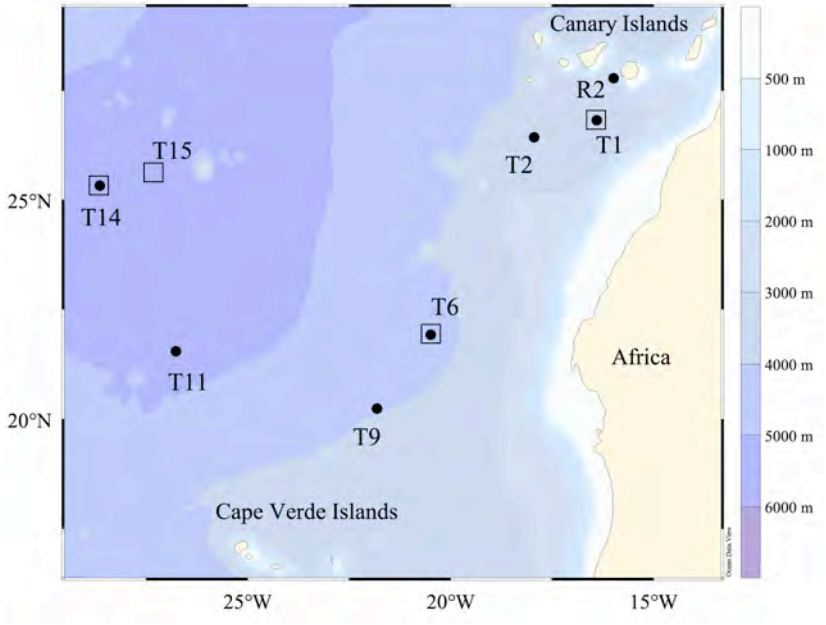


Fig. 1. Position of sampling stations during the RODA II cruises in February 2007. Full circles indicate stations where experiments to determine prokaryotic growth efficiencies were performed. Open squares indicate stations where assays to determine leucine-to-total carbon conversion factors were performed

Table 1. Temperature (T, ºC), salinity, type of experiment performed, total incubation time (h), initial prokaryotic abundance (PA, 10^5 cell ml^{-1}), prokaryotic heterotrophic production (PHP, pmol leu l^{-1} h^{-1}), turnover time (Tt, d), and high nucleic acid content cells (HNA, %) in the ambient water collected at each station

Station	Depth (m)	T (ºC)	Salinity	Exp. type	Incubation time PGE/CF (h)	PA (10^5 cell ml^{-1})	PHP (pmol leu l^{-1} h^{-1})	Tt (d)	HNA (%)
R2	350	14.2	35.995	PGE	48	4.4	0.17	424	21
R2	1000	7.8	35.269	PGE	48	4.3	0.04	1346	25
T1	540	10.9	35.473	PGE / CF	48 / 218	0.9	0.13	112	36
T1	800	8.0	35.223	PGE / CF	48/ 218	0.7	0.11	107	33
T2	400	13.2	35.831	PGE	48	1	0.46	35	35
T2	800	8. 7	35.305	PGE	48	0.6	0.14	71	32
T6	400	12.8	35.670	CF	- / 190	1.8	0.33	88	39
T6	800	7.8	35.130	PGE / CF	48 / 190	0.9	0.03	418	35
T9	400	11.7	35.498	PGE	48	1	0.35	46	43
T9	800	7.3	34.989	PGE	48	0.6	0.21	47	41
T11	400	12.9	35.681	PGE	48	1	0.48	34	43
T11	800	7.3	35.016	PGE	48	0.6	0.36	27	41
T14	400	14.1	35.920	PGE / CF	48 / 141	0.8	0.09	147	42
T14	800	8.5	35.264	PGE / CF	48 / 141	0.4	0.35	37	39
T15	400	13.8	35.889	CF	- / 140	0.8	0.32	41	42
T15	800	8.6	35.296	CF	- / 140	0.5	0.21	38	37

Prokaryotic Respiration (PR) and Prokaryotic Growth Efficiency (PGE)

PR was measured by following the changes in dissolved oxygen concentrations during dark incubations. Water samples drawn into carboys were carefully siphoned into twelve biological oxygen demand (BOD) bottles, and three replicate bottles were immediately fixed with Winkler reagents to determine the initial oxygen concentration. Three replicate bottles were incubated in the dark at in situ temperature and fixed with Winkler reagents after 24 and 48 h. At each time step, one additional replicate bottle was used to determine prokaryotic abundance (PA) and prokaryotic heterotrophic production (PHP) as described below. Dissolved oxygen measurements were made by automated Winkler titrations on the basis of colorimetric end-point detection as described in Arístegui et al. (2005). PR was estimated from the difference in oxygen concentration at the start and the end of the incubation. To convert oxygen consumption to carbon respiration, a respiratory quotient of 1 was assumed (del Giorgio et al. 2006).

Prokaryotic growth efficiency (PGE) was calculated as (PHP / PHP+PR) *x* 100. PR was estimated as described above in 48 h incubations (see Fig. 2 for an example). PHP was estimated in four different ways: (1) based on Leu incorporation at the start of the incubation using the theoretical conversion factor (1.5 kg C mol^{-1} Leu assuming no isotope dilution), (2) the integrated PHP over the 48-h incubation (see below) using the theoretical conversion factor (1.5 kg C mol^{-1} Leu), (3) the change in prokaryotic biomass, estimated from the increase in cell numbers during the incubation and using a conservative factor of 12 fg C $cell^{-1}$ (Fukuda et al. 1998), and (4) the integrated PHP over the 48 h incubation using the empirically determined conversion factor of each sample in which it was determined.

Prokaryotic abundance (PA) and prokaryotic heterotrophic production (PHP)

Picoplankton cells were enumerated using flow cytometry on a FACSCalibur (Becton Dickinson) with a laser emitting at 488 nm wavelength. Samples (1.5 ml) were fixed with paraformaldehyde (1% final concentration), incubated at 4°C for 15 to 30 min and then stored frozen in liquid nitrogen until analysis. Prior to counting the cells, 200 µl of sample were stained with a DMS-diluted SYTO-13 (Molecular Probes) stock (10:1) at 2.5 µM final concentration. Prokaryotes were identified by their signature in a plot of side scatter (SSC) versus green fluorescence (FL1). High and low nucleic acid cells (HNA, LNA) were separated in the scatter plot of SSC-FL1 (Gasol et al. 1999). HNA cells exhibited higher FL1 than LNA cells.

PHP was estimated by the incorporation of tritiated leucine (specific activity 171 Ci $mmol^{-1}$, Amersham) using the centrifugation method (Smith & Azam 1992). Four replicates and two TCA-killed blanks were incubated with 40 nmol l^{-1} of ^{3}H-Leu for 3 to 7 h. Precipitation was done with ice-cold TCA. Although the use of 40 nmol l^{-1} as a saturating concentration was justified by concentration kinetics done on board, the dilution of the ambient leucine could have diluted the tracer by 10-20%, introducing some variability on the data. Furthermore, not rinsing with ethanol may lead to the detection of unspecific ^{3}H labeling. That is why ethanol rinsing is often used to remove this labeling (Wicks & Robarts 1988, Ducklow et al. 2002, Kirchman et al. 2005). However, ethanol rinsed did not show any significant change in the results of other studies (Van Wambeke et al. 2002, Granéli et al. 2004, Van Wambeke et al. 2008)

Determination of the Leucine to carbon conversion factor (CF)

Eight experiments were performed to determine the Leu-to-carbon CFs for deep-water heterotrophic prokaryotic communities (see Fig.2 for an example). Each water sample was gently filtered through a 0.6 μm polycarbonate filter (Millipore, GTTP), and incubated in 2-l acid-clean polycarbonate bottles in the dark at in situ temperature. Subsamples were taken for PHP and PA measurements every 12–24 h until prokaryotes reached the stationary growth phase. CFs were calculated with the cumulative method (Bjørnsen & Kuparinen 1991), which maximizes the use of available data.

Statistical analyses

Data were log transformed and normality was checked with a Shapiro-test before Pearson correlations were calculated using the freely available R software (Ihaka & Gentleman 1996).

Results and Discussion

Deep-ocean variability in the leucine to carbon conversion factor (CF)

We empirically estimated the CF at the different stations and obtained a 7-fold variation (0.13-0.85 kg C mol^{-1} Leu) (Table 2, Fig. 2). The empirically obtained CFs were consistently lower than the theoretical CF for open ocean surface waters (1.55 kg C mol^{-1} Leu assuming no isotope dilution) (e.g. Ducklow et al. 1992, Carlson & Ducklow 1996, Zubkov et al. 2000). Our results are in agreement with the range of CFs

(0.02-1.29 kg C mol^{-1} Leu, mean = 0.32 kg C mol^{-1} Leu) reported by Alonso-Sáez et al. (2007) for the oligotrophic surface waters of the NE Atlantic Ocean. Furthermore, in the only other study where deep ocean (>200 m depth) CFs are reported (Gasol et al. 2009), CF ranged between 0.39-2.38 kg C mol^{-1} Leu, the latter value was the only one higher than the theoretical CF out of 5 measurements with no significant difference between epi- and mesopelagic CFs. Our average CF (0.5 ± 0.2 kg C mol^{-1} Leu) is similar to the mean CF of 0.4 ± 0.2 kg C mol^{-1} Leu reported by Gasol et al. (2009) and the mean CF of 0.3 ± 0.3 kg C mol^{-1} Leu obtained by Alonso-Sáez et al. (2007). Although it is known that CFs can substantially deviate from the theoretical CF (Kirchman 1992), the underlying reason for this is still unclear.

Table 2. Empirically obtained leucine-to-carbon prokaryotic conversion factors (CFs)

Station	Depth (m)	Leu to C CF (kg C mol^{-1} Leu)
T1	540	0.67
T1	800	0.55
T6	400	0.59
T6	800	0.54
T14	400	0.27
T14	800	0.13
T15	400	0.66
T15	800	0.85

Alonso-Sáez et al. (2007) analyzed the cause for these lower-than-theoretical CFs in oligotrophic surface waters of the NE Atlantic by measuring the respiration and assimilation of ^{14}C-labeled leucine along a productivity gradient. These authors found an increasing fraction of leucine respired and decreasing CFs towards offshore, suggesting that leucine catabolism might be related to low CFs. Thus, a proportion of the leucine taken up is not used for protein synthesis, hence biomass production, but respired. We suggest that similar processes as reported for oligotrophic surface waters take place also in the dark ocean, where carbon limitation and a slower prokaryotic growth than in surface waters are generally reported. The slow growth of deep ocean prokaryotes could also involve a relatively higher protein turnover than in surface waters, further reducing the deep water CFs as compared to the theoretical one. Alonso-Sáez et al. (2007) specifically tested the possibility of protein turnover and did not find

a significant influence of this process on the low CFs measured in oligotrophic surface waters, but similar measurements in the dark ocean are lacking. Overall, our results suggest a possible overestimation of carbon production (and concomitantly of PGE) in deep waters if calculated from leucine incorporation measurements using the commonly applied theoretical CF.

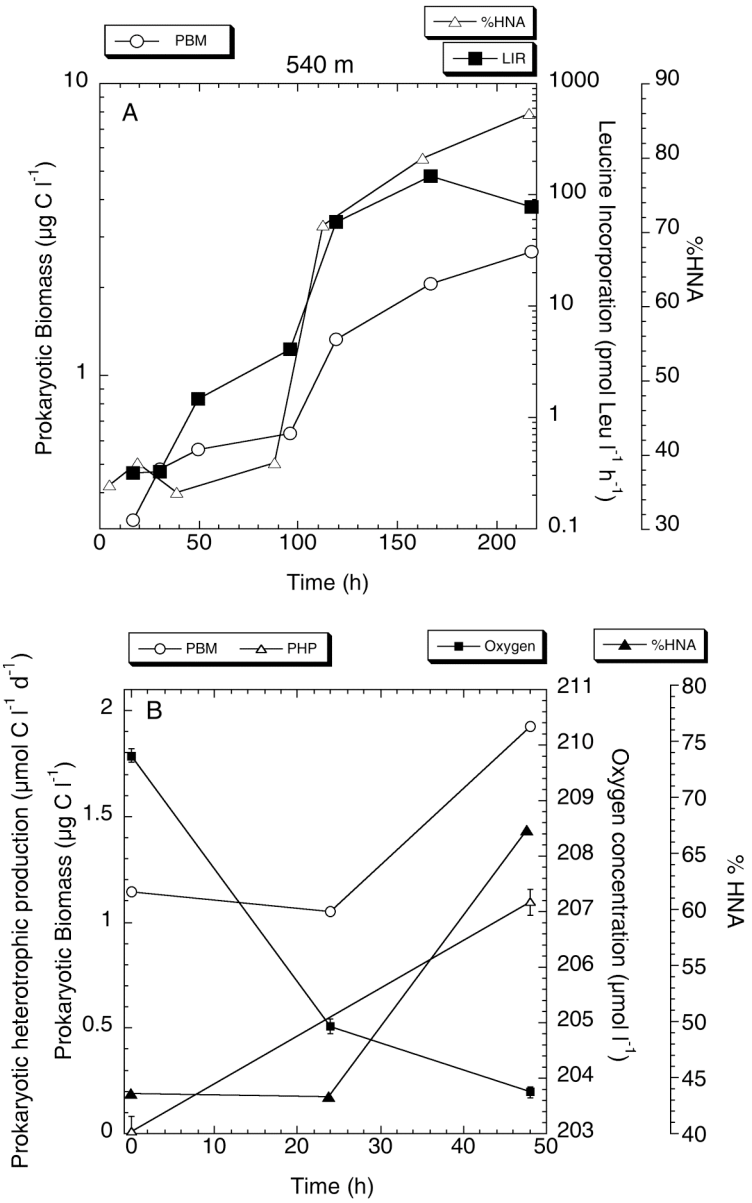


Fig. 2. (A) Mean (± SD) of prokaryotic biomass (PBM, µg C l^{-1}), high nucleic acid content cells (HNA, %) and leucine incorporation (LIR, pmol leu l^{-1} h^{-1}) during the carbon-to-leucine conversion factor experiment at station T1 (540 m). (B) Variation in prokaryotic heterotrophic production (PHP, µmol C l^{-1} d^{-1}) biomass (PBM, µg C l^{-1}), high nucleic acid content cells (HNA, %) and oxygen concentration (µmol l^{-1}) during the prokaryotic growth efficiency (PGE) experiment at station T1 (540 m). In most of the parameters plotted standard deviations are not visible since they are smaller than the symbols

Variability of prokaryotic growth efficiencies in the dark ocean

The use of different approaches for estimating PHP in the incubation experiments resulted in a considerable range of deep water PGEs (Table 3). A similar conclusion was reached by Alonso-Sáez et al. (2007) for the oligotrophic surface waters of the same study area (NE subtropical Atlantic) and by del Giorgio et al. (pers. com.) for North Pacific waters. Using the PHP measured at the start of the incubation (PHP t0 CF_t) resulted in one order of magnitude lower PGEs (PGE ranging between < 1 – 2 %), as compared to the integrated PHP (PHP Int CF_t), determined over the course of the incubation (PGE ranging between < 1 – 34 %), or by based on the increase in prokaryotic biomass (PBM; PGE ranging between < 1 – 33 %) (Table 3). Averaging all the different approaches, PGEs ranged from <1 to 24% (Table 3). The highest PGE estimates for deep waters are similar to the average PGE of surface water prokaryotes (del Giorgio & Cole, 2000), and are within the range reported by Alonso-Sáez et al. (2007) for the surface waters of the same study region (1-56%). Although there are only a few reports of PGE available for the dark ocean, the range obtained in the present study also falls within the PGE obtained in other studies. Arístegui et al. (2005) estimated an average PGE of 18 ± 3% and 13 ± 2% at 600 m and 1000 m depth, respectively, for the same study region. In an experimental simulation at the Bermuda Atlantic Time-Series Study (BATS) site, Carlson et al. (2004) estimated PGE to be 8 ± 4% for the upper mesopelagic prokaryotic assemblage after convective overturn. Reinthaler et al. (2006) found an average PGE of 2 ± 1% in the meso- and bathypelagical realm of the North Atlantic Ocean. Tanaka & Rassoulzadegan (2004), report an annual PGE of 19-39% for the mesopelagic layers of the Northwest Mediterranean Sea. Zaccone et al. (2003) determined a PGE range of 6-21% for the water column of the Ionian Sea derived from ETS (electron transport system) measurements, the highest PGE corresponding to the deepest water mass (2000-3500 m depth). Therefore, all the above results (except Reinthaler et al. 2006) suggest that PGEs in the mesopelagic realm are not always as low as predicted from the empirical function proposed by del Giorgio & Cole (1998), although there is a high variability of PGE estimates depending on the approach to compute PHP (Table 3). Arístegui et al. (2005) claimed, based on the PGE obtained as well as on other metabolic proxies for prokaryotes, that the mesopelagic prokaryotic assemblage in the subtropical Northeast Atlantic is as active as the surface waters community, even though prokaryotic

abundance is lower in the mesopelagic realm than in surface waters. Overall, although some uncertainties remain on the actual PGEs, deep ocean PGEs appear to be comparable to the PGEs of oligotrophic surface waters suggesting that also dark ocean prokaryotes play a substantial role in marine carbon flux.

Table 3. High nucleic acid content cells (HNA) % range, prokaryotic respiration (PR), biomass production (PBM) estimated from the change in abundance assuming a carbon content per cell of 12 fg C $cell^{-1}$ (Fukuda et al. 1998), leucine-based (using theoretical CF of 1.55 kg C mol^{-1} Leu) prokaryotic heterotrophic production at the initial incubation time (PHP t0 CF_t) and integrated over 48 h of incubation (PHP Int CF_t), prokaryotic growth efficiency (PGE, %) range and average (see text for details) and PGE calculated from the empirically obtained carbon-to-leucine conversion factor (PGE CF_e). Metabolic rates are in µmol C m^{-3} d^{-1}

Station	Depth (m)	HNA (%)	PR	PBM	PHP t0 CF_t	PHP Int CF_t	PGE range	PGE AVG.	PGE CF_e
R2	350	43-43	480	8	<1	1	<1-2	1	
R2	1000	39-43	970	4	<1	1	<1	<1	
T1	540	44-67	1180	33	6	277	1-19	7	9
T1	800	-	450	-	<1	114	<1-20	10	8
T2	400	45-56	1040	13	4	260	<1-20	7	
T2	800	43-59	120	22	2	38	2-24	14	
T6	800	48-61	170	18	1	28	<1-14	7	4
T9	400	55-60	590	13	8	70	1-11	4	
T9	800	41-69	490	52	2	110	<1-18	10	
T11	400	45-57	190	9	2	38	1-17	7	
T11	800	47-77	90	44	1	47	1-34	24	
T14	400	46-51	190	25	2	27	1-16	8	3
T14	800	47-56	190	29	2	6	1-13	6	<1

Relationship between CFs and PGE in the dark ocean

As shown above, CFs variability is high in the dark ocean influencing the PHP and PGE estimates of deep ocean prokaryotes (Table 3). In experiments with surface waters, decreases in empirically determined CFs along decreasing trophic gradients have been reported for the Mediterranean Sea (Pedrós-Alió et al. 1999), the NE Pacific Ocean (Sherr et al. 2001) and for the NE Atlantic Ocean (Alonso-Sáez et al. 2007). These trends suggest that CFs are ecologically constrained and not just confinement artifacts caused by bottle enclosures (Massana et al. 2001). However, for deep waters this is not so clear due to the low number of CF experiments carried out thus far.

We found a significant correlation (Pearson $r = 0.93$, $p = 0.019$, $n = 5$, log-log transformed) (Fig. 3) between the empirically derived CFs and the PGEs estimated using the integrated PHP and the theoretical CF, but no significant relation with the

PGE obtained with the PHP measured at the start of the incubation and with the PHP calculated from the increase in prokaryotic abundance over time. It is noteworthy that autocorrelation is not responsible for this relation because PGE was estimated with the theoretical CF (Fig. 3). Alonso-Sáez et al. (2007) also reported a correlation (Pearson r = 0.86, p < 0.0004, n = 12, log-log transformed) between empirically determined CFs and PGEs (averaging biomass increase and leucine incorporation at the start of the incubation) for the oligotrophic surface NE Atlantic Ocean. Our findings support the idea that both parameters (CF and PGE) reflect basically the same physiological processes as reported for surface water prokaryotes (Alonso-Sáez et al. 2007, del Giorgio et al. pers. com.) also in the dark ocean.

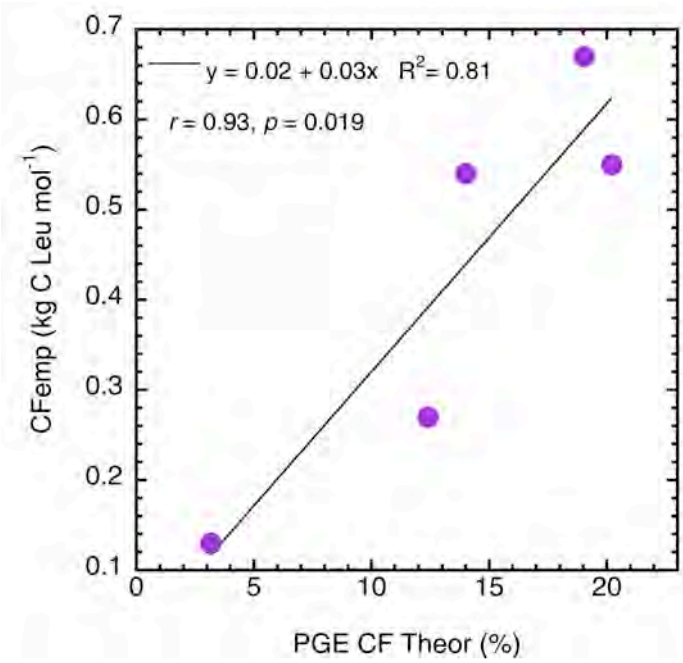


Fig. 3. Relationship between empirically derived leucine-to-carbon conversion factors (CFs) and prokaryotic growth efficiency (PGE) estimated via integrated PHP using the theoretical CF

Estimating prokaryotic carbon utilization in the ocean

Although PGE is known to be a key ecological variable in the analyses of oceanic carbon flux, and that empirically obtained CFs are required to obtain an accurate PGE, estimating these two parameters is far from trivial (see Gasol et al. 2008). There is a temporal scale problem in PGE determinations, because PHP can be measured in incubations over a time frame of minutes to hours while measurements of prokaryotic respiration require at least incubation times of ≈24 h in oceanic waters (cf.

Arístegui et al. 2005). Although there is an instantaneous estimate (without incubation) for respiration available such as the ETS measurement, this is a rather indirect way depending on poorly constrained and potentially variable conversion factor (e.g. ETS and the R:ETS ratio). The most essential issues that affect both PGE and CFs during long incubations is related to this time scale and related to bottle confinement. After a certain incubation period, leucine incorporation rates increase followed by increases in biomass (e.g. Ducklow & Hill 1985). This casts doubts whether the community that is actively growing is representative from the in situ assemblage. When this shift in community composition takes place, cells with high DNA (HNA) content start to be more abundant relative to the low DNA fraction of the prokaryotic community (e.g. Vaqué et al. 2001). This issue, however, is more significant in incubation experiments to determine CFs, as the incubation times are generally days. Nevertheless, the relation found between two different processes (Fig. 3) incubated at different time-scales (CF up to ~220 h and PGE 24-48 h) might indicate that processes remain representative for the in situ conditions. It is also possible that, as suggested by del Giorgio et al. (pers. com.), these empirical CFs and PGEs are simply different expressions of the same basic limitation. In this case, the CF would be effective in accounting for the decoupling of leucine incorporation and biomass production over short-term incubations, which is, however, greatly overcompensated if applied to long-term incubations. Following this argument, correcting the PGE with empirically obtained CFs would result in an underestimation of the actual PGE. However, this assumption remains to be tested for the dark ocean.

Another related problem affecting PGE and CF estimates is the possible variation in the carbon content per cell, which also shows a rather wide range, and can also change during the course of the incubations (Ducklow 2000). An additional difficulty, also related to deep water analyses, is the potential effect of incubating under depressurized conditions. However, thus far no consistent pattern has emerged from studies on the effect of hydrostatic pressure on deep ocean prokaryotic activity. There is evidence that deep water prokaryotic activity is overestimated if measured under decompressed conditions (Jannasch & Wirsen 1982), while other studies report an increasing energy demand associated with decompression suggesting an underestimation of PGE values determined under decompressed conditions (Tamburini et al. 2003). To be able to determine the magnitude of carbon flux mediated by prokaryotes in the dark ocean further testing and methodological improvements are

needed, and especial efforts should be directed to evaluate the conversion factors involved in the prokaryotic rate measurements under in situ conditions.

Acknowledgments

This research was supported by the Spanish "Plan Nacional de I+D" (MEC) under the RODA (CTM2004-06842-C03-03 / MAR) and CAIBEX (CTM2007-66408-C02-02) grants to J.A., a grant of the Earth and Life Science Division of the Dutch Science Foundation (ALW-NWO; ARCHIMEDES project, 835.20.023) to G.J.H. and a predoctoral Fellowship of the Spanish Ministry of Education and Science (AP2005-3932) to F.B. Project MODIVUS (CTM2005-04795/MAR) supported J.M.G. The work was carried out within the frame of the EU 'Networks of Excellence' MarBef and EurOceans. We thank the captain and crew of R/V Hespérides for their support at sea, and M. Espino and I. Alonso-González for their help with the respiration estimates. We also thank three anonymous reviewers for their comments and suggestions to improve the manuscript.

References

Alonso-Saéz L, Gasol JM, Arístegui J, Vilas JC, Vaqué D, Duarte CM, Agusti S (2007) Large-scale variability in surface bacterial carbon demand and growth efficiency in the subtropical northeast Atlantic Ocean. Limnol. Oceanogr. 52:533-546

Arístegui J, Duarte CM, Gasol JM, Alonso-Sáez L (2005) Active mesopelagic prokaryotes support high respiration in the subtropical northeast Atlantic Ocean. Geophys. Res. Lett. 32:L03608, doi:03610.01029/02004GL021863

Arístegui J, Gasol JM, Duarte CM, Herndl GJ (2009) Microbial Oceanography of the dark ocean's pelagic realm. Limnol. Oceanogr. 54:1501-1529

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10:257-263

Bell RT (1990) An explanation for the variability in the conversion factor deriving bacterial cell production from incorporation of [^{3}H]thymidine. Limnol. Oceanogr. 35:910-915

Bjørnsen PK, Kuparinen J (1991) Determination of bacterioplankton biomass, net production and growth efficiency in the Southern Ocean. Mar. Ecol. Prog. Ser. 71:185-194

Buesing N, Marxsen J (2005) Theoretical and empirical conversion factors for determining bacterial production in freshwater sediments via leucine incorporation. Limnol. Oceanogr. 3:101-107

Calvo-Díaz A, Morán XAG (2009) Empirical Leucine-to-Carbon Conversion Factors for Estimating Heterotrophic Bacterial Production: Seasonality and Predictability in a Temperature Coastal Ecosystem. Appl. Environ. Microbiol. 75:3216-3221

Carlson CA, Ducklow HW (1996) Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. Aquat. Microb. Ecol. 10:69-85

Carlson CA, Giovannoni SJ, Hansell DA, Goldberg SJ, Parsons R, Vergin K (2004) Interactions among dissolved organic carbon, microbial processes, and community structure in the mesopelagic zone of the northwestern Sargasso Sea. Limnol. Oceanogr. 49:1073-1083

Carlson CA, Giorgio PAd, Herndl GJ (2007) Microbes and the Disipation of Energy and Respiration: From Cells to Ecosystems. Oceanography 20:89-100

Carlucci AF, Craven DB, Robertson KJ (1986) Microheterotrophic utilization of dissolved free amino acids in depth profiles of Southern California Borderland basin waters. Oceanolog. Acta 9:89-96

del Giorgio PA, Cole JJ (1998) Bacterial growth yield efficiency in natural aquatic systems. Annu. Rev. Ecol. Syst. 29:503-541

del Giorgio PA, Cole JJ (2000) Bacterial energetics and growth efficiency. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, p 289-325

del Giorgio PA, Williams PJleB (2005) The global significance of respiration in aquatic ecosystems: from single cells to the biosphere. In: Williams PJleB & del Giorgio PA (eds) Respiration in Aquatic Ecosystems. Oxford University Press, New York

del Giorgio PA, Pace ML, Fischer D (2006) Relationship of bacterial growth efficiency to spatial variation in bacterial activity in the Hudson River. Aquat. Microb. Ecol. 45:55-67

Ducklow H (2000) Bacterial production and biomass in the oceans. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, p 85-120

Ducklow HW, Carlson CA, W. O. Smith J (1992) Bacterial growth in experimental plankton assemblages and seawater cultures from *Phaeocystis antartica* bloom in the Ross Sea. Aquat. Microb. Ecol. 19:215-227

Ducklow HW, Kirchman DL, Anderson TR (2002) The magnitude of spring bloom bacterial production in the North Atlantic Ocean. Limnol. Oceanogr. 47:1684-1693

Fukuda R, Ogawa H, Nagata T, Koike I (1998) Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. Appl. Environ. Microbiol. 64:3352-3358

Gasol JM, Zweifel UL, Peters F, Fuhrman JA, Hagström Å (1999) Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl. Environ. Microbiol. 65:4475-4483

Gasol JM, Pinhassi J, Alonso-Sáez L, Ducklow H, Herndl GJ, Koblízek M, Labrenz M, Luo Y, Morán XAG, Reinthaler T, Simon M (2008) Towards a better understanding of microbial carbon flux in the sea. Aquat. Microb. Ecol. 53:21-38

Gasol JM, Alonso-Sáez L, Vaqué D, Baltar F, Calleja ML, Duarte CM, Arístegui J (2009) Mesopelagic prokaryotic bulk and single-cell heterotrophic and community composition in the NW Africa-Canary Islands coastal-transition zone. Progr. Oceanogr. doi:10.1016/j.pocean.2009.07.014

Granéli W, Carlsson P, Bertilsson S (2004) Bacterial abundance, production and organic carbon limitation in the Southern Ocean (39-62º S, 4-14º E) during the asutral summer 1997/1998. Deep-Sea Res. Pt. II 51:2569-2582

Ihaka R, Gentleman R (1996) R: A language for data analysis and graphics. J. Comp. Graph. Stat. 5:299-314

Jannasch HW, Wirsen CO (1982) Microbial activities in undecompressed and decompressed deep-seawater samples. Appl. Environ. Microbiol. 43:1116-1124

Kirchman DL (1992) Incorporation of thymidine and leucine in the subarctic Pacific: application to estimating bacterial production. Mar. Ecol. Prog. Ser. 82:301-309

Kirchman DL, Malmstrom RR, Cottrell MT (2005) Control of bacterial growth by temperaturer and organic matter in the Western Arctic. Deep-Sea Res. Pt. II 52:3386-3395

Massana R, Pedrós-Alió C, Casamayor EO, Gasol JM (2001) Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters. Limnol. Oceanogr. 46:1181-1188

Pedrós-Alió C, Calderon-Paz J-I, Guixa-Boixereu N, Estrada M, Gasol JM (1999) Bacterioplankton and phytoplankton biomass and production during summer stratification in the northwestern Mediterranean Sea. Deep Sea Res. I 46:985-1019

Pulido-Villena E, Reche I (2003) Exploring bacterioplankton growth and protein synthesis to determine conversion factors across a gradient of dissolved organic matter. Microb. Ecol. 46:33-42

Reinthaler T, Aken Hv, Veth C, Williams PJl, Arístegui J, Robinson C, Lebaron P, Herndl GJ (2006) Prokaryotic respiration and production in the meso- and bathypelagic realm of the eastern and western North Atlantic basin. Limnol. Oceanogr. 51:1262-1273

Rivkin RB, Legendre L (2001) Biogenic carbon cycling in the oceans: effect of microbial respiration. Science 291:2398-2400

Robinson C, Williams PJleB (2005) Respiration and its measurements in surface marine waters. In: Williams PJleB & del Giorgio PA (eds) Respiration in Aquatic Ecosystems. Oxford University Press, New York

Sherr EB, Sherr BF, Cowles TJ (2001) Mesoscale variability in bacterial activity in the Northeast Pacific Ocean off Oregon, USA. Aquat. Microb. Ecol. 25:21-30

Sherry ND, Imanian B, Sugimoto K, Boyd PW, Harrison PJ (2002) Seasonal and interannual trends in heterotrophic bacterial processes between 1995 and 1999 in the subarctic NE Pacific. Deep-Sea Res. II 49:5775-5791

Smith DC, Azam F (1992) A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. Mar. Microb. Food Webs 6:107-114

Steinberg DK, B. A. Van Mooy, K. Buesseler, P. W. Boyd, T. Kobari, Karl DM (2008) Bacterial vs. zooplankton control of sinking particle flux in the ocean's twilight zone. Limnol. Oceanogr. 53:1327-1338

Tamburini C, Garcin J, Bianchi A (2003) Role of deep-sea bacteria in organic matter mineralization and adaptation to hydrostatic pressure conditions in the NW Mediterranean Sea. Aquat. Microb. Ecol. 32:209-218

Tanaka T, Rassoulzadegan F (2004) Vertical and seasonal variations of bacterial abundance and production in the mesopelagic layer of the NW Mediterranean Sea: bottom-up and top-down controls. Deep Sea Res. I 51:531-544

Van Wambeke F, Christaki U, Giiannakorou A, Moutin T, Souvemerzoglou K (2002) Longitudinal and vertical trends of bacterial limitation by phosphorus in the Mediterranean Sea. Microb. Ecol. 43:119-133

Van Wambeke F, Obernosterer I, Moutin T, Duhamel S, Ulloa O, Claustre H (2008) Heterotrophic bacterial production in the eastern South Pacific: longitudinal trends and coupling with primary production. Biogeosciences 5:157-169

Wicks RJ, Robarts RD (1988) Ethanol extraction requirement for purification of protein labeled with [^{3}H]leucine in aquatic bacterial production studies. Appl. Environ. Microbiol. 54:3191-3193

Zaccone R, Monticelli LS, Seritti A, Santinelli C, Azzaro M, Boldrin A, LaFerla R, D'Alcala MR (2003) Bacterial processes in the intermediate and deep layers of the Ionian Sea in winter 1999: vertical profiles and their relationship to the different water masses. J. Geophys. Res. 108:8117

Zubkov MV, Seigh MA, Burkill PH, Leakey RJG (2000) Bacterial growth and grazing loss in contrasting areas of North and South Atlantic. J. Plankton Res. 22:685-711

DISCUSSION

SYNTHESIS OF RESULTS AND GENERAL DISCUSSION

Dark ocean prokaryotic activity and assemblage structure: homogeneous or heterogeneous distribution?

Due to the low availability of labile organic matter, and the typically more stable abiotic conditions (temperature, salinity, pH, oxygen,…) of the dark ocean as compared to the surface waters, it is reasonably to infer that the prokaryotic assemblage and their associated activity should follow a rather homogeneous distribution through the deep waters. Besides, the low temperature and the low organic matter concentration characteristic of the dark ocean may also lead to the perception of the deep ocean as a site holding negligible biological activity. However, contrasting horizontal (lateral) and vertical (in depth) differences were found in prokaryotic activity (**Chapters I, II, III, IV, V, VI**) and community structure (**Chapter I and V**) within the dark ocean. A striking lateral substitution of *Bacteria* by *Archaea* was found for the epi- and mesopelagic waters of the NW Africa-Canaries coastal transition zone (**Chapter I**). Strong variations were also found in the prokaryotic abundance (PA) and heterotrophic production (PHP) and percentage of high nucleic content cell along that trophic gradient (**Chapter I**). These variations were related to the presence of different water masses, reflecting a possible influence of different organic matter pools on the prokaryotic assemblage structure and activity. This hypothesis was later confirmed when a significant correlation was found between the suspended particulate organic carbon and the prokaryotic respiration along a latitudinal gradient in the meso- and bathypelagic realm of the (sub)tropical North Atlantic (**Chapter II**). The relation between the prokaryotic activity and the organic matter distribution was further demonstrated by the significant correlations between the extracellular enzymatic activity (dissolved and particulate fractions), PHP, and the suspended POC concentration in the deep Central Atlantic Ocean (**Chapter IV**). Moreover, the extracellular enzymatic activity (EEA) distribution was also affected by the changes in the organic matter (OM) composition with depth (**Chapter III**), agreeing with a hypothetical “bottom-up” control of the prokaryotic activity by the OM distribution. In fact, the increase in cell-specific EEA, half saturation constant (K_m) and the V_{max}/K_m ratio with depth suggests an adaptation to the lower availability of labile organic matter with increasing depth. This conclusion

agrees with the higher rates of amino acid respiration in deep waters (Carlucci et al. 1986), and the increasing ratio of *D*-/-*L* amino acid uptake by the bulk prokaryotic community (Pérez et al. 2003). Using comparative environmental genomics, DeLong et al. (2006) also found a high abundance of genes responsible for glyoxylate and dicarboxylate metabolism, that correspond to the relatively more oxidized and degraded DOM present in the deep ocean as compared to surface-waters (Benner 2002). It also agrees with the first genome sequence obtained from a low temperature-high pressure adapted deep-ocean microbe (a psychropiezophile organism, *Photobacterium profundum* strain SS9) (Vezzi et al. 2005, Bartlett et al. 2007, 2008), where an amino acid fermentation pathway previously known only in strict anaerobes (the Strickland reaction) was found along with an enzymatic system similar to those for the utilization of various complex polymers including xenobiotics, chitin, pullulan and cellulose. All these findings imply an adaptation of deep-sea microbes to use more recalcitrant organic carbon (Carlson et al. 2004). However, this adaptation to the difference in organic matter "quality" via the production of extracellular enzymes produces a concomitant decrease in the prokaryotic growth yield in the dark ocean as compared to the surface-water. This inverse relation between EEA and growth yield is similar to the situation in developed versus developing countries. In the developed countries (i.e., surface waters) the metabolic energy (walking, running, working…) required to obtain food is not much because you can find it everywhere (shops, restaurants…). Therefore in these countries it is easier to gain biomass (it is clearly reflected by the high body mass indexes in those populations). However, in the developing countries (i.e., dark ocean), the metabolic energy required to obtain the same amount of food as in the developed countries is higher (more working hours required per unit food obtained), leading to higher respiration rates per amount of food taken up and thereby, not gaining biomass. In analogy, the metabolic energy required by heterotrophic prokaryotes to metabolize the less labile deep-ocean organic matter is related to a lower growth yield.

Other recent evidence also suggests a heterogeneous and active community of prokaryotes in the dark ocean. Some studies have shown seasonality in prokaryotic abundance in the dark ocean. A twofold change in the prokaryotic abundance was reported by Nagata et al. (2000) at a station sampled twice over few years in the deep Pacific. In the mesopelagic layer of the Mediterranean Sea, Tanaka & Rassoulzadegan (2002, 2004) also reported relatively large seasonal changes. Sherry et al. (1999) found

a fivefold seasonal variation in PHP in the NE Pacific. Church et al. (2003) detected a higher proportion of cells at depth than near the surface by fluorescence in situ hybridization (FISH), assumed to correspond to high rRNA content. Arístegui et al. (2005b) obtained a very similar proportion of actively respiring cells, and higher cell-specific respiration in the mesopelagic NW Atlantic than in the epipelagic. In the same region (NW Africa–Canary Islands coastal-transition zone), Gasol et al. (2009) found that the relationship between prokaryotes and their main predators (heterotrophic nanoflagellates) remained constant with depth, further contradicting the assumption that dark ocean prokaryotes are mostly inactive. Moreover, Teira et al. (2006) reported that 16-20% of the heterotrophic prokarayotic community of the dark North Atlantic are metabolically active taking up leucine, a percentage similar to that reported by del Giorgio & Gasol (2008) for the epipelagic waters. Hewson et al. (2006) reported considerable variability in bacterial composition among adjacent deep-water stations. The latter authors explained their findings as a result of episodic input of organic matter from the surface waters.

We also showed how the presence of island-eddies (presumably pumping organic matter to the dark ocean) produced an increase in the prokaryotic abundance and activity in the mesopelagic layer, but not in the bathypelagic (**Chapter V**). Besides, another interesting pattern related to the heterogeneity of the prokaryotic activity with depth was the presence of mesopelagic peaks of PA and bulk and single-cell activities (**Chapter V**). The presence of peaks in the mesopelagic layer has been reported before associated with water mass discontinuities (Sorokin 1971, Karl 1980, Carlucci et al. 1986) or being caused by lateral organic carbon supply (Nagata et al. 2000). Like observed for PA, peaks in heterotrophic production have been reported at density interfaces (Sorokin 1973). Karl (1980) described a generally higher activity at ca. 500-600 m depth than in the adjacent water layers.

In summary, we can conclude that the dark ocean is far from being a homogenous inactive ecosystem. The heterogeneity seems to be controlled by a "bottom-up" effect, where the organic matter distribution (particularly the suspended particulate organic carbon) modulates the prokaryotic activity. Furthermore, the dark ocean plays a key role in the carbon mineralization processes, with per-cell levels as high or even higher than in the epipelagic waters. Higher cell-specific EEA have been found in the dark ocean than in surface-waters (**Chapters III and IV**), and a range of

mesopelagic leucine-to-carbon conversion factors and prokaryotic growth efficiencies comparable to common oligotrophic surface waters (**Chapter VI**).

Preferential particle-attached life mode of dark-ocean prokaryotes

It is generally assumed that only less than 5% of the prokaryotes live associated with suspended and sinking particles in the dark ocean (Cho & Azam 1988, Turley & Mackie 1994). Nevertheless, a significant relation between suspended POC and respiration was found following a long-distance transect in the bathypelagic North Atlantic (**Chapter II**), suggesting a strong coupling between particles and prokaryotic metabolism. Furthermore, a very high proportion of dissolved EEA was present in the Central Atlantic (**Chapter IV**). Significant relations were also found between the dissolved EEA and the total fraction of EEA with suspended particulate organic matter and prokaryotic heterotrophic production, suggesting a strong link of prokaryotic metabolism to suspended organic matter in the dark ocean. As explained in **Chapter IV**, the high contribution of free EEA to total EEA might be interpreted as an indication of a particle-related prokaryotic life mode, taking into account the previous notion that the fraction of dissolved EEA is higher in particle-attached than in free-living microbes. Therefore, bearing in mind the strong relation between suspended POC and prokaryotic metabolism, we suggest that the actual percentage of attached prokaryotes may have been substantially underestimated in previous research in the deep ocean. This underestimation might be due to the very fragile nature of a significant portion of the bathypelagic particles, being disrupted during sampling. For instance, gels are abundant and important components of aggregates throughout the water column (Verdugo et al. 2004, 2008), spanning over a large size spectrum, from colloids to marine snow, and converting DOM to POM via self-assembly of polymers. Therefore, self-assembly of micro-gels may be a key mechanism of aggregate generation in the bathypelagic zone. However, disaggregation of these gels can easily occur during sampling. In any case, the strong relation found between suspended particulate organic matter and prokaryotic metabolism in the dark ocean suggests an association between suspended particles and prokaryotes stronger than assumed hitherto.

Beside the in situ activity-related associations we are reporting, other recent environmental genomic evidence also suggests a preferential particle-attached life mode for dark-ocean prokaryotes (DeLong et al. 2006, Martín-Cuadrado et al. 2007, Ivars-Martínez et al. 2008). In the North Pacific subtropical gyre, DeLong et al. (2006) found that deep-water prokaryotes are more enriched in genes for transposases, polysaccharide and antibiotic synthesis, indicating a potentially greater role of a particle-attached life style in dark-ocean prokaryotes than hitherto assumed. Also in the Mediterranean Sea, Martín-Cuadrado et al. (2007) found a high abundance of genes encoding transporters for dipeptides and oliopeptides and branched-chain amino acids, indicating that proteins (potentially associated with marine snow or colloidal particles) are important carbon sources for bathypelagic prokaryotes. Ivars-Martínez et al. (2008) reported differences in the genomic structure of two different ecotypes (one typical from surface and other from deep waters) of the common heterotrophic gammaproteobacterium *Alteromonas macleodii*. The latter authors found that the surface ecotype had a greater potential to utilize sugars and amino acids, whereas the deep-ocean ecotype harbored genes indicative of life under microaerophilic conditions, and for particle attachments. Overall, all these (meta)genomic studies also indicate that microbial life in the meso- and bathypelagic realm is more centered around particles than thought thus far.

Taking into account the low reactivity of the deep-waters DOM, prokaryotes might prefer to live attached to particles, where carbon and energy sources are available at higher concentrations than in the ambient water. Moreover, living in these “oases” surrounded by the oceanic desert of refractory dissolved OM might facilitate synergistic interactions in the cycling of matter in the dark ocean. Therefore, it is possible to conclude that the heterogeneous nature of deep-water microbes and their related activity seems to be governed by the presence and heterogeneity of colloidal and particulate matter distribution. Although the potential implications of such microzones have been mentioned before, most measurements do not take into account the potential non-random distribution of prokaryotes in the oceanic water column (Azam & Malfatti 2007). Therefore, new sampling techniques need to be developed in order to cope with the fragile nature of bathypelagic detrital matter and the apparently non-random distribution of prokaryotes in the dark ocean.

Significance of non-sinking particulate organic carbon and dark CO_2 fixation to heterotrophic carbon demand in the mesopelagic Atlantic

It is generally accepted that the deep-water heterotrophic food web relies on the organic matter (OM) generated by primary production in the sun-lit surface waters. On a global average, about 30% of the surface water's primary production is exported into the dark ocean as sedimenting particles (Arístegui et al. 2005a, Buesseler & Boyd 2009). Although the concentration of dissolved organic carbon (DOC) is generally about 10 times higher than that of particulate organic carbon (POC), the DOC pool only accounts for about 10% of the apparent oxygen utilization in mesopelagic waters (Arístegui et al. 2002). Hence, passively sinking particles originating from the euphotic zone are considered as the principal source of organic carbon available for the heterotrophic food web in the ocean's interior (Buesseler et al. 2007). The majority of the POC exported from the euphotic zone is remineralized in the mesopelagic layer (between 100-1000 m depth), leading to a typical exponential attenuation of the sinking POC concentration with depth (Martin et al. 1987). A correlation between the deep-water microbial activity and the sinking POC flux has been observed in the subarctic Pacific (Nagata et al. 2000). Also, in the Atlantic sector of the Southern Ocean, the prokaryotic production in the mesopelagic waters correlated with primary production and surface chlorophyll concentrations (Simon et al. 2004). In the Arabian Sea, prokaryotic abundance but not activity was related to the sedimenting POC flux, interpreted that deep-ocean prokaryotic abundance is not reflecting the most recent POC flux but, instead, being an indicator of the long-term average POC flux (Hansell & Ducklow 2003).

Assuming mass balance, the supply rate of organic carbon (generally calculated from sinking POC collected by sediment traps) reaching the ocean's interior should match the carbon demand of the heterotrophic biota inhabiting the meso- and bathypelagic realm. The deep-water biota is vastly dominated by prokaryotes, in terms of abundance and biomass. However, the prokaryotic organic carbon demand (determined as respiration plus production) has been shown to continuously exceed the POC flux into the dark ocean (Burd et al. in press). Recent estimates report a mismatch between deep-water prokaryotic carbon demand (PCD) and sinking POC flux by up to 2-3 orders of magnitude (Reinthaler et al. 2006, Steinberg et al. 2008, **Chapter II**). Moreover, a global budgeting exercise indicated that even the highest estimate of

sinking POC flux from surface waters would only explain about 50% of the measured oxygen consumption in the dark ocean (del Giorgio & Duarte 2002). This paradoxical imbalance has been shown to vary spatially. PCD was 3-4 times and around 10 times greater than the sinking POC flux in the subtropical and subarctic Pacific, respectively (Steinberg et al. 2008). This strong imbalance between POC supply and demand represents one of the great challenges in contemporary biological oceanography and marine biogeochemistry, and indicates major gaps in our understanding of the deep ocean carbon flux. It is apparent that some major components and aspects of organic carbon stocks and fluxes have not been taken into account adequately.

One of the major POC pools in the ocean's interior not adequately taken into account in the oceanic carbon budgets is suspended, non-sinking POC (nsPOC) collected with oceanographic bottles but, due to their buoyant nature, not accumulating in sediment traps used to determine sinking POC flux (Arístegui et al. 2009). In this section we use our data collated from the meso- and bathypelagic North Atlantic on sedimenting particulate organic matter (POM) flux, POM standing stock (thus including the resident nsPOC) and prokaryotic carbon demand (PCD) to re-evaluate the apparent discrepancy between prokaryotic organic matter demand and supply in the ocean's interior.

In contrast to the exponential decline in sinking POC with depth (Martin et al. 1987), the concentrations of nsPOC and nsPON remained fairly constant with depth down to bathypelagic waters (4000m depth) of the (sub)tropical North Atlantic (Fig. 1A, B). Generally, the prokaryotic contribution to the nsPOC and nsPON decreases with depth (Fig. 1C, D). Subtracting the prokaryotic contribution from the bulk nsPOC and nsPON pool, we obtain the amount potentially available for heterotrophic utilization of nsPOC (nsPOC_Av) and nsPON (nsPON_Av) (Fig. 1E, F). The amount of nsPOC and nsPON potentially available did not exhibit a general depth-related trend (Fig. 1E,F). The ratio nsPOC_Av : nsPON_Av was also fairly constant throughout the water column suggesting that there are no major shifts in the C:N ratio of nsPOM in deep-waters (Fig. 2), in contrast to the reported increasing C:N ratios with depth of sedimenting POM and DOM (Schneider et al. 2003, Hopkinson & Vallino 2005). This stability in the elemental composition of suspended POM, in combination with its fairly constant concentration throughout the (sub)tropical North Atlantic water column

suggests that it is either not utilized biotically or that its utilization by the deep-sea biota is matched by in situ production of nsPOM.

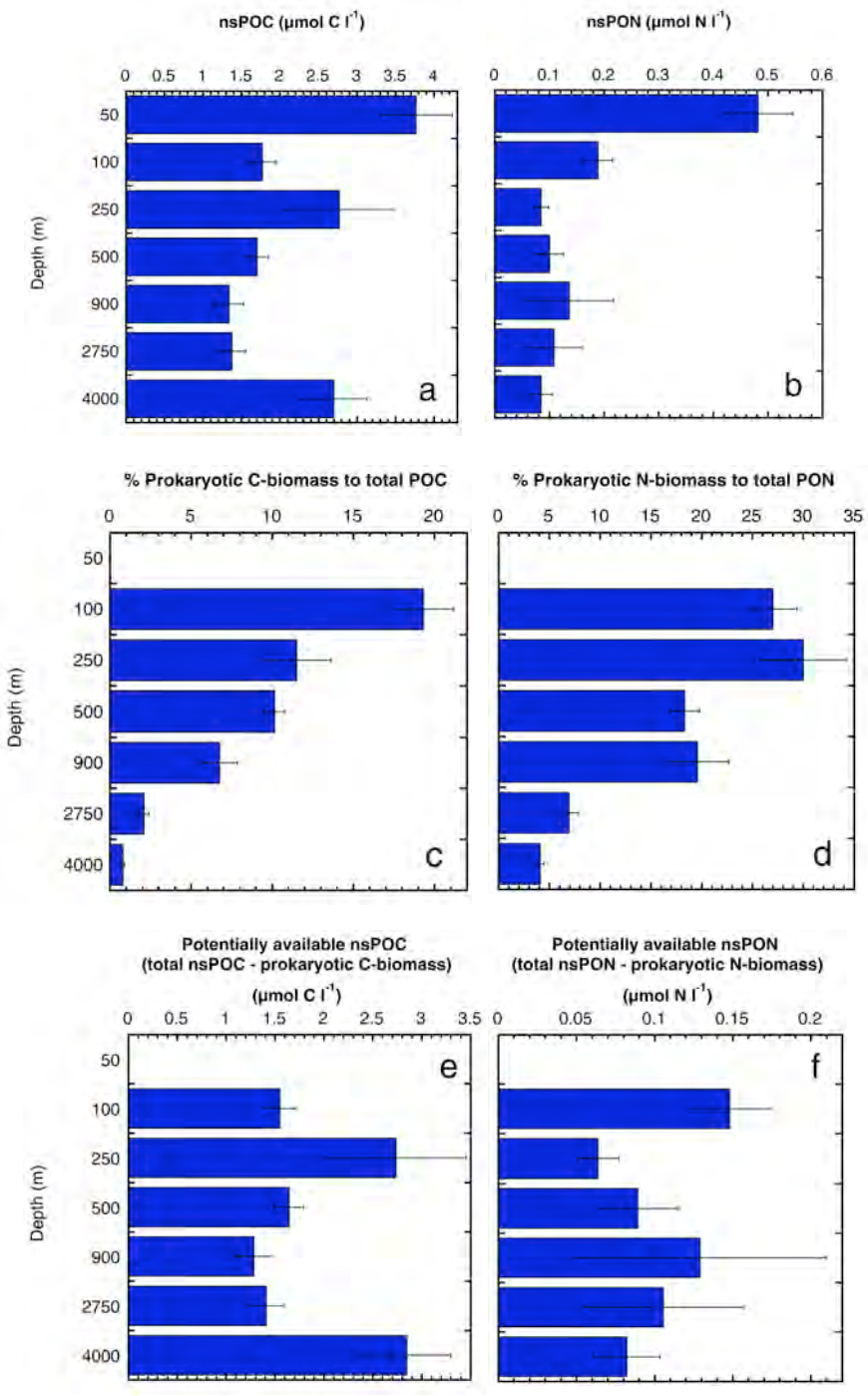


Fig. 1. Average (± SE, n = 22 – 48) vertical distribution of (A) bulk non-sinking particulate organic carbon (nsPOC) and (B) nitrogen (nsPON), contribution of the prokaryotic biomass to the bulk nsPOC (C) and nsPON (D), and potentially available nsPOC (E) and nsPON (F) after subtracting the prokaryotic C- and N-biomass, respectively. All organic matter concentrations are in µmol l^{-1}

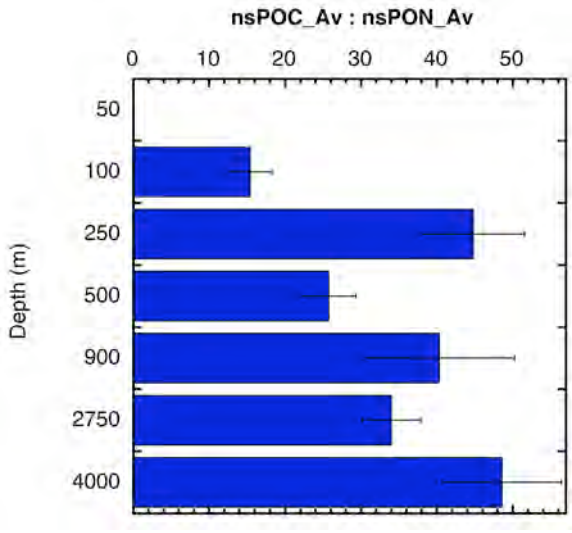


Fig. 2. Average (± SE, n = 20 – 46) vertical distribution of the ratio of potentially available nsPOC to nsPON in the core of the main water masses of the (sub)tropical NE Atlantic Ocean

Prokaryotic respiration (R) is the main parameter affecting the prokaryotic carbon demand (PCD) in the deep Atlantic, as it is typically 1-2 orders of magnitude higher than prokaryotic heterotrophic production (Reinthaler et al. 2006, **Chapter II**). Therefore, our PCD estimates greatly rely on the conversion of measurements of the electron transport system (ETS) to R, i.e., the R:ETS ratio used. Our PCD estimates were bracketed based on an R:ETS ratio of 0.086 and 0.6, derived from bacterial cultures in senescent phase (Christensen et al. 1980) and active mesopelagic prokaryotes of boundary regions (Arístegui et al. 2005b), respectively. The PCD decreased by one order of magnitude from the base of the euphotic zone towards the bathypelagic zone independent of the R:ETS ratio used (Table 1). The oxygen utilization rates (OUR) reported for the North and South Atlantic (Jenkins 1982, Jenkins & Wallace 1992, Brea 2008) are within the range of our PCD estimates (Table 1), suggesting that our PCD estimates are realistic. Based on the PCD and the concentration of nsPOC_Av, the turnover time (nsPOC_Av/PCD) of nsPOC_Av can be calculated. The turnover time of the nsPOC_Av increases from near-surface (9 d and 34 d using an R:ETS of 0.6 and 0.086, respectively) to bathypelagic waters (200 d and 1372 d) (Fig. 3A). In other terms, PCD potentially utilizes between 6-0.2% d^{-1} of the nsPOC_Av pool in the 100 m and 4000 m layer, respectively (using an R:ETS of 0.086) and 34-6% d^{-1} (using an R:ETS of 0.6) (Fig. 3B). Even assuming the high R:ETS ratio (R:ETS = 0.6), on average only 8 ± 3% of the present nsPOC_Av is used per day in the mesopelagic realm (Fig. 3B). Hence, in order to maintain steady state concentrations of buoyant nsPOC in the mesopelagic Atlantic waters, the loss of less than 8% in nsPOC daily

needs to be compensated. From where could this nsPOC pool be replenished in the mesopelagic waters?

Table 1. Comparison of the variation in the different depth layers of the prokaryotic carbon demand (PCD) assuming an R:ETS ratio of 0.086 and 0.6, oxygen utilization rates (OUR) reported for the North Atlantic Subtropical Gyre (OUR NAtl) (Jenkins 1982), and for the South Atlantic Subtropical Gyre (OUR SAtl) (Brea 2008), and dark DIC fixation rates (autotrophic production - AP). Metabolic rates in µmol C m^{-3} d^{-1}

Depth (m)	PCD (R:ETS=0.086)	PCD (R:ETS=0.6)	OUR NAtl	OUR SAtl	AP
100	58.9	353.4			41.3
250	18.3	109.5	36.5	26.4	32.4
500	11.9	71.5	14.7	16.8	3.7
900	4.2	25.2	6.5	12.3	0.3
2750	3.3	19.9			0.2
4000	4.7	28.5			0.3

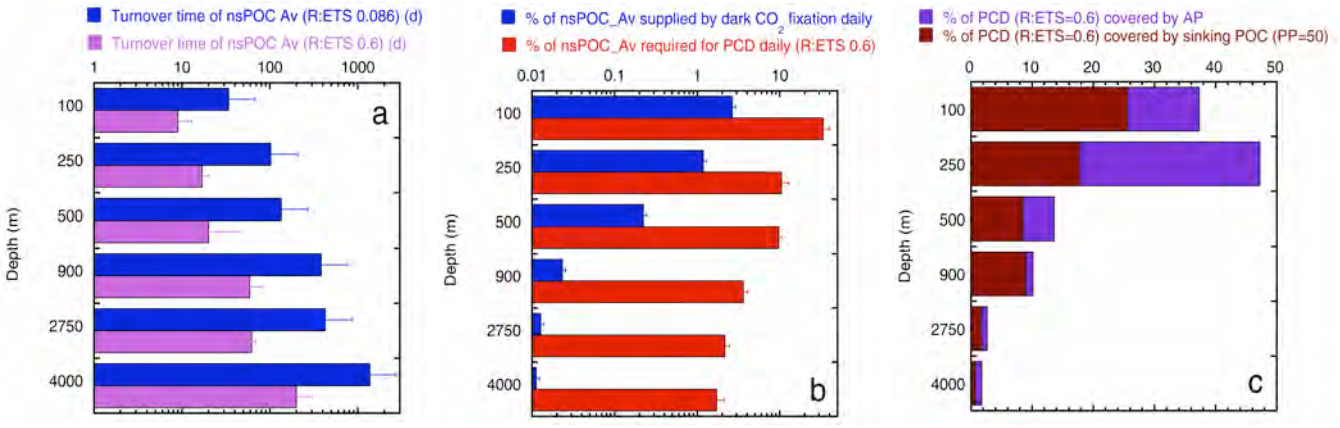


Fig. 3. Variation of (A) the mean (± SE, n= 18 – 39) turnover time of the potentially available suspended nsPOC (POC_Av) (in d) with depth (assuming a R:ETS ratio of 0.086 and 0.6), (B) the percentage of this nsPOC_Av required to meet the daily prokaryotic carbon demand (PCD) (using a conservative R:ETS of 0.6) and percentage of daily dark DIC fixation contributed to the POC_Av, and (C) the percentage of total PCD covered by dark CO_2 fixation (autotrophic production-AP) and by sinking POC (assuming a conservative R:ETS of 0.6 and surface primary production of 50 mmol C m^{-2} d^{-1})

Dissolved inorganic carbon (DIC) fixation by chemoautotrophic microbes occurs throughout the deep waters of the Atlantic (Herndl et al. 2005) amounting, on average 12 ± 5 µmol C m^{-3} d^{-1} in the mesopelagic realm of the (sub)tropical North

Atlantic (Table 1). This freshly produced organic carbon represents a nsPOC source in the meso- and bathypelagic waters, as microbial cells are too small to sediment. This autochthonously produced nsPOC potentially accounts for 72% to 12% (assuming an R:ETS of 0.086 and 0.6, respectively) of the required nsPOC to meet the daily heterotrophic prokaryotic carbon demand in the (sub)tropical North Atlantic's mesopelagic realm (Table 2, Fig. 3B).

Table 2. Potential contribution (in %) of dark CO_2 fixation (AP) and of the available sinking POC to the PCD (assuming an R:ETS of 0.086 and 0.6). The contribution from the sinking POC was inferred from a POC flux model (Antia et al. 2001) assuming a surface primary production (PP) of 28 mmol C m^{-2} d^{-1} (NE Atlantic gyre (Longhurst et al. 1995)), 35 mmol C m^{-2} d^{-1} (western gyre (Steinberg et al. 2001)) and of 50 mmol C m^{-2} d^{-1} (N Atlantic (Longhurst et al. 1995))

% contribution to PCD assuming R:ETS=0.086				
Depth	**AP**	**Sinking POC (PP=28)**	**Sinking POC (PP=35)**	**Sinking POC (PP=50)**
100	70	55	82	154
250	177	38	57	106
500	31	18	27	51
900	7	19	29	54
2750	5	4	6	10
4000	7	1	2	4
mesop avg	**72**	**25**	**37**	**70**
bathy avg	**6**	**3**	**4**	**7**
Mesop AP: Sinking ratio		2.8	1.9	1.0
Bathyp AP: Sinking ratio		2.4	1.6	0.9
% contribution to PCD assuming R:ETS=0.6				
Depth	**AP**	**Sinking POC (PP=28)**	**Sinking POC (PP=35)**	**Sinking POC (PP=50)**
100	12	9	14	26
250	30	6	9	18
500	5	3	5	8
900	1	3	5	9
2750	1	1	1	2
4000	1	0	0	1
mesop avg	**12**	**4**	**6**	**12**
bathy avg	**1**	**0**	**1**	**1**
Mesop AP: Sinking ratio		2.8	1.9	1.0
Bathyp AP: Sinking ratio		2.4	1.6	0.9

For comparison, the sinking POC flux based upon a model from a compilation of sediment trap data of the North Atlantic (Antia et al. 2001) was used to estimate the potential contribution of the sinking POC to nsPOC_Av. We calculated the sinking POC using the surface primary production (PP) estimates from the NE Atlantic gyre (Longhurst et al. 1995) (28 mmol C m^{-2} d^{-1}), from the western gyre (Steinberg et al. 2001) (35 mmol C m^{-2} d^{-1}) and as an upper limit 50 mmol C m^{-2} d^{-1} (for the entire North Atlantic (Longhurst et al. 1995) including temperate regions where PP is higher). The potential contribution of the sinking POC amounts to 4-6% to the PCD per day when using a surface PP of 28 and 35 mmol C m^{-2} d^{-1}, respectively (Table 2). Only when using the unrealistically high surface PP of 50 mmol C m^{-2} d^{-1} for this (sub)tropical mid-oceanic region, the contribution of the sinking POC to the mesopelagic PCD equals the contribution (12%) from the dark CO_2 fixation (Fig.3C, Table 2). Despite the rather low DIC fixation rates in the bathypelagic realm, even in this layer the contribution of the sinking POC to the PCD does not exceed the contribution derived from DIC fixation (Fig. 3C, Table 2). Thus, a significant fraction (at least similar to the sinking POC contribution) of the organic carbon required by the meso- and bathypelagic microbial community could be supplied by chemoautotrophic CO_2 fixation in the (sub)tropical North Atlantic's interior.

As shown previously, the major energy source for prokaryotic DIC fixation is likely derived from ammonia oxidation by *Crenarchaeota*, as archaeal *amo*A genes are by far more abundant than bacterial *amo*A genes in the mesopelagic realm of the North Atlantic (Agogué et al. 2008). Based on the radiocarbon signature of archaeal lipids, it has been shown recently that 83% of the archaeal carbon is derived by autotrophy in the subtropical mesopelagic North Pacific (at 670 m depth) (Ingalls et al. 2006). The radiocarbon signature of archaeal DNA collected from mesopelagic Pacific waters (670 – 915 m) allowed to differentiate between the three major carbon pools that are potentially available to prokaryotes: fresh DOC released from POC ($\Delta^{14}C$ > +50‰), ambient DIC ($\Delta^{14}C$ ~ -200 to -100 ‰), and aged bulk DOC ($\Delta^{14}C$ = -525‰) (Hansman et al. 2009). These authors concluded that both DIC and fresh DOC (presumably released from sinking POC) are utilized substantially, while ambient DOC is not a major substrate for mesopelagic prokaryotes (Hansman et al. 2009). There is also evidence of considerable chemolithotrophic production on sinking POC collected by sediment traps deployed in the mesopelagic (100-750 m depth) North Pacific (Karl et al.

1984). There, chemolithotrophic contribution ranged between 7-90% of the total microbial production, again indicating the potential importance of chemolithotrophic carbon production as a source of newly available carbon and energy in the mesopelagic realm.

Taken together, the current perception on the dependence of the dark ocean's heterotrophic microbial activity on sedimenting POC and the resulting mismatch between organic carbon supply and demand (Reinthaler et al. 2006, Steinberg et al. 2008, Burd et al. in press, **Chapter II**) needs revision. The large and fairly constant stock of buoyant, nsPOC needs to be considered as a potentially available pool of POC for heterotrophic deep-water microbes. We have shown that only a small proportion of the available suspended nsPOC is required per day to sustain the carbon demands of deep-ocean heterotrophic microbes. About 12-72% of the amount of nsPOC required by microheterotrophs in the mesopelagic ocean is potentially supplied by deep-water chemolithotrophs via DIC fixation. Therefore, the apparent gap between heterotrophic organic matter demand and supply in the mesopelagic realm might be substantially smaller than previously thought. Future research should focus on refining the nutritive quality of buoyant nsPOC versus sedimenting POC and, on a more refined view of chemolithoautotrophic production of non-sinking new organic carbon in the ocean's interior. The refined pathways of organic matter supply and demand in the dark ocean might allow us to arrive at a mechanistic understanding of deep ocean food web structure and activity and ultimately, should lead to improved models on the dark ocean's role in global carbon cycling.

CONCLUSIONS

The main conclusions that arise from this thesis are:

1. A high variability in prokaryotic assemblage structure and activity exists in the surface and deep waters along the NW Africa – Canary Islands Coastal Transition Zone, suggesting a heterogeneous nature of the dark ocean.

    a. There was a clear substitution of *Bacteria* (mainly SAR11) for *Archaea* (mainly Crenarchaeota) along trophic gradients (offshore and in depth).

    b. Different deep-water masses exert a key role in structuring the prokaryotic metabolism. An example was found in the intermediate water masses, with lower leucine incorporation rates in layers where patches of Antarctic Intermediate Waters were detected.

2. A strong relation was found between the suspended particulate organic carbon and the prokaryotic respiration in the dark realm of the (sub)tropical North Atlantic. This finding, together with the strong imbalance between the prokaryotic carbon demand and the sinking particulate organic carbon (POC) flux, suggests: i) a "bottom up" control of the deep-ocean prokaryotic metabolism by the suspended particulate organic matter pool, and ii) a preferentially particle- attached life mode of dark-ocean prokaryotes.

3. An increase in the cell-specific extracellular enzymatic activity (EEA) was found from the lower euphotic layer to the bathypelagic waters of the (sub)tropical North Atlantic.

    a. The increase in cell-specific EEA with depth was paralleled by an increase in cell-specific respiration and a decrease in cell-specific leucine incorporation. This finding, together with the increase in the half saturation constant (K_m) of the ectoenzymes and in the cell-specific V_{max} / K_m ratio, suggests an adaptation of the EEA to the refractory nature of the dark-ocean organic matter.

   b. An increase with depth was found in the cell-specific alkaline-phosphatase (APase) activity hence, not related to phosphate availability in the dark ocean. In fact, APase seems to be utilized by deep-water prokaryotes to access organic C moieties from organic matter, rather than phosphate directly.

4. EEA exists mainly in dissolved form in the deep waters of the Central Atlantic Ocean, where a relation was found between the EEA (dissolved and total fractions) and prokaryotic heterotrophic production and the particulate organic matter pool. These findings suggest again a preferential particle-attached life style of dark-ocean prokaryotes.

5. Mesoscale island-induced eddies have a profound impact on the distribution and function of prokaryotes in the ocean. However, even in these mesoscale features characterized by higher productivity in the surface-water than outside the eddies, the effect on the prokaryotes' distribution and function was restricted to the epi- and mesopelagic. This is probably due to the consumption of the major part of sinking POC in the top 1000 m water column, suggesting a rapid attenuation of the influence of the sinking POC on the bathypelagic microbes.

6. Like other metabolic parameters determined in this thesis, the prokaryotic growth efficiency (PGE) and the empirically obtained leucine-to-carbon conversion factor (LeuCF) showed a strong variability in the mesopelagic waters of the Northeast Atlantic Ocean, exhibiting similar ranges to those reported for surface waters of oligotrophic regions.

7. The suspended particulate organic carbon and the dark fixation of dissolved inorganic carbon by deep-water prokaryotes can help to reduce the imbalance between the organic carbon required by microbes and the supply of organic matter to the dark ocean. Assuming a conservative high prokaryotic carbon demand (using a R : ETS of 0.6), the consumption of less than 8% of the non-sinking POC pool per day is required to meet the heterotrophic microbial carbon demand in the mesopelagic waters. Our numbers also indicates that the

mesopelagic dark fixation of dissolved inorganic carbon (DIC) can supply 12% to 72% of this daily required organic carbon, whereas the sinking POC could only account for 4-12%. Thus, the actual imbalance between organic carbon supply and demand is not as strong as assumed hitherto due to the thus far uncounted dark ocean's autochthonous production of particulate organic matter.

FUTURE RESEARCH

From the work reported in this thesis, there are several issues that need to be resolved in the future regarding the dark-ocean microbial oceanography.

1. The main target should be the relation between particles and deep-water prokaryotes. Several lines of evidence point towards a particle-attached life style of prokaryotes (DeLong et al. 2006, **Chapter II**, **Chapter IV**). The recently recognized abundance of gels in the oceans might allow spatially close interaction between prokaryotes and the organic matter continuum. However, with the currently available sampling techniques it is not possible to determine the extent of this interaction due to the fragile nature of these dark-ocean particles. New sampling techniques are needed to distinguish between really free-living and the particle-associated microbes of the dark ocean.

2. Conversion factors are needed in order to better constrain the carbon fluxes mediated by prokaryotes in the dark ocean. We have reported the first prokaryotic growth efficiencies (PGE) calculated with empirically obtained leucine-to-carbon conversion factor (LeuCF) for the dark ocean (**Chapter VI**). Due to the high variability found in both parameters (PGE and LeuCF), and the important role that the dark ocean plays in the global carbon fluxes, it is imperative to greatly increase the number of determinations of the conversion factor. Only then, we will be able to more accurately determine the prokaryotic metabolism and thereby, better constrain the global carbon biogeochemical cycle.

3. More efforts should also be directed to study the effect of hydrostatic pressure on the metabolic rates of prokaryotes inhabiting the dark ocean, because contradictory results have been reported thus far (Jannasch & Wirsen 1982, Tamburini et al. 2002). This pressure effect should be accounted for not only in the routine metabolic rate measurements but also in the determinations of the conversion factors involved.

4. Although it was not the main topic of this thesis, more effort should also be diverted to studying the microbial food webs of the dark ocean. There are very

few reports concerning the consumption and lysis rates of prokaryotes by heterotrophic nanoflagellates or viruses, respectively. It is very likely that the interactions between the individual components of microbial food web and the dark-ocean particulate and colloidal material are very different from those of surface-water assemblages. These interactions are crucial to model the nutrient and carbon cycling in the dark ocean.

5. A better budgeting of the sources and sinks of carbon in the dark ocean is required to better understand the global carbon fluxes. Maybe some of the issues raised in this section may help to close this gap (e.g. pressure effect on metabolic rates, microbial life concentrated on particles, different conversion factors, novel metabolic pathways,…). A more refined view of chemolithoautotrophic production of non-sinking new organic carbon in the ocean's interior would be recommended. Anyway, it would certainly be a topic of paramount importance to try to close the apparent imbalance between the microbial carbon demand and the organic matter supply in the meso- and bathypelagic realms.

6. A general increase in the database of the function and diversity parameters should be achieved to allow for a refined view on the large-scale spatial trends in the regulation of deep-water microbial processes. One option might be to follow Longhurst's model of biogeographical provinces, defining the different compartments that form the dark ocean.

7. It should also be recommended to better link the recent advances in the molecular approaches to actual fluxes measurements. Connecting diversity to function, relating the genetic information with the deep-ocean biogeochemical cycles. Finally, the developments in molecular techniques should also be used to study the adaptations of several members of the community to the biotic (e.g. mortality…) and abiotic (e.g. pressure, temperature, nutrients…) conditions of the dark ocean.

SPANISH SUMMARY

RESUMEN EN ESPAÑOL

INTRODUCCIÓN GENERAL

"Las bacterias representan la historia vital de mayor éxito del mundo. Ellas son hoy, y han sido siempre los organismos más importantes en la Tierra; no pueden ser llevadas al olvido y nos sobrevivirán a todos nosotros. Este es su momento, no la "era de los mamíferos" como nuestros libros de textos proclaman de un modo chovinista. Pero el precio que pagan por dicho éxito es una relegación permanente a un micromundo, y no pueden conocer el gusto y el dolor de la conciencia. Vivimos en un universo de compensaciones; la complejidad y la persistencia no trabajan bien como compañeras."

Stephen Jay Gould, "An Earful of Jaw", *Eight Little Piggies: Reflections in Natural History* (1993).

Papel de los procariotas en el ecosistema oceánico

Los procariotas juegan un papel clave en la ecología de los océanos, mediando la mayoría de los ciclos biogeoquímicos, y actuando como un eslabón trófico básico a través del bucle microbiano (Pomeroy 1974, Williams 1981, Azam et al. 1983). Ellos son el principal grupo responsable de convertir la materia orgánica disuelta (MOD) a materia orgánica particulada (MOP) viva; supliendo de este modo, con materia orgánica, que sería inaccesible de otra forma, a los niveles tróficos superiores de la cadena alimenticia. Este concepto de "bucle microbiano" ha sido redefinido durante las últimas décadas (Azam 1998, Fuhrman 1999), incorporando entre otras, las ideas de que los procariotas pueden competir con el fitoplancton eucariota por los nutrientes inorgánicos (Obernosterer & Herndl 1995, Thingstad 2000), consumir la mitad o más de la producción primaria (Ducklow 2000, Robinson 2008), sufrir lisis por mediación de virus (Fuhrman 2000, Breitbart et al. 2008) y ser consumidos por protistas (Jurgens & Massana 2008). Por ello, hoy en día se acepta que el bucle microbiano ejerce una influencia primordial en la distribución de los flujos de carbono y nutrientes en el océano (Nagata 2008). Dicha noción se ha basado en una serie de avances tecnológicos.

Inicialmente, el número de procariotas en los sistemas marinos estaba subestimado severamente. Las primeras estimas de abundancia de procariotas se basaban en el número de células capaces de crecer en placas de agar (e. g. Zobell 1946); una técnica que, como fue luego demostrado, subestimaba la abundancia *in situ* de procariotas en varios órdenes de magnitud (Jannasch & Jones 1959). En los años 70, después del desarrollo de las técnicas de microscopía de fluorescencia, se encontró que la abundancia de procariotas en las aguas superficiales rondaba los 10^5 - 10^6 ml^{-1} (Francisco et al. 1973, Hobbie et al. 1977, Porter & Feig 1980). Esta discrepancia entre los resultados obtenidos mediante el uso de placas y la microscopía fue posteriormente denominada como la "Gran anomalía de contaje en placa" (Staley & Konopka 1985). Además, la mayoría de los descubrimientos realizados concernientes al bucle microbiano fueron efectuados usando una aproximación de "caja-negra"; esto es, sin tener en cuenta los distintos grupos filogenéticos y funcionales que se encontraban en los componentes individuales de la comunidad microbiana. Una vez más, el desarrollo de técnicas (en esta ocasión de metodologías moleculares) a principio de los años 90 nos permitió empezar a responder a preguntas como quién y dónde está haciendo el qué en el océano.

Debido a este papel fundamental que juegan los procariotas en el océano, siendo responsables de hasta >90% de la respiración comunitaria (Robinson & Williams 2005), la estimación de sus actividades metabólicas se tornan como un imperativo básico si queremos estudiar los flujos de carbono. De hecho, la cantidad de carbono orgánico final que fluye a través de los procariotas hacia niveles tróficos superiores y/o la concentración de carbono respirada en el océano dependerá en gran medida de la eficiencia de crecimiento procariota (ECP) — la proporción de carbono orgánico incorporado como biomasa (producción) comparada con la incorporación total de carbono (producción más respiración) (Carlson et al. 2007). Sin embargo, obtener las tasas metabólicas involucradas en la estimación de la ECP no es tarea simple. Debido a las dificultades relacionadas con la medida de la respiración procariota (RP), la ECP es calculada frecuentemente midiendo la producción heterotrófica procariota (PHP) a través de la incorporación de leucina o timidina aplicando un factor de conversión obtenido empíricamente para convertir la incorporación de leucina o timidina a producción de carbono (del Giorgio & Cole 1998). Sin embargo, este factor de

conversión (LeuFC) de leucina a carbono no es constante, pudiendo variar hasta en un orden de magnitud dentro de un mismo sistema (e. g. Sherry et al. 2002, Alonso-Sáez et al. 2007). Consecuentemente, se suele recomendar estimar empíricamente los LeuFCs para una mejor cuantificación de los flujos de carbono (Bell 1990, Pulido-Villena & Reche 2003, Buesing & Marxsen 2005, Calvo-Díaz & Morán 2009). Finalmente, la eficiencia de convertir MOD a producción de biomasa o remineralizarla dependerá de la calidad de la materia orgánica y del balance estequiométrico entre el carbono, nitrógeno y fósforo disponible (Goldman et al, 1987, Obernosterer & Herndl 1995, del Giorgio & Cole 1998, 2000). Por ello, para entender mejor y poder predecir el efecto de la variabilidad metabólica de los procariotas en el ecosistema, es necesario relacionar dicha variabilidad metabólica con las variaciones ambientales (e. g. pool de materia orgánica) y con la composición de la comunidad procariota, intentando así llegar a un conocimiento mecanicista del bucle microbiano en el océano.

Principales fuentes de carbono para los procariotas en el océano

El reservorio de materia orgánica en el océano está divido funcionalmente en dos compartimentos (disuelto y particulado), aunque representan reservorios conectados entre sí (Simon et al. 2002, Engel et al. 2004, Verdugo et al. 2004). Se considera materia orgánica particulada (MOP) cuando puede ser retenida en filtros con un tamaño de poro de entre 0.2-0.7 μm. El reservorio de MOP se subdivide a su vez en dos tipos de partículas atendiendo a sus propiedades de hundimiento: flotantes y partículas que se hunden. La mayor parte de la MOP está comúnmente formada por partículas suspendidas pequeñas, con flotabilidad neutra (Kepkay 2000, Verdugo et al. 2004). Estas partículas suspendidas son generalmente más lábiles que las partículas que se hunden recogidas a las mismas profundidades (Repeta & Gagosian 1984, Wakeham & Canuel 1988, Druffel et al. 1998). Además, geles mayores (i. e. de varios cientos de μm o mayores) pueden formarse a partir de la unión de macromoléculas o de material coloidal, transformando moléculas orgánicas a través del espectro de tamaños hasta convertirlas en partículas que se hunden (Verdugo et al. 2004, Engel et al. 2004). Las partículas exopoliméricas transparentes (PET; Alldredge et al. 1993, Passow 2002) y

otros macrogeles son los principales responsables de la formación de los grandes agregados (> 500 μm) que se hunden ("nieve marina") (Suzuki & Kato 1953, Alldredge & Silver 1988, Alldredge & Gotschalk 1988, Thornton 2002). Se ha demostrado que estas grandes partículas albergan un micrcoambiente complejo dando cobijo a una comunidad microbiana particularmente activa (Alldredge et al. 1986, Kaltenböck & Herndl 1992, Müller-Niklas et al. 1994).

La MOD se genera fundamentalmente por las exudaciones extracelulares del fitoplancton y por procesos de pastaje (e infecciones víricas), ya sea directamente sobre el fitoplancton ("sloppy feeding") o dentro de la cadena trófica microbiana (Nagata 2000). Además, los procariotas heterotróficos también liberan copiosas cantidades de MOD a través de la envoltura de polisacáridos que rodea las células (Heissenberger & Herndl, 1994; Heissenberger et al., 1996; Stoderegger & Herndl, 1998). Sin embargo, no todo esta MOD es accesible para los procariotas debido a que generalmente está compuesta de una mezcla de MOD muy vieja y refractaria y una fracción menor formada por MOD jóven y bio-disponible (Benner 2002). La mayor parte de la MOD producida en los procesos de pastaje y de infecciones víricas se libera como moléculas de alto peso molecular (>1000 Da). También el fitoplancton senescente libera fundamentalmente MOD de alto peso molecular (Lignell 1990). La concentración de azúcares libres y aminoácidos es generalmente baja (<10% del total del carbono orgánico), mientras que las formas combinadas (i. e. oligómeros y polímeros) son más comunes. Además, los procariotas utilizan preferentemente MOD de alto peso molecular, debido a su mayor bio-reactividad comparada con la mayoría de la MOD de bajo peso molecular (Amon & Benner 1994, Benner 2002). Sin embargo, los procariotas son capaces de asimilar directamente sólo compuestos de bajo peso molecular (i. e. monómeros y oligómeros). Para poder utilizar substratos de alto peso molecular tienen que segmentarlos antes de incorporarlos, porque sólo las moléculas <600 Da pueden ser transportadas a través de la pared celular de los procariotas (Weiss et al. 1991). Hoppe et al. (2002) demostró que la hidrólisis ectoenzimática de MOD se encuentra íntimamente ligada a la incorporación del correspondiente oligómero o monómero. Consecuentemente, la medida del tipo y de la cantidad de actividad enzimática

extracelular (AEE) en el océano sirve como un indicador muy valioso de la utilización de substratos y para estimar la regulación del flujo de carbono a través de los procariotas.

Procariotas en el océano oscuro: el hábitat mayor, pero menos conocido, de la biosfera

Aunque es el habitat acuático menos estudiado del planeta, el océano oscuro (>200 m de profundidad) es un "plusmarquista" de entre los habitats de la Tierra. Es el mayor habitat (~ 1.3 x 10^{18} m^3) y la mayor reserva de carbono orgánico activo (fundamentalmente en la forma de carbono orgánico disuelto) en la biosfera (Libes 1992, Hansell & Carlson 1998, Benner 2002), conteniendo también más del 98% del reservorio global de carbono inorgánico disuelto (COD) (Gruber et al. 2004). El océano profundo también contiene el mayor reservorio de microbios de los sistemas acuáticos (Whitman et al. 1998), albergando cerca del 75% y 50% de la biomasa y de la producción procariota, respectivamente, del océano global (ver Arístegui et al. 2009 para una revisión).

Sin embargo, generalmente la abundancia de procariotas (AP) y su producción de biomasa disminuyen exponencialmente en profundidad alrededor de 2-3 órdenes de magnitud (Reinthaler et al. 2006, Arístegui et al. 2009). A pesar de que ese decremento en la AP existe, la riqueza de la comunidad procariota disminuye sólo alrededor de un 30% desde la capa epipelágica hasta la zona batipelágica (Moeseneder et al. 2001a, Hewson et al. 2006). Otra característica típica de los procariotas del océano profundo es la estratificación de dicha comunidad que tiene lugar en las distintas masas de agua (DeLong et al. 2006); la cual ha sido atribuida a cambios en la materia orgánica que ocurren en profundidad (Moeseneder et al. 2001a, DeLong et al. 2006), y a adaptaciones a la presión y a la temperatura (Vezzi et al. 2005, Lauro et al. 2006). La contribución relativa de *Bacterias* a la AP total disminuye en profundidad, mientras que la de las *Crenarchaeas* aumenta (Karner et al. 2001, Moeseneder 2001b, Teira et al. 2006). Hay evidencias recientes que sugieren que una fracción substancial de las *Crenarchaeas* utilizan CO_2 como fuente de carbono, y amonio como donador de energía, a través de la

oxidación de amonio (Herndl et al. 2005, Könneke et al. 2005, Ingalls et al. 2006, Wutcher et al. 2006). Sin embargo, la comunidad de *Crenarchaeas* batipelágicas parece ser fundamentalmente heterotrófica (Teira et al. 2006, Kirchman et al. 2007). En cualquier caso, se ha estimado que la fijación de CO_2 que llevan a cabo las *Crenarchaeas* del océano profundo se encuentra en torno a 1 mmol C m^{-2} d^{-1} en el Atlántico Norte (Herndl et al. 2005). Este es un suplemento substancial al flujo de carbono de la materia orgánica generada en aguas superficiales, aportando materia orgánica producida recientemente, con un valor nutricional mayor que la materia orgánica refractaria típica de aguas profundas. Consecuentemente, esta fijación de CO_2 ha sido considerada como la "producción primaria del océano oscuro" (Herndl et al. 2008). Pero, *¿cuál es el papel de esta nueva fuente de materia orgánica profunda en la red trófica del océano oscuro?*

Generalmente se asume que el reservorio de MOD no es el que mantiene el metabolismo de los procariotas del océano oscuro. La fracción disuelta de la materia orgánica se hace más refractaria en profundidad, provocando un incremento sucesivo en los ratios COD:NOD:FOD (Benner 2002). Por ello, se supone que la mayor parte del reservorio de MOD del océano oscuro está compuesto por productos de degradación, de bajo peso molecular y ricos en carbono, provenientes de la remineralización de materia orgánica (Benner 2002). De hecho, aunque la mayoría de la MOD del océano profundo sigue sin haberse podido caracterizar a nivel molecular, se ha demostrado que alrededor del 25% del reservorio de carbono detrítico (disuelto y particulado), y aproximadamente un 50% del reservorio de nitrógeno detrítico de la MOD de aguas profundas, son de origen procariota (Kaiser & Benner 2008). Entonces, *¿cómo afecta el cambio que se produce en la cantidad y en la calidad de la materia orgánica (MO) en profundidad en el metabolismo (respiración, producción, AEE, ECP) de los procariotas del océano profundo? ¿A caso dichas diferencias en la composición de MO generan variaciones en los factores de conversión de carbono a leucina (LeuFC) que se utilizan para la estimación de la producción heterotrófica de biomasa de los procariotas?*

Aunque la MOD del océano profundo sea de naturaleza refractaria, se asume que más del 95% de los procariotas viven como organismos libres en el océano oscuro, mientras que se piensa que la fracción restante vive asociada con partículas, ya sea

suspendidas o con tendencia a hundirse (Cho & Azam 1988, Turley & Mackie 1994). Sin embargo, algunos datos recientes de genómica ambiental ponen a prueba esta hipótesis de "vida libre" y sugieren un modo de vida preferentemente pegado a partículas para los procariotas del océano oscuro (López-López et al. 2005, DeLong et al. 2006, Martín-Cuadrado et al. 2007). En cualquier caso, la cuantificación de este material particulado no es nada sencilla, probablemente porque alteramos parte de este material coloidal mientras muestreamos con las técnicas rutinariamente empleadas. Son necesarias evidencias directas que puedan apoyar esta hipótesis de un estilo de vida predominantemente asociado a partículas para los procariotas del océano oscuro. *¿Existe alguna relación directa entre las partículas suspendidas y el metabolismo de los procariotas en el océano batipelágico?*

Actualmente se acepta que la fuente de materia orgánica disuelta requerida para mantener el metabolismo de los procariotas del océano oscuro no proviene de MOD exportada directamente de las aguas superficiales, sino que se origina a partir de la transformación y solubilización de partículas. Arístegui et al. (2002) estimó que el COD residente podía explicar solamente entre un 10-20% de la utilización aparente de oxígeno global del océano oscuro. Kart et al. (1988) postularon la paradoja de la "descomposición de partículas" basadas en la noción de que los procariotas asociados a partículas solubilizan una cantidad de COP mayor de la que requieren para sostener su propio crecimiento, liberando así MOD a las aguas circundantes. Coincidiendo con esta paradoja, Cho & Azam (1998) y Smith et al. (1992) encontraron que los procariotas que viven asociados a la nieve marina presentan una alta AEE, transformando MOP en MOD con una tasa superior a la propia incorporación del substrato resultante. Este desacople entre la solubilización y la incorporación ha sido postulado como un mecanismo bioquímico clave para la transferencia a gran escala de MOP que se hunde a materia orgánica disuelta (Smith et al. 1992). Por ello, la AEE tiene de nuevo un rol crucial; ya que es justamente el paso intermedio, "conectando" el reservorio de materia orgánica con el metabolismo de los procariotas del océano oscuro. Teniendo en consideración la tendencia de los procariotas que viven asociados a partículas a liberar enzimas extracelulares a los alrededores (Smith et al. 1992), es posible sugerir que el

hecho de encontrar una alta proporción de AEE disuelta podría ser indicativo de un posible modo de vida pegado a partículas. Debido a ello se puede proponer la siguiente cuestión: *¿Existe una alta proporción de AAE libre en el océano oscuro? ¿Acaso esta AAE disuelta está relacionada con el pool de MOP suspendida?*

Poco se conoce sobre como responden los procariota del océano profundo a los cambios en la composición, aporte y distribución de carbono orgánico (Azam 1998). Está generalmente aceptado que la mayor parte del carbono orgánico es generado en la zona eufótica y posteriormente transportado hacia aguas profundas, aunque la mayor parte (>90% del flujo exportado) es respirado en la capa mesopelágica (Arístegui et al. 2005a). De hecho, un tercio de la respiración biológica de la MO oceánica tiene lugar en el océano oscuro (del Giorgio & Duarte 2002, Arístegui et al 2005a). Sin embargo, cálculos de estimas globales y estudios locales intensivos sugieren que la estimaciones de la actividad metabólica (demanda de carbono procariota) en el océano oscuro excede el aporte de substratos orgánicos (generalmente calculados como el flujo de COP que se hunde) (Carlson et al. 1994, Reinthaler et al. 2006, Steinberg et al. 2008). Este desequilibrio indica, o una sobreestimación de la actividad metabólica de los procariotas del océano profundo, o la existencia de fuentes de carbono orgánico no tenidas en cuenta (Arístegui et al. 2005a, Burd et al. en prensa). La alta abundancia de COP suspendido en el océano (Kepkay 2000, Verdugo 2004) ha sido generalmente obviada en los cálculos globales de carbono (Arístegui et al. 2005a). *Es entonces posible que el COP suspendido y/o la fijación de CO_2 oscura disminuyan ese desajuste existente entre la demanda de carbono procariota y el aporta de materia orgánica en el océano oscuro?*.

El océano profundo ha sido mucho menos estudiado que las aguas superficiales, aún cuando existen evidencias crecientes de que el océano oscuro juega un papel central en la biogeoquímica oceánica, y de que acoge un reservorio único de una alta diversidad genética y metabólica microbiana (ver revisión de Arístegui et al. 2009). Ello se debe básicamente a la dificultad relacionada con el muestreo del océano profundo (costes y tiempo involucrados) así como al hecho, de que hasta fechas recientes, el océano oscuro se suponía como un lugar que poseía solamente una insignificante actividad biológica. Sólo en las últimas dos décadas se ha ido desarrollando una noción del océano profundo

como un lugar que alberga una comunidad activa y diversa de procariotas (Bacterias and Archaeas) (Karner et al. 2001, López-García 2001, Kirchman et al. 2007). Durante mucho tiempo existió la percepción generalmente aceptada del océano oscuro como un sistema homogéneo con una comunidad procariota estable y de baja actividad. Pero, *¿realmente se encuentran la actividad y la composición de la comunidad procariota homogéneamente distribuida en el océano oscuro?*.

OBJETIVOS DE LA INVESTIGACIÓN

El objetivo del trabajo de esta tesis fue responder a varias cuestiones e incertidumbres mencionadas en la Introducción General. Estas se pueden resumir básicamente en las siguientes preguntas:

1. ¿Cómo es la variabilidad de los gradientes en profundidad de la actividad y la composición de la comunidad procariota en el océano oscuro? **Capítulo I**.

2. ¿Están estos gradientes relacionados con variaciones en el reservorio de materia orgánica del océano profundo?. Si fuese así, ¿tendrá este control "bottom-up" del océano profunda alguna influencia en el balance global de carbono? **Capítulo II**.

3. ¿Existen tasas de hidrólisis de materia orgánica significativamente importante en el océano oscuro?. ¿Cómo afecta la variabilidad en la calidad y cantidad de materia orgánica en el metabolismo de los procariotas (PHP, RP, AEE)? **Capítulo III**.

4. ¿Se encuentran esas enzimas preferentemente pegadas a la pared celular de los procariotas o son liberadas activamente?. ¿Cuál sería la consecuencia de esto? **Capítulo IV**.

5. ¿Afectan los procesos a mesoescala (e. g. remolinos generados por islas) a la variabilidad en la diversidad y función de los procariotas?. Si fuera así, ¿se prolongaría dicha influencia a la comunidad del océano oscuro? **Capítulo V**.

6. ¿Son los factores de conversión de carbono a leucina del océano profundo distintos de los de las aguas superficiales?. Si fuese así, ¿sería comparable el flujo de carbono canalizado a través de los procariotas del océano profundo con el que realizan los procariotas de la capa epipelágica? **Capítulo VI**.

Para responder a la primera cuestión se realizó un estudio de la distribución de la abundancia de procariotas (AP), actividad y composición de la comunidad de los principales grupos de procariotas (*Crenarchaeota* Grupo I, *Euryarchaeota* Grupo II, SAR 11, *Roseobacter*, *Gammaproteobacteria* y *Bacteroidetes*) desde las aguas superficiales hasta los 2000 m de profundidad. Esta investigación se llevó a cabo siguiendo un transecto a largo de una zona costera de transición (desde el afloramiento del Noroeste africano a las aguas abiertas de la región de las Islas Canarias) donde un gradiente trófico puede ser presupuesto, al menos en las aguas superficiales (**Capítulo I**).

Para responder a la segunda pregunta, más de 9000 km fueron recorridos en el Atlántico Norte (sub)tropical analizando gradientes regionales y la distribución en profundidad de los pools de materia orgánica (disuelta y particulada) y del metabolismo microbiano (producción heterotrófica procariota [PHP] y respiración procariota [RP]) en el núcleo de las principales masas de agua (desde la capa de mezcla superficial hasta los 4500 m de profundidad) (**Capítulo II**). A su vez, también realizamos una comparación entre la demanda de carbono de los procariotas y el aporte calculado de carbono orgánico particulado que se hunde.

Para estudiar la relación entre el metabolismo procariota y la actividad enzimática extracelular (AEE) en el océano oscuro, se midieron la AP, PHP, RP y la AAE de dos enzimas glicolíticas (α-glucosidasa y β-glucosidasa), una enzimas proteolítica (leucina aminopeptidasa) y fosfatasa alcalina en la zona meso- y batipelágica del Atlántico (sub)tropical (**Capítulo III**).

Con el objetivo de cuantificar la proporción de AAE libre en la columna de agua, las dinámicas de la AAE total y disuelta fueron investigadas a través del Atlántico Central desde la parte baja de la zona eufótica hasta las capas bati- y abisopelágicas (hasta los 7000 m de profundidad) (**Capítulo IV**).

El efecto de cuatro remolinos oceánicos inducidos por islas (2 ciclónicos y 2 dos

anticiclónicos) en la diversidad y en la función de procariotas fue analizado en el campo de remolinos que se encuentra al sur de las Islas Canarias. La abundancia, contenido relativo de ácidos nucleicos, viabilidad, actividades totales y a nivel celular, y la estructura de la comunidad procariota fueron analizadas para investigar si los posibles efectos en la diversidad y en la función generados en las aguas superficiales se transmitían hacia las comunidades procariotas más profundas de las zonas meso- y batipelágicas debajo de los remolinos (**Capítulo V**).

Trece experimentos se llevaron a cabo en la capa mesopelágica del Atlántico Norte subtropical para estudiar como las diferentes condiciones bióticas y abióticas de las aguas profundas afectan a la eficiencia de crecimiento procariota (ECP) y al factor de conversión carbono a leucina (LeuFC), así como para comprobar como el LeuFC afecta a las estimas de la ECP del océano profundo (**Capítulo VI**).

PLANTEAMIENTO Y METODOLOGÍA

Se ha empleado un amplio abanico de metodologías con el ánimo de alcanzar los objetivos que perseguidos con esta tesis (ver Objetivos de la Investigación); abarcando desde técnicas de biología molecular, hasta la ecología y la biogeoquímica marina. Las metodologías específicas utilizadas en cada estudio de esta tesis se encuentran descritas rigurosamente en detalle en los capítulos respectivos. Las principales metodologías de muestreo y experimentales utilizadas se explican brevemente en esta sección.

Las muestras fueron obtenidas a bordo de buques de investigación oceanográficos, mediante la utilización de una roseta equipada con veinticuatro botellas Niskin o NOEX de 10-12 l de capacidad. También montado en esta roseta se encontraba un sistema de CTD que nos permitió estimar la temperatura, salinidad y fluorescencia a través de la columna de agua. Después de obtener las muestras de las botellas, diversos procedimientos fueron llevados a cabo dependiendo del objetivo fundamental de cada estudio.

La **abundancia de procariotas (AP)** fue determinada mediante la utilización de un citómetro de flujo FACSCalibur (Becton Dickinson) equipado con un láser emitiendo a una longitud de onda de 499 nm. Los procariotas fueron identificados atendiendo a sus características en los gráficos de dispersión lateral frente a la fluorescencia verde.

A su vez, las células con **alto** y con **bajo contenido de ácidos nucleicos** (**A-AN** y **B-AN** respectivamente) fueron también separadas en el gráficos de fluorescencia verde frente a la dispersión lateral tal y como se explica en el estudio de Gasol et al. (1999).

Las células procariotas dañadas y las viables fueron estimadas en muestras no fijadas siguiendo el protocolo de la doble tinción de ácidos nucleicos (**NADS** del inglés *Nucleic Acid Double-Staining*) (Gregori et al. 2001, Falcioni et al. 2008).

La **actividad heterotrófica de los procariotas (AHP)** fue estimada a partir de las tasas de síntesis de proteínas determinadas por la incorporación de leucina marcada con tritio (Kirchman et al. 1985). A su vez, la **producción heterotrófica de los procariotas (PHP)** fue calculada a partir de las tasas de AHP, mediante la utilización del factor de conversión teórico (de 1.55 kg C mol^{-1} Leu incorporado), asumiendo que la dilución isotópica interna era despreciable en las muestras (Kirchman & Ducklow 1993).

La **respiración procariota (RP)** fue medida siguiendo los cambios producidos en la concentración de oxígeno disuelta durante incubaciones oscuras (protegidas de la luz), dentro de botellas de demanda de oxígeno (BOD del inglés *Biological Oxygen Demand*). Ello fue realizado mediante valoraciones automáticas del método Winkler, a partir de detecciones colorimétricas del punto final de valoración, tal y como se describe en Arístegui et al. (2005b). Por otro lado, la respiración de procariotas fue también medida a través del análisis de la actividad respiratoria del sistema de **transporte de electrones** (**ETS** del inglés *Electron Transport System*), siguiendo las modificaciones de la técnica de la reducción del tetrazolio como se describe en Arístegui & Montero (1995).

Los análisis del **carbono** y del **nitrógeno orgánico particulado** (**COP** y **NOP** respectivamente) fueron realizados en un analizador elemental Perkin-Elmer 2400 CHN (carbono, hidrógeno, nitrógeno) según el protocolo JGOFS (Joint Global Ocean Flux Study) (UNESCO 1994).

Los análisis del **carbono orgánico total (COT)** fueron realizados usando el método de combustión a alta temperatura mediante la utilización de un analizador Shimadzu TOC-5000A. La absorbancia del CO_2 generado por la combustión de COT fue detectada con una célula infrarroja externa (LiCor Model LI-6252) y fueron cuantificados mediante el área de los picos con el integrador Shimadzu (Benner & Strom 1993).

El análisis del **nitrógeno orgánico disuelto (NOD)** fue llevado a cabo siguiendo el método desarrollado por Valderrama (1981) mediante la utilización de un autoanalizador (TRAACS).

La **actividad enzimática extracelular (AEE)** de los procariotas de la α-glucosidasa, β-glucosidasa, aminopeptidasa y fosfatasa alcalina fue estimada analizando la hidrólisis de los substratos análogos fluorogénicos 4-metilcoumarinil-7-amida (MCA)-L-leucina-7-amida-4-metilcoumarin, 4-metilumbelliferil (MUF)-α-D-glucósido, 4-MUF-β-D-glucósido and MUF-fosfato (Hoppe 1983). La fluorescencia resultante de la ruptura del MUF o el MCA fue detectada espectrofluorométricamente utilizando un fluorómetro Fluorolog-3 con un lector de placas de microporos MicroMax 384 (Horiba) a unas longitudes de onda de excitación y de emisión de 365 nm y 445 nm respectivamente.

Las técnicas de **hibridación in situ fluorescentes con deposición catalizada** (**CARD-FISH** del inglés *Fluorescence in situ hybridization and catalyzed reporter deposition*), y de **MICRO-CARD-FISH** (**hibridación in situ fluorescentes con deposición catalizada** combinado con **micro-autoradiografía**) fueron realizadas para la cuantificación de las *Bacterias* y *Archaeas* marinas hibridando mediante la utilización de sondas compuestas por oligonucleótidos marcados con HRP y utilizando la tiramida-Alexa488 para la amplificación de la señal, según el protocolo descrito en Teira et al. (2004).

También se llevaron acabo diversas técnicas de **"fingerprinting" de ADN** con el objetivo de estudiar variaciones en la estructura de las comunidades procariotas (tanto de *Bacterias* como de *Archaeas*) entre muestras de distinto origen. Para ello se desarrolló la extracción de ADN utilizando el UltraClean Soil DNA Isolation Kit MoBio kit (MoBIO laboratories, Carlsbad, CA, USA) siguiendo el protocolo que le acompaña. La técnica de **T-RFLP** (del inglés *Terminal-restriction fragment length polymorphism*) se llevo a cabo siguiendo el protocolo de Moeseneder et al. (2001b). A su vez, el análisis automatizado del espacio intergénico ribosómico (**ARISA** del inglés *Automated ribosomal intergenic spacer analysis*) se realizó de acuerdo con Fisher & Triplett (1999) y con Hewson & Fuhrman (2004). Ambas técnicas de fingerprinting (T-RFLP y ARISA) fueron efectuadas empleando un secuenciador capilar ABI Prism 310 (Applied Biosystem) y los electroferogramas resultantes fueron analizados usando el software ABI Genescan. Los resultados obtenidos de dicho software eran luego transferidos al programa Fingerprinting II (BioRad) para determinar el área de los picos y para la estandarización usando marcadores de tamaño. La matriz obtenida era luego

analizada con el software Primer (Primer-E) para determinar similitudes entre los fingerprintings de distintas muestras.

RESULTADOS

CAPÍTULO I. *Gradientes fuertes de costa a océano y de superficie a profundidad en la estructura y actividad de la comunidad procariota en una región de zona costera de transición.*

La mayoría de los estudios realizados que describen la variabilidad de la composición del picoplanton se han llevado a cabo en aguas oceánicas superficiales. Solamente unos cuantos han cuantificado las abundancias de los diferentes grupos de procariotas del océano oscuro. Dentro de estos, algunos estudios utilizaron técnicas basadas en PCR (del inglés *Polymerase Chain Reaction*) para identificar los componentes de la comunidad procariota (Hewson et al. 2006), mientras que otros emplearon técnicas independientes de la PCR (Karner et al. 2001, Teira et al. 2004, 2006, Herndl et al. 2005). Estos últimos, sin embargo, utilizaron únicamente dos o tres sondas de procariotas marcando a las *Bacteria*, *Crenarchaeota* and *Euryarchaeota*. Además, la información concerniente a la actividad de los procariotas de aguas profundas es muy escasa si la comparamos con toda la que hay disponible para los procariotas de aguas superficiales (Reinthaler et al. 2006). La variabilidad en la actividad de los procariotas puede ser debida a cambios en la abundancia relativa de grupos específicos. Algunos estudios han mostrado una conexión clara entre la distribución de los principales grupos de procariotas y las actividad específicas de grupos de procariotas en el océano oscuro (Teira et al. 2004, 2006, Herndl et al. 2005). Dicha relación puede ser más evidente en regiones donde existan gradientes ambientales marcados y una relativamente alta actividad del picoplancton. Arístegui et al. (2005b) encontró una alta actividad procariota en las aguas mesopelágicas del Atlántico NE, una región caracterizada por una fuerte variabilidad hidrológica, con los gradientes más pronunciados cerca del afloramiento costero del NO de África.

La distribución de *Crenarchaeotas* marinas (Grupo I), de *Euryarchaeotas* marinas (Grupo II) y algunos de los principales grupos de *Bacterias* (SAR11,

Roseobacter, *Gammaproteobacteria* and *Bacteroidetes*) fueron estudiadas en la columna de agua del Atlántico Norte (de superficie a 2000 metros de profundidad) a lo largo de un transecto desde las aguas costeras del afloramiento del NO de África hasta las aguas abiertas de la Zona de Transición Costera (ZTC) canaria. Se utilizo una hibridación *in situ* fluoresecente (CARD-FISH del inglés *Catalyzed reporter deposition-fluorescence in situ hybridization*) para identificar a la comunidad procariota. Asimismo, se determinó la abundancia del picoplancton y la incorporación total de leucina. Se observaron unos cambios pronunciados en la composición de la estructura de la comunidad procariota desde la costa hacia el océano abierto en el máximo profundo de clorofila (MPC) mientras la actividad heterotrófica disminuía.

Las abundancias absolutas de todos los grupos bacterianos disminuyeron de costa a océano abierto, mientras que ambos grupos de *Archaeas* aumentaban hacia océano abierto. La abundancia y la actividad de los procariotas disminuyó entre 2 y 3 órdenes de magnitud, respectivamente, desde la superficie hasta los 2000 metros de profundidad. Las tasas de crecimiento de los procariotas eran altas en la zona mesopelágica (~0.13 d^{-1}). La abundancia total de SAR11 decreció desde un 42% en el MPC a un 4% a 2000 metros, mientras que las *Crenarchaeotas* marinas (Grupo I) aumentaron desde un 1% en el MPC a un 39% en la capa del mínimo de oxígeno. Se encontró una influencia clara de las distintas masas de agua intermedias sobre la actividad heterotrófica del picoplancton, encontrando valores menores de tasas de incorporación de leucina en las zonas donde se detectaron intrusiones de Agua Antártica Intermedia. Cabe destacar que los gradientes encontrados de costa a océano y de superficie a profundidad para la abundancia y la producción total de procariotas y para la estructura de la comunidad eran comparables con los cambios observados en otros estudios a escala de grandes cuencas oceánicas, mostrando a las ZTC como regiones que presentan una alta variabilidad en la diversidad y en el metabolismo microbiano.

CAPÍTULO II. *Evidencias de metabolismo procariota en materia orgánica particulada suspendida en las aguas oscuras del Atlántico Norte (sub)tropical.*

Se determinó la abundancia de procariotas (AP), la actividad respiratoria (ETS), la producción heterotrófica de procariotas (PHP), y la concentración de materia orgánica particulada suspendida (MOP) y disuelta (MOD) en las aguas meso- y batipelágicas del Atlántico Norte (sub)tropical. Teniendo en cuenta que la MOD es prácticamente toda refractaria por debajo de la capa de mezcla de las regiones (sub)tropicales, donde la renovación de aguas por hundimiento está muy restringida, y que los flujos de carbono que se hunde son muy pequeños en el océano abierto, es posible pensar que el metabolismo de los procariotas del océano oscuro debe depender en gran medida de las partículas suspendidas (que mediríamos como MOP). Debido a ello, variaciones en las distribución de la MOP recogida con botellas oceanográficas, considerado ser principalmente material detrítico suspendido, deberían de estar relacionadas con variaciones en el metabolismo de los procariotas del océano oscuro.

La AP disminuyó en un orden de magnitud desde la base de la zona eufótica hasta las aguas batipelágicas, mientras que el ETS decreció en dos y la PHP en tres órdenes de magnitud en profundidad. En una sección siguiendo la dorsal centro-oceánica del Atlántico desde los 35ºN hasta los 5ºN, el ETS por debajo de los 1000 m de profundad incrementó hacia el sur multiplicando por tres sus tasas. Este gradiente latitudinal encontrado en las aguas profundas vino acompañado por un incremento de hasta seis veces en la concentración de carbono orgánico particulado (COP), mientras que la MOD no presentó ningún patrón de distribución aparente. Correlaciones significativas fueron encontradas entre la MOP y el ETS en las masas de agua que encontraban entre los 1000 m y los 3000 m: el Agua Antártica Intermedia y el Agua Profunda del Atlántico Nordeste. Además, se encontró un fuerte desequilibrio en el océano oscuro entre la demanda de carbono procariota (estimada mediante dos aproximaciones distintas) y el flujo de carbono que se hunde derivado de datos obtenidos con trampas de sedimento corregidos con ^{230}Th. Dicho desajuste era mayor cuanto más profundo en la columna de agua, sugiriendo que el reservorio de carbono

suspendido debe de ser el principal responsable de compensar la mayor parte del déficit de carbono. Nuestros resultados, junto con otras descubrimientos recientes discutidos en este capítulo, indican que la vida microbiana del océano profundo parece ser más dependiente de partículas suspendidas (de hundimiento lento) transportadas lateralmente de lo que se asumía hasta la fecha.

CAPÍTULO III. *Actividad enzimática extracelular procariota en relación a la producción de biomasa y a la respiración en las aguas meso- y batipelágicas del Atlántico (sub)tropical.*

Los procariotas necesitan utilizar la actividad enzimática extracelular para hidrolizar la materia orgánica, haciendo de estas actividades un parámetro ecológico fundamental. Sin embargo, la mayor parte de los estudios realizados con anterioridad concernientes a dicha actividad se han limitado a la zona eufótica y, en los ambientes marinos, a áreas costeras. Por ello, existen muy pocos perfiles verticales de actividades enzimáticas extracelulares para el océano abierto y que se extiendan desde la zona eufótica hasta las capas meso- y batipelágicas. Teniendo en cuenta que la actividad enzimática extracelular es el paso que une la materia orgánica con el metabolismo de los procariotas, es posible pensar que el cambio que ocurre en la disminución de la calidad y cantidad de la materia orgánica en profundidad tenga alguna repercusión sobre la actividad enzimática extracelular y consecuentemente sobre el metabolismo de los procariotas. Es probable que dicho incremento en la proporción de materia orgánica recalcitrante en profundidad conlleve un incremento paralelo en la actividad enzimática celular específica, produciendo, consecuentemente, una disminución en la producción de biomasa por parte de los procariotas.

La actividad enzimática extracelular de los procariotas, la abundancia, producción heterotrófica y respiración de los procariotas fueron determinadas en las capas meso- y batipelágicas del Atlántico Norte (sub)tropical. Mientras que la producción heterotrófica de procariotas (PHP) disminuyó desde la base de zona eufótica hasta las aguas batipelágicas en dos órdenes de magnitud, la abundancia de procariotas y la PHP específica (por célula) decreció solamente en un orden de magnitud. En contraste con la PHP específica, la actividad enzimática extracelular específica (alpha- y beta-glucosidasa, leucina aminopeptidasa y la fosfata alcalina) aumentaron en profundidad, tal y como le sucedió a la tasa de respiración específica. La actividad específica de la fosfatasa alcalina se multiplicó por cinco desde las masas de agua intermedias hasta las aguas más profundas. Sin embargo, la concentración de fosfato sólo varió en un factor de dos entre las distintas masas de agua, indicando que la

actividad de la fosfatasa no estaba relacionada con la disponibilidad de fosfato en las aguas profundas. También se encontró que las actividades enzimáticas extracelulares específicas estaban inversamente relacionadas con las tasas específicas de incorporación de leucina por parte de los procariotas. Relacionado con esto, se descubrió además que la constante de semisaturación (K_m) y el ratio V_{max}/K_m (que describe la habilidad de las enzimas para competir a bajas concentraciones de substratos) incrementaron en profundidad, poniendo de manifiesto unas adaptaciones del sistema enzimático de los procariotas para con la naturaleza recalcitrante de la materia orgánica del océano oscuro. Debido a todo ello, parece probable que la utilización de la materia orgánica más refractaria de las a aguas profundas conlleve una mayor actividad enzimática extracelular específica y respiración, y una menor PHP específica que en las aguas superficiales (donde la materia orgánica es menos recalcitrante).

CAPÍTULO IV. *Alta actividad enzimática extracelular disuelta en las aguas profundas del Océano Atlántico Central .*

La actividad enzimática extracelular del océano oscuro está relacionada con la composición de la materia orgánica particulada (MOP). Estudios anteriores indican que una parte substancial de la MOP que se hunde es solubilizada a materia orgánica disuelta (MOD), supliendo con materia orgánica a la producción procariota en las zonas meso- y batipelágicas (Cho & Azam 1988). La observación de que el carbono orgánico particulado decrece más rápidamente en profundidad que la demanda de carbono procariota, permitió la formulación de la "paradoja de descomposición de partículas" de Karl et al. (1988). Cho & Azam (1988) propusieron que la razón que explicaba dicha paradoja es una sobre-expresión de enzimas extracelulares por parte de la comunidad de procariotas que vive asociada a partículas, liberando enzimas extracelulares a la partícula. Dicha sobre-expresión de enzimas extracelulares disueltas resulta en un desajuste entre le procesos de hidrólisis y de incorporación del producto resultante, y consecuente, en la liberación potencial de estos productos a las aguas circundantes, proporcionando materia orgánica a los procariotas de vida libre (Smith et al. 1992). Basándonos en esta noción de que los procariotas que viven en partículas liberan enzimas extracelulares al ambiente, nuestra hipótesis se fundamentaba en que la contribución de la AEE disuelta con respecto a la total debería de ser mayor en el océano oscuro abierto si los procariotas de las aguas profundas eran más dependientes de la MOP como substrato que las comunidades de procariotas de aguas superficiales. Ello se debe a que las comunidades microbianas heterotróficas de aguas superficiales tienen accesos a MOD bio-reactiva, mientras que en las aguas profundas la mayoría de la MOD es refractaria.

Se determinó la abundancia de procariotas (AP), la producción heterotrófica procariota (PHP), y la materia orgánica particulada suspendida, a la vez que las actividades enzimáticas extracelulares (AEE) potenciales (alfa- y beta-glucosidasa [AGasa y BGasa], leucina aminopeptidasa [LAPasa], y fosfatasa alcalina [Fasa]) totales y disueltas (definidas operacionalmente como las que pasaban a través un filtro de 0.2 μm de tamaño de poro) en las aguas meso- y batipelágicas del Atlántico (sub)tropical a

lo largo de un transecto longitudinal trasatlántico y de otro transecto latitudinal. Se encontraron diferencias significativas entre ambos transectos en las concentraciones de MOP pero no en las AP, PHP (excepto en la capa subsuperficial y en el mínimo de oxígeno), y ni en las AEE disueltas ni totales. La PHP disminuyó en tres órdenes de magnitud desde la base de la zona eufótica hasta las aguas batipelágicas, mientras que la AP y la PHP específica (por célula) solamente decrecieron en uno y dos órdenes de magnitud respectivamente. La proporción de AEE disuelta respecto a la total fue mayor en el océano oscuro para todas las enzimas, estando comprendidas entre un 54-100%, 56-100%, 65-100% and 57-97% para la AGasa, BGasa, LAPasa y Fasa, respectivamente. Los parámetros cinéticos (V_{max} y K_m) de las fracciones disueltas y totales de la LAPasa y de la Fasa eran muy similares a lo largo de toda la columna de agua, sugiriendo un origen similar para las AEE disueltas y particuladas. Se encontraron correlaciones significativas entre ambas AEE (disueltas y totales) con el metabolismo de los procariotas y con el reservorio de MOP. Basándonos en nociones previas que indican que la fracción de AEE disuelta es mayor en microbios adheridos a partículas que en los que viven libremente, nuestros resultas sugieren, de acuerdo con recientes evidencias genómicas, que la actividad microbiana del océano oscuro se concentra fundamentalmente en material coloidal y particulado, que es fácilmente alterado durante en el proceso de muestreo. Debido a ello, técnicas de muestreo selectivas son necesarias para recoger específicamente esos agregados de aguas profundad, que probablemente representan puntos calientes de actividad microbiana en el océano profundo.

CAPÍTULO V. *Remolinos mesoescalares: puntos calientes de actividad procariota y modificadores de la estructura de las comunidades procariotas.*

Los remolinos mesoescalares son estructuras comunes en todos los océanos, con grandes implicaciones en la productividad y en la biogeoquímica regional. Los remolinos anticiclónicos acumulan materia orgánica en sus núcleos y presentan una alta respiración microbiana y producción heterotrófica. Los remolinos ciclónicos incrementan la entrada de nutrientes hacia las aguas superficiales aumentando la producción nueva y la concentración de clorofila. Debido a ello, los remolinos ejercen un control primordial en la generación, acumulación y hundimiento de la producción biogénica en los océanos, así como en los procesos asociados de remineralización mediados por los procariotas. Sin embargo, aunque el papel clave de los procariotas en los ciclos biogeoquímicos es ampliamente reconocido (e. g. Azam et al. 1983), sólo se han publicado un reducido número de estudios, algunas veces contradictorios, analizando la respuesta de los procariotas heterotróficos a la influencia de los remolinos. Incluso menos información hay disponible sobre cambios en la estructura de la comunidad procariota debido a la presencia de remolinos oceánicos.

Con el fin de investigar los efectos de los remolinos mesoescalares en la estructura de la comunidad procariota y en su actividad muestreamos 2 remolinos ciclónicos (RC) y 2 anticiclónicos (RA) en el campo permanente de remolinos que se encuentra en la región de las Islas Canarias. Las estaciones de remolinos fueron comparadas con dos estaciones fuera del campo de influencia (FF) de los remolinos también localizadas en la Corriente de Canarias. La distribución de la abundancia de procariotas (AP), la actividad heterotrófica procariota (AHP), de varios indicadores de actividad a nivel celular (como el contenido en ácidos nucleicos, la proporción de células vivas, y la fracción de células activamente incorporando leucina), a la vez que la estructura de las comunidades bacterianas y de *Archaeas* fueron determinadas desde las aguas superficiales hasta los 2000 metros de profundidad. En la capa epipelágica superficial (0-200 m), los efectos de los remolinos sobre la comunidad procariota eran más aparentes, tal y como se puso de manifiesto en una mayor AP, AHP, proporción de

células libres y porcentaje de células activamente incorporando leucina encontradas en las estaciones de remolinos al compararlas con las FF. La composición de la comunidad procariota también fue diferente entre los remolinos y las estaciones FF en la capa epipelágica. En la zona mesopelágica (200-1000 m) igualmente se encontraron diferencias significativas en la AP y la AHP entre los remolinos y las estaciones FF, aunque en general no se hallaron diferencias claras en la composición de la comunidad ni en la actividad a nivel celular. Los efectos sobre la actividad de los procariotas y la estructura de la comunidad fueron más agudos en los RA que en los RC, disminuyendo en intensidad en profundad en ambos tipos de remolinos. En resumen, las dos clases de remolinos presentaron distintas composiciones de las comunidades (al compararlas con las estaciones FF, en la capa epipelágica), y representan "puntos calientes" oceánicos de actividad procariota (en las zonas epi- y mesopelágicas).

CAPÍTULO VI. *Determinando la utilización de carbono procariótica en el océano oscuro: eficiencia de crecimiento, factores de conversión de leucina a carbono y sus relaciones.*

Un concepto clave en el estudio de la ecología de los ecosistemas es el balance entre los procesos catabólicos y anabólicos, habitualmente denominado como eficiencia de crecimiento. Este parámetro cuantifica la proporción de carbono orgánico incorporado a biomasa (producción) comparado con la obtención total de carbono orgánico (producción más respiración). En los ecosistemas oceánicos los procariotas son los principales reguladores de los flujos de carbono, jugando un rol crucial como el componente fundamental del bucle microbiano, controlando la cantidad de carbono que entra en la cadena trófica y la que se respirará. Sin embargo, la estimación de las eficiencias de crecimiento de los procariotas (ECP) no es trivial, y para ello, se necesita emplear factores de conversión cuya variabilidad prácticamente no se ha estudiado. Se ha comprobado como la utilización de factores de conversión teóricos de leucina a carbono conlleva grandes errores en las estimaciones metabólicas de la producción heterotrófica procariotra, y consecuentemente en la ECP. Por este motivo, normalmente se recomiendo determinar empíricamente los factores de conversión de leucina a carbono (LeuFC) a la hora de querer cuantificar de una manera precisa los flujos de carbono (Bell 1990, Pulido-Villena & Reche 2003, Buesing & Marxsen 2005, Calvo-Díaz & Morán 2009).

Para poder comprender plenamente los ciclos biogeoquímicos del carbono en el océano, es crucial tener en cuenta todo el rango de profundidades donde se lleven a cabo procesos metabólicos. Sin embargo, aunque recientemente el papel potencial de los procariotas del océano profundo para metabolizar substratos orgánicos se ha venido aceptando, así como la importancia de estimar las ECP para los estudios de flujos de carbono, sólo existen unos pocos estudios que hayan medido las ECP y los LeuFC del océano oscuro. Se ha encontrado que la respiración de aminoácidos marcados incrementa en profundidad, donde las ECP se piensan que son menores que en las aguas superficiales. Como la respiración de leucina ha sido relacionado con bajos LeuFC (Alonso-Sáez et al. 2007), puede ser postulado que la producción heterotrófica

procariota debe de presentar bajos LeuFC. Aunque la importancia de determinar empíricamente los LeuFC para una estimación precisa de la ECP se encuentra comúnmente aceptada, sólo hay disponible un estudio (Gasol et al. 2009) para el océano profundo donde se hayan estimados los LeuFC (5 estimas para aguas mesopelágicas, pero no se calcularon las ECP). Debido a ello, la influencia de los LeuFC sobre la ECP sigue siendo desconocida para el océano oscuro, donde tiene lugar una parte substancial del procesado de carbono orgánico.

Se realizaron experimentos en la zona mesopelágica del Océano Atlántico Nordeste subtropical con el objetivo de determinar la variabilidad de los factores de conversión de leucina a carbono y de la eficiencia de crecimiento procariota. El modo en que la producción heterotrófica procariota (PHP) fue calculada tenía una influencia directa sobre la ECP (se encontraron variaciones de ECP de entre 1-31% para una misma muestra). Los LeuFC empíricamente obtenidos para aguas profundas mostraron una variabilidad de hasta 7 veces en magnitud (entre 0.13-0.85 kg C mol^{-1} Leu), siendo siempre menor que el LeuFC teórico de 1.55 kg C mol^{-1} Leu asumiendo que no existía dilución isotópica. Las LeuFC determinados empíricamente estaban altamente correlacionados con la ECP, sugiriendo que ambos parámetros son representaciones de los mismos procesos metabólicos básicos. En resumen, las ECP obtenidas en este estudio indican que la comunidad procariota mesopelágica puede ser igual de importante en el procesamiento de carbono que los procariotas epipelágicos, al menos en alguna regiones.

SÍNTESIS DE RESULTADOS Y DISCUSIÓN GENERAL

Estructura de la comunidad y actividad de los procariotas del océano oscuro: ¿distribución homogénea o heterogénea?

Debido a la poca disponibilidad de materia orgánica lábil, y a las condiciones abióticas (temperatura, salinidad, pH, concentración de oxígeno,…) típicamente más estables en el océano oscuro que en las aguas superficiales, es razonable inferir que la estructura de la comunidad procariota y la actividad asociada a ella debería de seguir una distribución más o menos homogénea a lo largo de las aguas profundas. Además, la baja temperatura y concentración de materia orgánica características del océano profundo pueden también llevar a la percepción del océano profundo como un lugar donde la actividad biológica es prácticamente despreciable. Sin embargo, se encontraron diferencias horizontales (laterales) y verticales (en profundidad) en la actividad de los procariotas (**Capítulos I, II, III, IV, V, VI**) y en la estructura de la comunidad (**Capítulos I y V**) en el océano oscuro. Una interesante sustitución lateral de *Bacterias* por *Archaeas* fue encontrada en las aguas epi- y mesopelágicas de la zona costera de transición del NO África-Canarias (**Capítulo I**). También se encontraron fuertes diferencias en la abundancia de procariotas (AP), en la producción heterotrófica de procariotas (PHP) y en el porcentaje de células con alto contenido en ácido nucleicos a lo largo de ese gradiente trófico (**Capítulo I**). Estas variaciones estaban relacionadas con la presencia de distintas masas de agua, reflejando una probable influencia de la variación de los reservorios de materia orgánica sobre la estructura de la comunidad de los procariotas y su correspondiente actividad. Dicha hipótesis se confirmó posteriormente al encontrarse una correlación significativa entre el carbono orgánico particulado suspendido y la respiración procariota a lo largo de un gradiente latitudinal en las zonas meso- y batipelágicas del Atlántico Norte (sub)tropical (**Capítulo II**). La relación existente entre la actividad de los procariotas y la distribución de materia orgánica quedó aún más patente por las correlaciones significativas encontradas entre la actividad enzimática extracelular (tanto la fracción disuelta como la particulada), la PHP, y la concentración de COP suspendido en las aguas profundas del Océano Atlántico Central (**Capítulo IV**). Además, la distribución de la actividad enzimática

extracelular (AEE) estaba asimismo afectada por cambios en la composición de la materia orgánica (MO) en profundidad (**Capítulo III**), también de acuerdo con un hipotético control "bottom-up" de la actividad procariota por la distribución de la MO. De hecho, el incremento en profundidad de la AAE por célula, de la constante de semisaturación (K_m) y del ratio V_{max}/K_m apunta hacia una adaptación a la menor disponibilidad de materia orgánica lábil que existe al aumentar la profundidad. Esta conclusión concuerda con las mayores tasas de respiración de aminoácidos encontradas en las aguas profundas (Carlucci et al. 1986), y con el incremento en el ratio de incorporación de *D*-/-*L* aminoácidos por parte de la comunidad procariota (Pérez et al. 2003). Usando genómica ambiental comparativa, DeLong et al. (2006) también halló una alta abundancia de genes responsable del metabolismo del glioxilato y dicarboxilato, que corresponde con la MOD relativamente más oxidada y degrada presente en el océano profundo al compararla con las aguas superficiales (Benner 2002). Igualmente está de acuerdo con la primera secuencia genómica obtenida de un psicropiezófilo (microbio adaptado a vivir a bajas temperaturas y alta presión) del océano profundo (*Photobacterium profundum* SS9) (Vezzi et al. 2005, Bartlett et al. 2007, 2008), donde una vía para la fermentación de aminoácidos, que previamente sólo se conocía en anaerobios estrictos (la reacción de Strickland), junto con un sistema enzimático similar a los que se utilizan para utilizar varios polímeros complejos (incluyendo xenobióticos, quitina y celulosa), fueron encontrados. Todos estos descubrimientos implican una adaptación de los microbios del mar profundo para utilizar un carbono orgánico más recalcitrante (Carlson et al. 2004). Sin embargo, esta adaptación a las diferencias en la "calidad" de la materia orgánica a través de la producción de enzimas extracelulares lleva consigo una disminución en la eficiencia de crecimiento de los procariotas comparada con la de aguas superficiales. Esta relación inversa entre la AEE y la eficiencia de crecimiento es similar a la situación que tiene lugar en los países subdesarrollados al compararlos con los desarrollados. En las naciones desarrolladas (i. e. aguas superficiales) la energía metabólica (caminar, correr, trabajar,…) requerida para obtener alimento no es mucha porque es sencillo encontrar comida en numerosos lugares (tiendas, restaurantes,…). Debido a ello, en estos países es más sencillo incrementar la biomasa (algo que queda reflejado por el alto índice de masa corporal que presentan dichas poblaciones). Sin embargo, en los países subdesarrollados (i. e. océano oscuro), la energía metabólica necesaria para obtener la misma cantidad de alimento que en los países desarrollados es mucho mayor (se

requieren más horas de trabajos por unidad de comida obtenida). Esto provoca una mayor tasa de respiración por unidad de alimento ingerida, y consecuentemente, una mayor dificultad para que se produzca un incremento en biomasa. Equivalentemente, la energía metabólica requerida por los procariotas heterotróficos para metabolizar la materia orgánica menos refractaria típica del océano profundo se relaciona con una menor eficiencia de crecimiento de los procariotas.

Otras evidencias recientes apuntan también hacia una comunidad de procariotas activa y heterogéneamente distribuida en el océano oscuro. Algunos estudios han mostrado también una estacionalidad en la abundancia de procariotas en el océano oscuro. Nagata et al. (2002) encontraron como la abundancia procariota se doblaba en una estación que fue muestreada dos veces a lo largo de varios años en las aguas profundas del Pacífico. En la capa mesopelágica del Mar Mediterráneo, Tanaka & Rassoulzadegan (2002, 2004) hallaron unos cambios estacionales relativamente importantes. Sherry et al. (1999) encontraron un incremento de cinco veces en la variación de PHP en Pacífico NE. Church et al. (2003) detectaron una mayor proporción de células en profundidad que cerca de la superficie mediante hibridación in situ fluorescente, asumiendo que correspondía con un alto contenido de rRNA. Arístegui et al. (2005b) obtuvo una proporción muy similar de células con actividad respiratoria, y una mayor respiración por células en la capa mesopelágica que en la epipelágica del Atlántico NO. En la misma región (zona costera de transición del NO de África–Islas Canarias), Gasol et al. (2009) encontró que la relación entre los procariotas y sus principales depredadores (nanoflagelados heterotróficos) permanecía constante en profundidad, lo que contradice la idea de que los procariotas del océano profundo se encuentran generalmente inactivos. Además, Teira et al. (2006) indicó que el 16-20% de la comunidad heterotrófica procariota de las aguas profundas del Atlántic Norte se encuentran activos desde un punto de vista metabólico incorporando leucina. Dicho porcentaje es similar al que del Giorgio & Gasol (2008) sugirieron para las aguas epipelágicas. Hewson et al. (2006) halló una variabilidad considerable en la composición bacteriana entre estaciones adyacentes de aguas profundas. Estos últimos autores justificaron sus descubrimientos como resultado de hundimientos episódicos de materia orgánica proveniente de aguas superficiales.

Nosotros también hemos mostrado como la presencia de remolinos generados por islas (probablemente bombeando materia orgánica hacia el océano oscuro) producen un incremento en la abundancia de procariotas y en su actividad en la zona mesopelágica, pero no en la batipelágica (**Capítulo V**). Asimismo, otro descubrimiento interesante relacionado con la distribución heterogénea de la actividad de los procariotas en profundidad fue la presencia de picos mesopelágicos de AP y de actividades totales y normalizadas por células (**Capítulo V**). La presencia de picos en la capa mesopelágica ha sido referenciada con anterioridad asociada a discontinuidades entre masas de agua (Sorokin 1971, Karl 1980, Carlucci et al. 1987) o causadas por aportes laterales de carbono orgánico (Nagata 2000). Como se ha observado para la AP, también se han encontrado picos de producción heterotrófica en frentes de densidad (Sorokin 1973). Karl (1980) describió un mayor actividad sobre los 500-600 m de profundidad que en las aguas circundantes.

En resumen, podemos concluir que el océano oscuro no es ni mucho menos un ecosistema homogéneo e inactivo. La heterogeneidad dominante parece ser controlada por un efecto "bottom-up", donde la distribución de la materia orgánica (concretamente del carbono orgánico particulado suspendido) modula la actividad de los procariotas. Globalmente, el océano oscuro juega un papel clave en los procesos de mineralización de carbono, con tasas por célula igual de altas o mayores que las que se dan en las aguas epipelágicas. Mayores AEE específicas han sido encontradas en el océano oscuro que en las aguas superficiales (**Capítulos III y IV**), y un rango de factores de conversión empíricos de leucina a carbono y de eficiencias de crecimiento mesopelágicos comparables con los que se dan en aguas superficiales han sido descubiertos (**Capítulo VI**).

Preferencia de los procariotas del océano oscuro a vivir adheridos a partículas

Generalmente se asume que sólo menos del 5% de los procariotas viven asociados con partículas que se hunden o suspendidas en el océano oscuro (Cho & Azam 1988, Turley & Mackie 1994). Sin embargo, una relación significativa fue encontrada entre el COP suspendido y la respiración a lo largo de un transecto de larga

distancia en el Atlántico Norte batipelágico (**Capítulo II**), lo que sugiere un fuerte vínculo entre el metabolismo de los procariotas y las partículas. Asimismo, una proporción muy alta de AEE disuelta se encontraba presente en el Atlántico Central (**Capítulo IV**). Igualmente, se encontraron relaciones significativas entre la AEE disuelta y la fracción total de AEE con la materia orgánica particulada suspendida y la producción heterotrófica de procariotas, sugiriendo también un fuerte link entre metabolismo de los procariotas y la materia orgánica suspendida en el océano oscuro. Como se explicó en el **Capítulo IV**, la alta proporción de AAE libre con respecto a la total puede ser interpretada como un indicador de un modo de vida de los procariotas relacionados con partículas, teniendo en cuenta las nociones previas que demuestran como la fracción de AEE disueltas es mayor en microbios que viven pegados a partículas que en los que viven de forma libre. Por ello, teniendo en mente la fuerte relación encontrada entre el COP suspendido y el metabolismo de los procariotas, sugerimos que el verdadero porcentaje de procariotas adheridos puede haber sido considerablemente subestimado en anteriores investigaciones del océano profundo. Dicha subestimación puede ser debida a una naturaleza muy frágil de un proporción significativa de las partículas batipelágicas, pudiendo verse alteradas durante el muestreo. Por ejemplo, los geles son componentes abundantes e importantes dentro de los agregados que se encuentran a lo largo de la columna de agua (Verdugo et al. 2004, 2008), distribuyéndose sobre una largo espectro de tamaños, desde coloides hasta nieve marina, y convirtiendo MOD a MOP a través del auto-ensamblaje de polímeros. Debido a ello, el auto-ensamblaje de microgeles puede ser un mecanismo clave en la generación de agregados en la zona batipelágica. Sin embargo, la desagregación de estos geles puede ocurrir fácilmente durante el muestreo. En cualquier caso, la fuerte relación encontrada entre la materia orgánica particulada suspendida y el metabolismo de los procariotas en el océano oscuro indica una mayor asociación entre las partículas suspendidas y los procariotas de lo que se asumía hasta ahora.

Conjuntamente con las asociaciones que hemos encontrado *in situ*, existen otras evidencias genómicas recientes que también apuntan hacia un modo de vida preferentemente pegado a partículas para los procariotas del océano profundo (DeLong et al. 2006, Martín-Cuadrado et al. 2007, Ivars-Martínez et al. 2008). En el giro subtropical del Pacífico Norte, DeLong et al. (2006) encontraron que los procariotas de aguas profundas estaban enriquecidos en genes relacionados con la síntesis de

polisacáridos y antibióticos, indicando un papel potencialmente mayor de lo que presumía hasta la fecha del estilo de vida adherido a partículas para los procariotas del océano profundo. También en el Mar Mediterráneo, Martín-Cuadrado et al. (2007) hallaron una alta abundancia de genes que codificaban transportadores de dipéptidos y oligopéptidos y aminoácidos en cadena, indicando que las proteínas (potencialmente asociadas con la nieve marina y con las partículas coloidales) son importantes fuentes de carbono para los procariotas batipelágicos. Ivars-Martínez et al. (2008) encontraron diferencias en la estructura genómica de dos distintos ecotipos (uno típico de las aguas superficiales y el otro habitual de aguas profundas) de la común gammaproteobacteria *Alteromonas macleodii*. Estos últimos autores descubrieron que el ecotipo de aguas superficiales tenía un mayor potencial para la utilización de azúcares y aminoácidos, mientras que el ecotipo de aguas profundas albergaba genes que reflejaban una vida bajo condiciones microaerofílicas, y para la adsorción a partículas. En resumen, todos estos estudios (meta)genómicos indican también que la vida microbiana en las capas meso- y batipelágicas se encuentra más agrupada alrededor de partículas de lo que se pensaba hasta hace poco tiempo.

Teniendo en cuenta la baja reactividad de la MOD de las aguas profundas, los procariotas pueden preferir vivir adheridos a partículas, donde las fuentes de carbono y energía se encuentran más disponibles que en las aguas circundantes. De esta manera, el hecho de vivir en estos “oasis” en medio del gran desierto oceánico de MO disuelta refractaria puede facilitar interacciones sinérgicas en el ciclo de materia del océano oscuro. Por ello, es posible concluir apuntando que la naturaleza heterogénea de los microbios de las aguas profundas y sus consecuentes actividades parecen estar controladas por la presencia y heterogeneidad de la distribución de materia coloidal y particulada. Aunque las implicaciones potenciales de dichas micro-zonas han sido mencionadas con anterioridad, la mayoría de las medidas no tienen en cuentan la distribución potencialmente no aleatoria de los procariotas en la columna de agua oceánica (Azam & Malfatti 2007). Debido a ello, es necesario el desarrollo de nuevas técnicas de muestreo con el objetivo de lidiar con la naturaleza frágil de la materia detrítica batipelágica y con la aparente distribución no azarosa de los procariotas del océano oscuro.

Importancia del carbono orgánico particulado suspendido y de la fijación de CO_2 para la demanda de carbono heterotrófica en el Atlántico mesopelágico

Generalmente se acepta que la cadena trófica heterotrófica de las aguas profundas depende de la materia orgánica (MO) generada por la producción primaria en las aguas superficiales (donde hay luz suficiente para poder realizar la fotosíntesis). En conjunto, alrededor del 30% de la producción primaria de las aguas superficiales se exporta al océano oscuro en forma de partículas que se hunden (Arístegui et al. 2005a, Buesseler & Boyd 2009). Aunque la concentración de carbono orgánico disuelto (COD) es generalmente 10 veces mayor que la del carbono orgánico particulado (COP), el reservorio de COD sólo puede explicar alrededor del 10% de la utilización aparente de oxígeno en las aguas mesopelágicas (Arístegui et al. 2002). Debido a ello, se considera al hundimiento pasivo de partículas originadas en la zona eufótica como la fuente principal de carbono orgánico disponible para red trófica heterotrófica del océano interior (Buesseler et al. 2007). La mayoría del COP exportado desde la zona eufótica se remineraliza en la capa mesopelágica (entre los 100-1000 m de profundidad), conllevando una típica atenuación exponencial en profundidad en la concentración de COP que se hunde (Martin et al. 1987). Se ha observado una correlación entre la actividad microbiana de las aguas profundas y en flujo de COP que se hunde en el Pacífico subártico (Nagata et al. 2000). Asimismo, en el sector Atlántico del Océano Sur, la producción procariota en las aguas mesopelágicas se correlaciona con la producción primaria y con la concentración de clorofila superficial (Simon et al. 2004). En el Mar Arábico, la abundancia de procariotas, y no la actividad, se relacionó con el flujo de COP que se hunde, indicando que la abundancia procariota del océano profundo no refleja el flujo más reciente de COP pero que, en cambio, es un indicador del promedio a larga escala temporal del flujo de COP (Hansell & Ducklow 2003).

Asumiendo una conservación de masas, la tasa de aporte de carbono orgánico (generalmente calculada como el COP que se hunde y se colecta con trampas de sedimento) que alcanza el océano interior debería de coincidir con la demanda de carbono de la biota heterotrófica que habita las capas meso- y batipelágicas. La biota de aguas profundas está fundamentalmente dominada por procariotas, tanto en términos de abundancia como de biomasa. Sin embargo, se ha demostrado continuamente como la demanda de carbono orgánico de los procariotas (calculada como la producción más la

respiración) excede el flujo de COP que llega al océano oscuro (Burd et al. en prensa). Estimas recientes muestran un desacople de entre 2-3 órdenes de magnitud entre la demanda de carbono de los procariotas (DCP) y el flujo de COP que se hunde (Reinthaler et al. 2006, Steinberg et al. 2008, **Capítulo II**). Además, en un ejercicio de balances globales, del Giorgio & Duarte (2002) indicaron que incluso la mayor estima posible del flujo de COP que se hunde desde las aguas superficiales explicaría solamente alrededor de un 50% del consumo de oxígeno medido en el océano oscuro. Se ha visto como este paradójico desequilibrio varía también espacialmente. La DCP fue entre 3-4 veces y alrededor de 10 veces mayor que el flujo de COP que se hunde en el Pacífico subtropical y en el subártico respectivamente (Steinberg et al. 2008). Este substancial desajuste entre el aporte de COP y la demanda representa uno de los mayores retos de la oceanografía biológica y de la biogeoquímica marina contemporáneas, e indica un vacío central en nuestro entendimiento del flujo de carbono del océano profundo. Parece ser lógico que algún componente primordial o aspecto de los reservorios y flujos de carbono orgánico no ha sido atendido de manera adecuada.

Uno de los principales reservorios de COP en el océano interior que no se ha tenido en cuenta apropiadamente en los balances de carbono orgánicos oceánicos ha sido el COP suspendido, el que no se hunde (COPs). Dicho COPs se colecta con botellas oceanográficas pero, debido a su tendencia a flotar, no se acumula en las trampas de sedimento utilizadas para determinar el flujo de COP que se hunde (Arístegui et al. 2009). En esta sección utilizamos datos recogidos de las zonas meso- y batipelágicas del Atlántico Norte correspondientes a flujos de materia orgánica particulada (MOP) sedimentaria, pools de MOP (incluyendo el COPs residente) y a demandas de carbono de procariotas (DCP) para reevaluar la aparente discrepancia existente entre la demanda de materia orgánica procariota y el aporte al océano interior.

En contraste con la disminución exponencial típica del COP en profundidad (Martin et al. 1987), la concentración de COPs y de NOPs permaneció prácticamente constante en profundidad hasta las aguas batipelágicas (4000 m de profundidad) del Atlántico Norte (sub)tropical (Fig. 1A, B). Habitualmente, la contribución de los procariotas al COPs y al NOPs disminuye en profundidad (Fig. 1C, D). Restando la contribución de los procariotas a los reservorios de COPs y NOPs obtenemos la cantidad de COPs (COPs_Dp) y NOPs (NOPs_Dp) que se encuentra potencialmente disponible para una utilización heterotrófica (Fig. 1E, F).

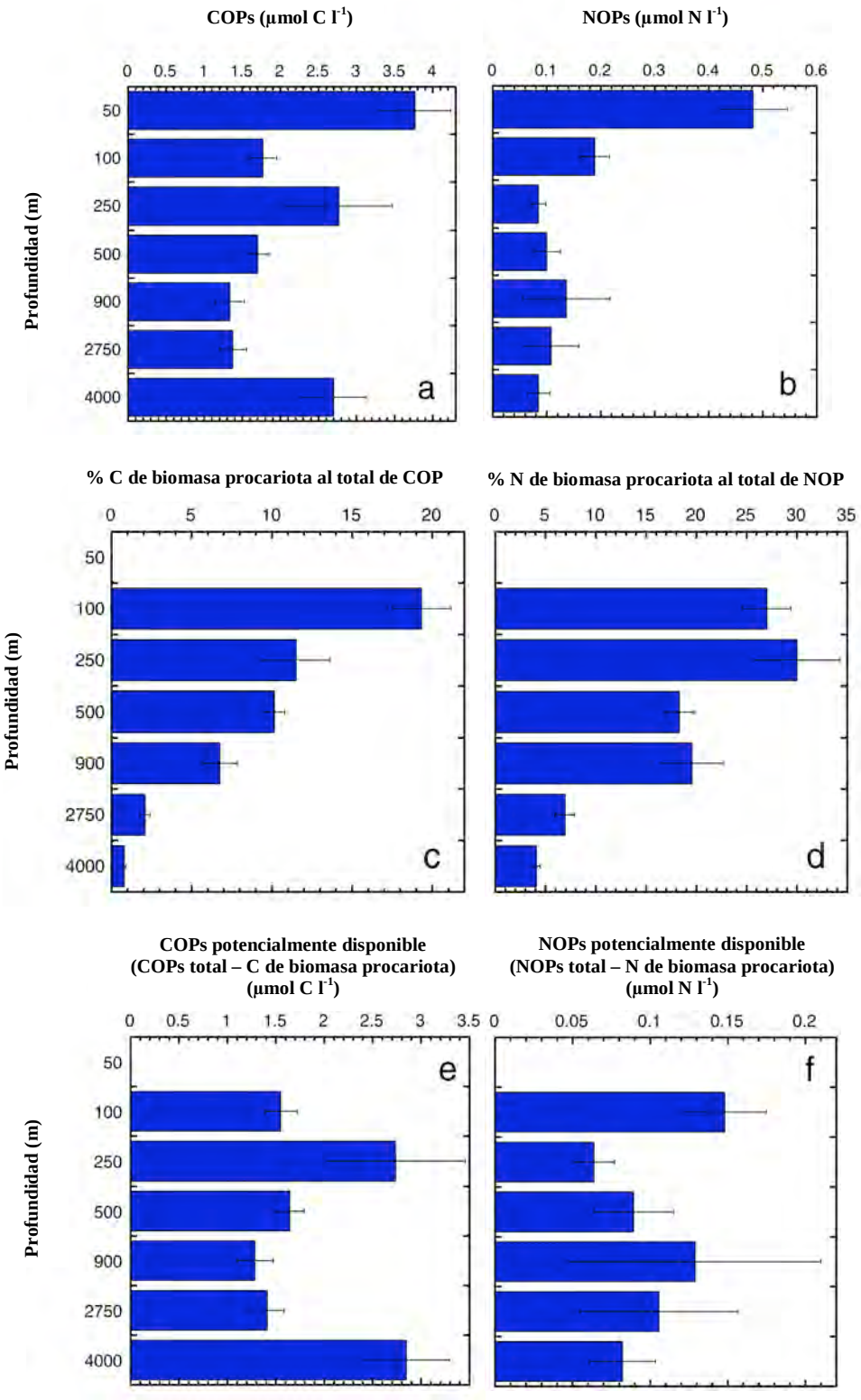


Fig. 1. Promedios (± ES, n = 22 – 48) de la distribución vertical de (A) el carbono orgánico particulado suspendido (COPs) (B) el nitrogeno orgánico particulado suspendido (NOPs), la contribución de la biomasa de procariotas al total de COPs (C) y NOPs (D), y el potencialmente disponible COPs (E) y NOPs (F) después de restarles la biomasa de C y N de los procariotas respectivamente. Todas las concentraciones de materia orgánica se encuentran en µmol l^{-1}

Las cantidades de COPs_Dp y de NOPs_Dp no mostraron una tendencia de disminución con la profundidad (Fig. 1E, F). El ratio COPs_Dp : NOPs_Dp se mantuvo prácticamente constante a través de la columna de agua, evidenciando que no existían cambios drásticos en los ratio C : N de la MOPs en las aguas profundas (Fig. 2), en contraste con el incremento del ratio C : N en profundidad que se ha encontrado en la MOP y MOD que se hunde (Schneider et al. 2003, Hopkinson & Vallino 2005). Esta estabilidad en la composición elemental de la MOP suspendida (MOPs), junto con su distribución prácticamente constante a través de la columna de agua del Atlántico Norte (sub)tropical indican que o no se utiliza de forma biótica o que su utilización por parte de la biota del mar profundo se acopla con una producción *in situ* de MOPs.

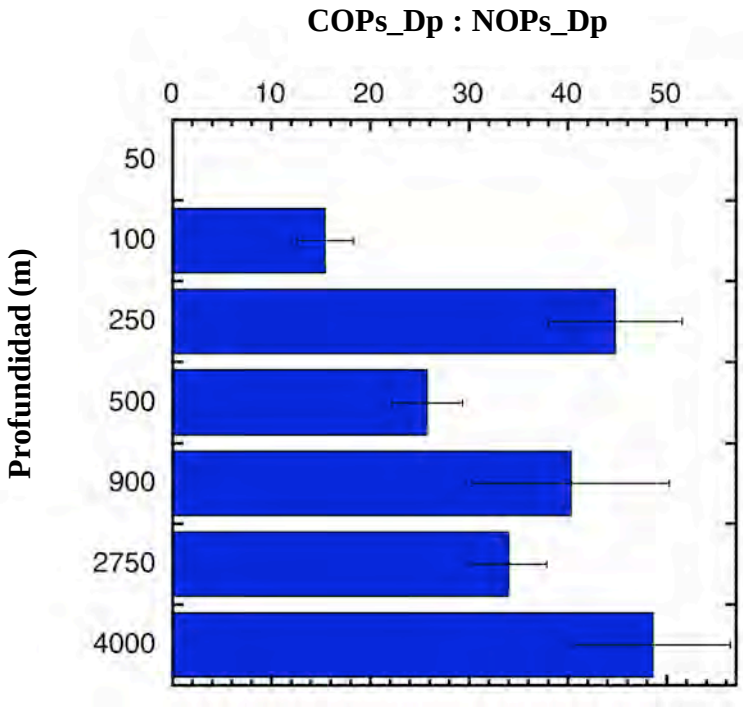


Fig. 2. Promedio (± ES, n = 20 – 46) de la distribución vertical del ratio entre el COPs y el NOPs potencialmente disponibles en los núcleos de las principales masas de agua del Océano Atlántico Norte (sub)tropical

La respiración de los procariotas (R) es el parámetro fundamental que afecta a la demanda de carbono procariota (DCP) en el Atlántico profundo. Esto se debe a que la R es típicamente entre 1-2 órdenes de magnitud mayor que la producción heterotrófica de los procariotas (Reinthaler et al, 2006, **Capítulo II**). Debido a ello, nuestras estimaciones de la DCP dependen en gran medida del factor de conversión empleado para la conversión de la actividad del sistema de transporte de electrones (ETS) a R; i. e. el ratio R : ETS utilizados. Nuestras estimas de DCP han sido calculadas incluyendo un rango de ratios R : ETS que van desde 0.086 a 0.6; obtenidos a partir de cultivos de bacterias en fase senescente (Christensen et al. 1980) y de procariotas activos de la capa mesopelágica de regiones de borde (Arístegui et al. 2005b) respectivamente. La DCP disminuyó en un orden de magnitud desde la base de la zona eufótica hasta la zona batipelágica independientemente del ratio R : ETS utilizado (Tabla 1). La utilización aparente de oxígeno (UAO) calculada para el Atlántico Norte y el Sur (Jenkins 1982, Jenkins & Wallace 1992, Brea 2008) se encuentran dentro del rango de nuestras estimas de DCP (Tabla 1), sugiriendo que nuestras estimaciones de DCP son realistas. El tiempo de renovación (COPs_Dp / DCP) del COPs_Dp puede ser calculado a partir de la DCP y de la concentración de COPs_Dp. El tiempo de renovación del COPs_Dp aumenta desde las aguas superficiales (9 d a 34 d usando un R : ETS de 0.6 y 0.086 respectivamente) a las aguas batipelágicas (200 d a 1372 d) (Fig. 3A). O lo que es lo mismo, la DCP potencialmente utiliza entre un 6-0.2% d^{-1} del reservorio de COPs_Dp a 100 m y a 4000 m de profundidad respectivamente (usando un R : ETS de 0.086) y un 34-6% d^{-1} (usando un R : ETS de 0.6) (Fig. 3B). Incluso si asumimos un R : ETS alto (R : ETS = 0.6), solamente se requeriría por día un promedio del 8 ± 3% del COPs_Dp presente en la capa mesopelágica. Por lo que para mantener en equilibrio la concentración de COPs en las aguas mesopelágicas del Atlántico, una pérdida de menos del 8% del COPs diario debe ser compensada. Pero, ¿de dónde puede provenir está renovación de COPs en las aguas mesopelágicas?.

Los microbios quimioautotróficos realizan una fijación de carbono inorgánico disuelto (CID) a lo largo de todas las aguas profundas del Atlántico (Herndl et al. 2005), contribuyendo con un promedio de 12 ± 5 µmol C m^{-3} d^{-1} en la capa mesopelágica del Atlántico (sub)tropical (Tabla 1). Esta materia orgánica recientemente producida representa una fuente de COPs en las aguas meso- y batipelágicas, debido a que las células microbianas son demasiado pequeñas para sedimentar. Dicho COPs producido

autóctonamente proporciona potencialmente entre un 72% a un 12% (asumiendo un R : ETS de 0.086 y de 0.6 respectivamente) del COPs requerido diariamente para suplir la demanda de carbono heterotrófica de los procariotas de la zona mesopelágica del Atlántico Norte (sub)tropical (Tabla 2, Fig. 3B).

Tabla 1. Comparación de la variación en las distintas profundidades de la demanda de carbono procariota (DCP) asumiendo un R : ETS de 0.086 y de 0.6 respectivamente, de la utilización aparente de oxígeno (UAO) reportadas para el giro subtropical del Atlántico Norte (UAO AtlN) (Jenkins 1982), y para el giro subtropical del Atlántico Sur (UAO AtlS) (Brea 2008), y de las tasas de fijación de CID (producción autotrófica de procariotas – PAP). Todas las tasas metabólicas de encuentran en in μmol C m^{-3} d^{-1}

Profundidad (m)	DCP (R:ETS=0.086)	DCP (R:ETS=0.6)	UAO AtlN	UAO AtlS	PAP
100	58.9	353.4			41.3
250	18.3	109.5	36.5	26.4	32.4
500	11.9	71.5	14.7	16.8	3.7
900	4.2	25.2	6.5	12.3	0.3
2750	3.3	19.9			0.2
4000	4.7	28.5			0.3

Por otro lado, se ha calculado el flujo de COP que se hunde basándonos en un modelo que recopilaba un gran número de datos de trampas de sedimentos del Atlántico Norte (Antia et al. 2001) para estimar la contribución potencial del COP que se hunde y compararlos con la del COPs_Dp. Calculamos el COP que se hunde utilizando las estimas de producción primaria (PP) del giro del Atlántico NE (Longhurst et al. 1995) (28 mmol C m^{-2} d^{-1}), del giro occidental (Steinberg et al. 2001) (35 mmol C m^{-2} d^{-1}) y usando un límite superior de 50 mmol C m^{-2} d^{-1} (obtenido para todo el Atlántico Norte (Longhurst et al. 1995) incluyendo las regiones templadas donde la PP es mayor). La contribución potencial del COP que se hunde contribuye alrededor de un 4-6% de la DCP diaria cuando se asume una PP de 28 y de 35 mmol C m^{-2} d^{-1} respectivamente (Tabla 2). Sólo cuando se utiliza una PP superficial claramente irreal para esta región centro-oceánica (sub)tropical de 50 mmol C m^{-2} d^{-1}, la contribución del COP que se hunde a la DCP se iguala (12%) a la de la fijación oscura de CO_2 (Fig. 3C, Tabla 2). Aunque en la capa batipelágica las tasa de fijación de CID son bajas, incluso en esta zona la contribución del COP que se hunde a la DCP no excede la contribución

proveniente de la fijación de CID (Fig. 3C, Tabla 2). Por lo que, una fracción significativa (al menos similar a la procedente del COP que se hunde) del carbono orgánico requerido por la comunidad microbiana de las zonas meso- y batipelágicas puede ser suministrada por la fijación quimioutotrófica de CO_2 en el interior del Atlántico Norte (sub)tropical.

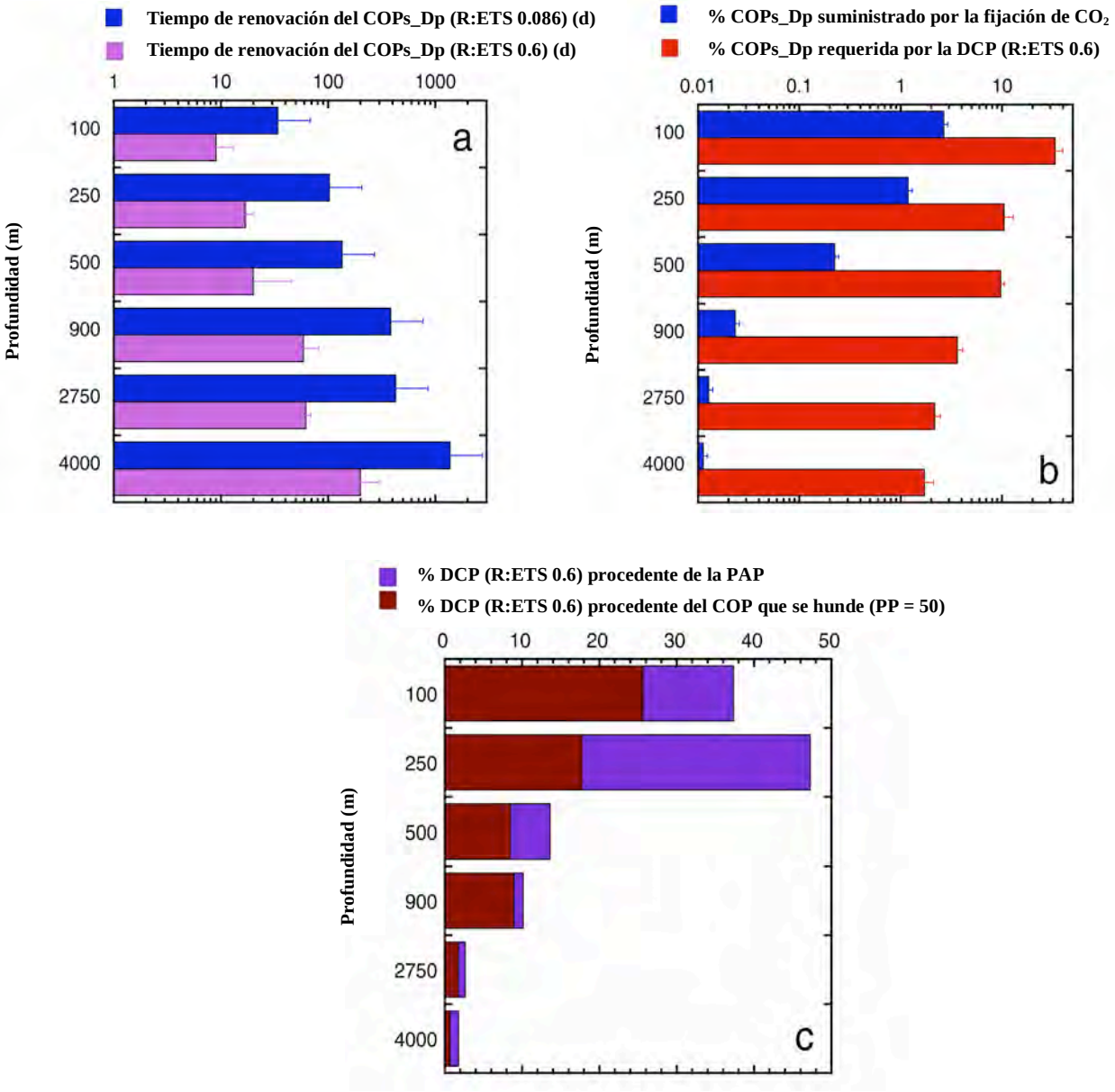


Fig. 3. Variación del (A) tiempo de renovación medio (± ES, n= 18 – 39) del COPs potencialmente disponible (COPs_Dp) en profundidad (asumiendo un R : ETS de 0.086 y de 0.6), (B) porcentaje de este COPs_Dp requerido para suplir la demanda de carbono procariota (DCP) diaria (asumiendo un R : ETS de 0.6), y (C) porcentaje de la DCP total cubierta por la fijación oscura de CO_2 (producción autotrófica de procariotas – PAP) y del COP que se hunde (asumiendo un R : ETS conservador de 0.6 y una producción primaria superficial de 50 mmol C m^{-2} d^{-1})

Tabla 2. Contribución potencial (en %) de la fijación oscura de CO_2 (APA) y del COP que se hunde disponible a la DCP (asumiendo un R : ETS de 0.086 y de 0.6). La contribución procedente del POC que se hunde fue estimada a partir de un modelo del flujo de COP (Antia et al. 2001) asumiendo unas producciones primarias superficiales (PP) de 28 mmol C m^{-2} d^{-1} (giro subtropical del Atlántico NE (Longhurst et al. 1995)), de 35 mmol C m^{-2} d^{-1} (giro occidental del Atlántico N (Steinberg et al. 2001)) y de 50 mmol C m^{-2} d^{-1} (Atlántico N (Longhurst et al. 1995))

% contribución a la DCP asumiendo un R : ETS = 0.086				
Profundidad	**PAP**	**COP que se hunde (PP=28)**	**COP que se hunde (PP=35)**	**COP que se hunde (PP=50)**
100	70	55	82	154
250	177	38	57	106
500	31	18	27	51
900	7	19	29	54
2750	5	4	6	10
4000	7	1	2	4
Promedio mesopelágico	**72**	**25**	**37**	**70**
Promedio batipelágico	**6**	**3**	**4**	**7**
Ratio mesopelágico entre PAP : COP que se hunde		2.8	1.9	1.0
Ratio batipelágico entre PAP : COP que se hunde		2.4	1.6	0.9
% contribución a la DCP asumiendo un R : ETS = 0.6				
Profundidad	**PAP**	**COP que se hunde (PP=28)**	**COP que se hunde (PP=35)**	**COP que se hunde (PP=50)**
100	12	9	14	26
250	30	6	9	18
500	5	3	5	8
900	1	3	5	9
2750	1	1	1	2
4000	1	0	0	1
Promedio mesopelágico	**12**	**4**	**6**	**12**
Promedio batipelágico	**1**	**0**	**1**	**1**
Ratio mesopelágico entre PAP : COP que se hunde		2.8	1.9	1.0
Ratio batipelágico entre PAP : COP que se hunde		2.4	1.6	0.9

Tal y como se ha demostrado previamente, es muy probable que la fuente principal de energía para la fijación de CID por parte de los procariotas provenga de la oxidación de amonio realizada por las *Crenarchaeotas*; ya que el gen *amoA* de las *Archaeas* es mucho más abundante que el gen *amoA* de las *Bacterias* en la capa mesopelágica del Atlántico Norte (Agogué et al. 2008). Se he mostrado recientemente, basándose en las características del radiocarbono de los lípidos de archaeas, que un 83% del carbono de las archaeas tiene un origen autotrófico en la zona mesopelágica subtropical del Pacífico Norte (a una profundidad de 670 m) (Ingalls et al. 2006). Las propiedades del radiocarbono del ADN de las *Archaeas* de la aguas mesopelágicas del Pacífico (entre 670 y 915 metros de profundidad) han permitido diferenciar entre los tres reservorios fundamentales de carbono que se encuentran potencialmente disponibles para los procariotas: COD fresco recientemente liberado a partir de COP ($\Delta^{14}C$ > +50‰), CID ambiental ($\Delta^{14}C$ ~ -200 to -100 ‰), y COD ambiental envejecido ($\Delta^{14}C$ = -525‰) (Hansman et al. 2009). Estos autores concluyeron que ambos, el CID y el COD fresco (presumiblemente generado a partir del COP), son utilizados en gran medida, mientras que el COD ambiental no un substrato importante para los procariotas mesopelágicos (Hansman et al. 2009). Existen también evidencias de una producción quimiolitotrófica considerable en el COP que se hunde recogido con trampas de sedimentos en el Pacífico Norte mesopelágico (entre 100-750 metros de profundidad) (Karl et al. 1984). En dicho estudio, la contribución quimiolitotrófica fue responsable de entre un 7-90% de la producción microbiana total, indicando otra vez la importancia potencial de la producción de carbono quimiolitotrófica como fuente de carbono y energía de reciente generación en la zona mesopelágica.

En resumen, la percepción actual de la dependencia de la actividad heterotrófica microbiana del océano oscuro en el COP que se sedimenta y el resultante desajuste entre el aporte de carbono orgánico y la demanda (Reinthaler et al. 2006, Steinberg et al. 2008, Burd et al. en prensa, **Capítulo II**) necesitan ser revisados. El gran y prácticamente constante stock de COPs necesita ser considerado como un reservorio potencialmente disponible de COP para los microbios heterotróficos de las aguas profundas. Hemos mostrado que sólo una pequeña proporción del COPs potencialmente utilizable es requerida diariamente para mantener la demanda de carbono de los microbios heterotróficos del océano profundo. Entre un 12-72% de la cantidad de COPs requerida por los microheterótrofos en el océano mesopelágico es potencialmente

suministrado por quimiolitótrofos de aguas profundas mediante la fijación de CID. Consecuentemente, el desajuste aparentemente existente entre el aporte y la demanda heterotrófica de materia orgánica en la capa mesopelágica puede ser considerablemente menor de lo que se pensaba. Futuras investigaciones deberían centrarse en redefinir la calidad nutritiva del COPs en comparación con el COP que se hunde, y en obtener una visión más refinada de la producción quimiolitoautotrófica de materia orgánica suspendida de reciente generación en el interior del océano. Estas rutas redefinidas de las fuentes y sumideros de materia orgánica en el océano oscuro nos podrían hacer llegar a un entendimiento mecanicista de la estructura y actividad de la red trófica del océano profundo y, en último lugar, nos permitiría mejorar los modelos sobre el papel del océano oscuro en el ciclo global del carbono.

CONCLUSIONES

Las principales conclusiones que se obtienen de esta tesis son:

1. Existe una alta variabilidad en la estructura de la comunidad procariota y en su actividad en las aguas superficiales y profundas de la Zona Costera de Transición del NO de África – Islas Canarias, lo que sugiere una naturaleza heterogénea del océano oscuro.

    a. Se encontró una sustitución clara de *Bacterias* por *Archaeas* (fundamentalmente Crenarchaeota) a lo largo de gradientes tróficos (hacia océano abierto y en profundidad).

    b. Las distintas masas de agua juegan un papel clave a la hora de estructurar el metabolismo de los procariotas. Un ejemplo fue encontrado en el caso de las masas de agua intermedias, hallando menores tasas de incorporación de leucina en las capas donde existían intrusiones de Agua Antártica Intermedia.

2. Se encontró una fuerte relación entre el carbono orgánico particulado suspendido y la respiración de los procariotas en las aguas oscuras del Atlántico Norte (sub)tropical. Este descubrimiento, junto con el intenso desajuste existente entre la demanda de carbono procatiota y el flujo de carbono orgánico particulado (COP) que se hunde, sugieren: i) un control "bottom-up" del metabolismo de los procariotas del océano profundo por el reservorio de materia orgánica particulada suspendida, y ii) un modo de vida de los procariotas del océano oscuro preferentemente adherido a partículas.

3. Se halló un incremento en la actividad enzimática extracelular (AEE) específica por célula desde la base de la capa eufótica hasta las aguas batipelágicas del Atlántico Norte (sub)tropical.

    a. Este aumento en la AEE específica en profundidad fue acompañado por un incremento en la respiración por célula y una disminución en la incorporación de leucina por célula. Este descubrimiento, junto con el

aumento de la constante de semisaturación (K_m) de las enzimas extracelulares y del ratio V_{max} / K_m, indican una adaptación de la AEE para con la naturaleza refractaria de la materia orgánica del océano oscuro.

b. Se encontró un incremento en la actividad por célula de la fosfatasa alcalina en profundidad, indicando que dicha actividad no se relaciona con la disponibilidad de fosfato en el océano oscuro. De hecho, la fosfatasa alcalina parece ser utilizada por los procariotas de aguas profundas como un medio para conseguir C a partir de la materia orgánica, en vez de utilizarla para obtener fosfato directamente.

4. La AEE se encuentra fundamentalmente en forma disuelta en las aguas profundas del Océano Atlántico Central, donde se encontró una relación entre la AEE (las fracciones disueltas y particuladas) y la producción heterotrófica procariota y el reservorio de materia orgánica particulada. Dichos descubrimiento de nuevo sugieren un estilo de vida predominantemente pegado a partículas para los procariotas del océano profundo.

5. Los remolinos mesoescalares generados por islas oceánicas pueden tener un profundo impacto sobre la distribución y la función de los procariotas en el océano. Sin embargo, incluso en estas estructuras mesoscalares, caracterizadas por una mayor productividad en sus aguas superficiales que fuera de los remolinos, el efecto sobre la distribución y la función de los procariotas se restringe a las zonas epi- y mesopelágicas. Ello se debe probablemente a que la mayor parte del COP que se hunde se consume en los primeros 1000 m de la columna de agua, indicando una rápida atenuación de la influencia del COP que se hunde en los microbios batipelágicos.

6. Como fue el caso para otros parámetros determinados en esta tesis, la eficiencia de crecimiento procariota (ECP) y el factor de conversión de leucina a carbono determinado empíricamente (LeuFC) presentaron una alta variabilidad en las aguas mesopelágicas del Océano Atlántico Nordeste, exhibiendo rangos similares a los reportados para las aguas superficiales de regiones oligotróficas.

7. El carbono orgánico particulado suspendido y la fijación oscura de carbono inorgánico de los procariotas de aguas profundas pueden ayudar a reducir el desequilibrio existente entre el carbono orgánico requerido por los microbios y el aporte de materia orgánica al océano oscuro. Asumiendo una alta demanda de carbono procariota (empleando un R : ETS de 0.6), se requiere el consumo de menos del 8% del reservorio de COP suspendido diariamente para compensar la demanda de carbono heterotrófica microbiana de las aguas mesopelágicas. Nuestros números indican asimismo que la fijación oscura de carbono inorgánico disuelto (CID) puede aportar diariamente entre un 12% y un 72% de este carbono orgánico requerido, mientras que el COP que se hunde sólo puede aportar entre un 4-12%. Consecuentemente, el desequilibrio realmente existente entre el aporte de carbono orgánico y la demanda no es tan fuerte como se ha asumido previamente si incorporamos esta, previamente ignorada, producción oscura de materia orgánica particulada autóctona del océano oscuro.

FUTURAS LÍNEAS DE INVESTIGACIÓN

Partiendo de los resultados obtenidos en esta tesis hay varios aspectos concernientes a la oceanografía microbiana del océano oscuro que necesitarían ser resueltos en un futuro próximo.

1. El objetivo primordial debería ser el estudio de la relación entre las partículas y los procariotas de aguas profundas. Varias evidencias apuntan hacia un estilo de vida preponderantemente adherido a partículas para los procariotas (DeLong et al. 2006, **Capítulo II**, **Capítulo IV**). La recientemente descubierta alta abundancia de geles en los océanos puede permitir interacciones espaciales próximas entre procariotas y el continuo de la materia orgánica. Sin embargo, no es posible determinar la extensión de estas interacciones con las técnicas de muestreo de las que se dispone actualmente debido a la frágil naturaleza de estas partículas del océano profundo. Nuevas técnicas de muestreo son necesarias para distinguir entre microbios libres y asociados a partículas en el océano oscuro.

2. Los factores de conversión son necesarios si queremos comprender y cuantificar mejor los flujos de carbono mediados por los procariotas en el océano oscuro. Nosotros hemos reportado las primeras eficiencias de crecimiento procariotas (ECP) calculadas a partir de factores de conversión de leucina a carbono obtenidos empíricamente (LeuFC) para el océano profundo (**Capítulo VI**). Debido a la alta variabilidad presentada por estos dos parámetros (ECP y LeuFC), y al papel crucial que tiene el océano oscuro en los flujos de carbono globales, es imperativo incrementar en gran medida el número de determinaciones de factores de conversión. Solamente entonces seremos capaces de determinar de una forma precisa el metabolismo procariota, y con ello, el ciclo biogeoquímico global del carbono.

3. Un mayor esfuerzo debería dirigirse a estudiar el efecto de la presión hidrostática sobre las tasas metabólicas de los procariotas que habitan en el océano oscuro, debido a que, hasta la fecha, solamente se han referenciado resultados contradictorios (Jannasch & Wirsen 1982, Tamburini et al. 2002).

Dicho efecto de la presión debería de ser tenido en cuenta no sólo en la estimación rutinaria de tasas metabólicas, sino también en la determinación de los propios factores de conversión involucrados.

4. Aunque no fuera el tema fundamental de esta tesis, más esfuerzo debería de ser dirigido también hacia el estudio de la red trófica microbiana del océano oscuro. Existen muy pocos trabajos concernientes a las tasas de consumo y lisis de procariotas por parte de nanoflagelados heterotróficos y virus respectivamente. Es muy posible que las interacciones existentes entre los componentes individuales de la red trófica microbiana y la materia particulada y coloidal del océano oscuro sean muy diferentes de las que presentan los agregados de aguas superficiales. Dichas interacciones son cruciales para modelar los ciclos de nutrientes y de carbono en el océano oscuro.

5. Una mejor cuantificación de las fuentes y sumidero de carbono del océano oscuro sería necesaria para entender mejor los flujos globales de carbono. Quizás algunos de los asuntos mencionados en esta sección pueden ayudar a reducir el presente desequilibrio (e. g. efecto de la presión hidrostática sobre las tasas metabólicas, vida microbiana concentrada en partículas, distintos factores de conversión, rutas metabólicas novedosas,…). Una visión más clarificada de la producción quimiolitoautotrófica de carbono orgánico suspendido en el interior del océano sería recomendable. En cualquier caso, sería rotundamente un tema de importancia capital el intentar ajustar el desequilibrio aparentemente existente entre la demanda microbiana de carbono y el aporte de materia orgánica en las capas meso- y batipelágicas.

6. Debería de realizarse un incremento generalizado de las bases de datos de los parámetros de diversidad y función de los procariotas para poder permitirnos alcanzar una visión refinada de las distribuciones espaciales a gran escala en la regulación de los procesos microbianos de aguas profundas. Una opción podría ser seguir el modelo de Longhurst de las provincias biogeográficas, definiendo los diversos compartimentos que forman el océano oscuro.

7. Sería recomendable también conectar de una manera más apropiada los avances acometidos recientemente en las aproximaciones moleculares con las medidas

actuales de flujos. Conectando la diversidad con la función, relacionando la información genética con los ciclos biogeoquímicos del océano profundo. Finalmente, el desarrollo de técnicas moleculares debería de ser utilizado también para estudiar las adaptaciones de los distintos miembros de la comunidad a las condiciones bióticas (e. g. mortalidad,…) y abióticos (e. g. presión hidrostática, temperatura, nutrientes,…) del océano oscuro.

REFERENCES

(from introduction, discussion and Spanish Summary)

ANNEXES

REFERENCES / BIBLIOGRAFÍA

Agogué H, Brink M, Dinasquet J, Herndl GJ (2008) Major gradients in putatively nitrifying and non-nitrifying Archaea in the deep North Atlantic. Nature 456:788-791

Alldredge AL, Gotschalk C (1988) In situ settling behavior of marine snow. Limnol. Oceanogr. 33:339-351

Alldredge AL, Silver MW (1988) Characteristics, dynamics and significance of marine snow. Prog. Oceanogr. 20:41-82

Alldredge AL, Cole JJ, Caron DA (1986) Production of heterotrophic bacteria inhabiting macroscopic organic aggregates (marine snow) from surface waters. Limnol. Oceanogr. 31:68-78

Alldredge AL, Passow U, Logan BE (1993) The existence, abundance, and significance of large transparent exopolymer particles in the ocean. Deep Sea Res. I 40:1131-1140

Alonso-Saéz L, Gasol JM, Arístegui J, Vilas JC, Vaqué D, Duarte CM, Agusti S (2007) Large-scale variability in surface bacterial carbon demand and growth efficiency in the subtropical northeast Atlantic Ocean. Limnol. Oceanogr. 52:533-546

Amon RMW, Benner R (1994) Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. Nature 369:549-552

Antia AN, Koeve W, Fischer G, Blanz T, Schulz-Bull D, Scholten J, Neuer S, Kremling K, Kuss J, Peinert R, Hebbeln D, Bathmann U, Conte M, Fehner U, Zeitzschel B (2001) Basin-wide particulate carbon flux in the Atlantic Ocean: Regional export patterns and potential for atmospheric CO_2 sequestration. Glob. Biogeochem. Cy. 15:845-862

Arístegui J, Montero MF (1995) The relationship between community respiration and ETS activity in the ocean. J. Plankton Res. 17:1563-1571

Arístegui J, Duarte CM, Agusti S, Doval M, Alvarez-Sagado XA, Hansell DA (2002) Dissolved organic carbon support of respiration in the dark ocean. Science 298:1967

Arístegui J, Agustí S, Middelburg JJ, Duarte CM (2005a) Respiration in the mesopelagic and bathypelagic zones of the ocean, In: Williams PJleB & del Giorgio PA (eds) Respiration in Aquatic Ecosystems. Oxford University Press, New York

Arístegui J, Duarte CM, Gasol JM, Alonso-Sáez L (2005b) Active mesopelagic prokaryotes support high respiration in the subtropical northeast Atlantic Ocean. Geophys. Res. Lett. 32:L03608, doi:03610.01029/02004GL021863

Arístegui J, Gasol JM, Duarte CM, Herndl GJ (2009) Microbial Oceanography of the dark ocean's pelagic realm. Limnol. Oceanog. 54:1501-1529

Azam F (1998) Microbial control of oceanic carbon flux: the plot thickens. Science 280:694-696

Azam F, Malfatti F (2007) Microbial structuring of marine ecosystmems. Nat. Rev. Microbiol. 5:782-791

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10:257-263

Bartlett DH, Lauro FM, Eloe EA (2007) Microbial adaptation to high pressure. In: Gerday C, Glandsdorf N (eds) Physiology and biochemistry of extremophiles. American Society for Microbiology Press, Washington, D. C., p 333-348

Bartlett DH, Ferguson G, Valle G (2008) Adaptation of the psychrotolerant piezophile *Photobacterium profundum* strain SS9. In: Michiels C, Bartlett DH, Aertsen A (eds) High-pressure microbiology. ASM Press, Washington, D. C., p 319-337

Bell RT (1990) An explanation for the variability in the conversion factor deriving bacterial cell production from incorporation of [^{3}H]thymidine. Limnol. Oceanogr. 35:910-915

Benner R (2002) Chemical composition and reactivity. In: Hansell DA, Carlson CA (eds) Biogeochemistry of marine dissolved organic matter. Elsevier Science, New York, p 59-90

Benner R, Strom M (1993) A critical evaluation of the analytical blank associated with DOC measurements by high-temperature catalytic oxidation. Mar. Chem. 41:153-160

Brea S (2008) Circulación, mezcla, ventilación y mineralización de las aguas del Atlántico Sur. Ph. D. Thesis. Univ. of Vigo

Breitbart M, Middelboe M, Rohwer F (2008) Marine viruses: Community dynamics, diversity and impact on microbial processes. In: Kirchman DL (ed) Microbial Ecology of the Oceans, 2nd edition. John Wiley and Sons, Hoboken, N. J., p 443-479

Buesing N, Marxsen J (2005) Theoretical and empirical conversion factors for determining bacterial production in freshwater sediments via leucine incorporation. Limnol. Oceanog. 3:101-107

Buesseler KO, Boyd PW (2009) Shedding light on processes that control particle export and flux attenuation in the twilight zone of the open ocean. Limnol. Oceanog. 54:1210-1232

Buesseler KO, Lamborg CH, Boyd PW, Lam PJ, Trull TW, Bidigare RR, Bishop JKB, Casciotti KL, Dehairs F, Elskens M, Honda M, Karl DM, Siegel DA, Silver MW, Steinberg DK, Valdes J, Mooy BV, Wilson S (2007) Revisiting carbon flux through the ocean's twilight zone. Science 316:567-570

Burd AB, Hansell DA, Steinberg DK, Anderson TR, Arístegui J, Baltar F, Beaupré SR, Buesseler KO, DeHairs F, Jackson GA, Kadko DC, Koppelmann R, Lampitt RS, Nagata T, Reinthaler T, Robinson C, Robinson BH, Tamburini C, Tanaka T (in press) Assesing the apparent imbalance between geochemical and biochemical indicators of meso- and bathypelagic biological activity: What the @$#! is wrong with the present calculations of carbon budget? Deep-Sea Res.

Calvo-Díaz A, Morán XAG (2009) Empirical Leucine-to-Carbon Conversion Factors for Estimating Heterotrophic Bacterial Production: Seasonality and

Predictability in a Temperature Coastal Ecosystem. Appl. Environ. Microb. 75:3216-3221

Carlson CA, Ducklow HW, Michaels AF (1994) Annual flux of dissolved organic carbon from the euphotic zone in the northwestern Sargasso Sea. Nature 371:405-408

Carlson CA, Giovannoni SJ, Hansell DA, Goldberg SJ, Parsons R, Vergin K (2004) Interactions among dissolved organic carbon, microbial processes, and community structure in the mesopelagic zone of the northwestern Sargasso Sea. Limnol. Oceanogr. 49:1073-1083

Carlson CA, del Giorgio PA, Herndl GJ (2007) Microbes and the Disipation of Energy and Respiration: From Cells to Ecosystems. Oceanography 20:89-100

Carlucci AF, Craven DB, Robertson KJ (1986) Microheterotrophic utilization of dissolved free amino acids in depth profiles of Southern California Borderland basin waters. Oceanolog. Acta 9:89-96

Cho BC, Azam F (1988) Major role of bacteria in biogeochemical fluxes in the ocean's interior. Nature 332:441-443

Christensen JP, Owens TG, Devol AH, Packard TT (1980) Respiration and physiological state in marine bacteria. Mar. Biol. 55:267-276

Church MJ, DeLong EF, Ducklow HW, Karner MB, Preston CM, Karl DM (2003) Abundance and distribution of planktonic *Archaea* and *Bacteria* in the waters west of the Antarctic Peninsula. Limnol. Oceanogr. 48:1893-1902

del Giorgio PA, Cole JJ (1998) Bacterial growth yield efficiency in natural aquatic systems. Annu. Rev. Ecol. Syst. 29:503-541

del Giorgio PA, Cole JJ (2000) Bacterial energetics and growth efficiency. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, p 289-325

del Giorgio PA, Duarte CM (2002) Respiration in the open ocean. Nature 420:379-384

del Giorgio PA, Gasol JM (2008) Physiological structure and single-cell activity in marine bacterioplankton. In: Kirchman DL (ed) Microbial ecology of the ocean. Wiley-Liss, New York, p 243-298

DeLong EF, Preston CM, Mincer T, Rich V, Hallam SJ, Frigaard NU, Martínez A, Sullivan MB, Edwards R, Brito BR, Chisholm SW, Karl DM (2006) Community genomics among stratified microbial assemblages in the ocean's interior. Science 311:496-503

Druffel ERM, Griffin S, Bauer JE, Wolgast DM, Wang X-C (1998) Distribution of particulate organic carbon and radiocarbon in the water column for the upper slope to the abyssal northeastern Pacific Ocean. Deep-Sea Res. II 45:667-687

Ducklow H (2000) Bacterial production and biomass in the oceans. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, p 85-120

Engel A, Thoms S, Riebesell U, Rochelle-Newall E, Zondervan I (2004) Polysaccharide aggregation as a potential sink of marine dissolved organic carbon. Nature 428:929-932

Falcioni T, Papa S, Gasol JM (2008) Evaluating the flow-fytometric nucleic acid double-staining protocol in realistic rituations of planktonic bacterial death. Appl. Environ. Microb. 74:1767-1779

Fisher MM, Triplett EW (1999) Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Appl. Environ. Microbiol. 65:4630-4636

Francisco DE, Mah RA, Rabin AC (1973) Acridine orange-epifluorescence technique for counting bacteria in natural waters. Trans. Amer. Microsc. Soc. 92:416-421

Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. Nature 399:541-548

Fuhrman J (2000) Impact of viruses on bacterial processes. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, p 327-350

Gasol JM, Zweifel UL, Peters F, Fuhrman JA, Hagström Å (1999) Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl. Environ. Microbiol. 65:4475-4483

Gasol JM, Alonso-Sáez L, Vaqué D, Baltar F, Calleja ML, Duarte CM, Arístegui J (2009) Mesopelagic prokaryotic bulk and single-cell heterotrophic and community composition in the NW Africa-Canary Islands coastal-transition zone. Progr. Oceanogr. 83: 189-196

Goldman JC, Caron DA, Dennett MR (1987) Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. Limnol. Oceanogr. 32:1239-1252

Gregori G, Citterio S, Ghani A, Labra M, Sgorbati S, Brown S, Denis M (2001) Resolution of viable and membrane-compromised bacteria in freshwater and marine waters based on analytical flow cytometry and nucleic acid double staining. Appl. Environ. Microbiol. 67:4662-4670

Gruber DF, Simijouw J-P, Seitzinger SP, Taghon GL (2004) Dynamics and characterization of refractory dissolved organic matter produced by a pure bacterial culture in an experimental predator-prey system. Appl. Environ. Microbiol. 72:4184-4191

Hansell DA, Carlson CA (1998) Deep-ocean gradients of dissolved organic carbon. Nature 395:263-266

Hansell DA, Ducklow HW (2003) Bacterioplankton distribution and production in the bathypelagic ocean: Directly coupled to particulate organic carbon export? Limnol. Oceanogr. 48:150-156

Hansman RL, Griffin S, Watson JT, Druffel ERM, Ingalls AE (2009) The radiocarbon signature of microorganisms in the mesopelagic ocean. Proc. Natl. Acad. Sci. USA 106:6513-6518

Heissenberger A, Herndl GJ (1994) Formation of high molecular weight material by free-living marine bacteria. Mar. Ecol. Prog. Ser. 111:129-135

Heissenberger A, Leppard GG, Herndl GJ (1996) Relationship between the intracellular integrity and the morphology of the capsular envelope in attached and free-living marine bacteria. Appl. Environ. Microbiol. 62:4521-4528

Herndl GJ, Reinthaler T, Teira E, Aken Hv, Veth C, Pernthaler A, Pernthaler J (2005) Contribution of *Archaea* to total prokaryotic production in the deep Atlantic Ocean. Appl. Environ. Microbiol. 71:2303-2309

Herndl GJ, Agogué H, Baltar F, Reinthaler T, Sintes E, Varela MM (2008) Regulation of aquatic microbial processes: the "microbial loop" of the sunlit surface waters and the dark ocean dissected. Aquat. Microb. Ecol. 53:59-68

Hewson I, Fuhrman JA (2004) Richness and diversity of bacterioplankton species along an estuarine gradient in Moreton Bay, Australia. Appl. Environ. Microbiol. 70:3425-3433

Hewson I, Steele JA, Capone DG, Fuhrman JA (2006) Remarkable heterogeneity in meso- and bathypelagic bacterioplantkon assemblage composition. Limnol. Oceanogr. 51:1274-1283

Hobbie JE, Daley RJ, Jasper S (1977) Use of Nuclepore filters for counting bacteria by epifluorescence microscopy. Appl. Environ. Microbiol. 33:1225-1228

Hopkinson J, C.S., Vallino JJ (2005) Efficient export of carbon to the deep ocean through dissolved organic matter. Nature 433:142-145

Hoppe H-G (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Mar. Ecol. Prog. Ser. 11:299-308

Hoppe H-G, Arnosti C, Herndl GJ (2002) Ecological significance of bacterial enzymes in the marine environment. In: Burns RG, Dick RP (eds) Enzymes in the environment: activity, ecology, and applications. Marcel Dekker, Inc., New York, p 73-108

Ingalls AE, Shah SR, Hansman RL, Aluwihare LI, Santos GM, Druffel ERM, Pearson A (2006) Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. Proc. Natl. Acad. Sci. USA 103:6442-6447

Ivars-Martínez E, Martín-Cuadrado AB, D'Auria G, Mira A, Ferriera S, Johnson J, Friedman R, Rodriguez-Valera F (2008) Comparative genomics of two ecotypes of the marine planktonic copiotroph *Alteromonas macleodii* suggests alternative lifestyles associated with different kinds of particulate organic matter. The ISME J. doi:10.1038/ismej.2008.1074

Jannasch HW, Jones GE (1959) Bacterial populations in sea water as determined by different methods of enumeration. Limnol. Oceanogr. 40:436-441

Jannasch HW, Wirsen CO (1982) Microbial activities in undecompressed and decompressed deep-seawater samples. Appl. Environ. Microbiol. 43:1116-1124

Jenkins WJ (1982) Oxygen utilization rates in the North Atlantic Subtropical Gyre and primary production in oligotrophic systems. Nature 300:246-248

Jenkins WJ, Wallace DWR (1992) Tracer based inferences if new primary production in the Sea, Vol. Plenum, New York

Jürgens K, Massana R (2008) Protistan grazing on marine bacterioplankton. In: Kirchman DL (ed) Microbial Ecology of the Oceans, 2nd edition. John Wiley and Sons, Hoboken, N. J., p 383-441

Kaiser K, Benner R (2008) Major bacterial contribution to the ocean reservoir of detrital organic carbon and nitrogen. Limnol. Oceanogr. 53:99-112

Kaltenböck E, Herndl GJ (1992) Ecology of amorphous aggregations (marine snow) in the Northern Adriatic Sea: IV. Dissolved nutrients and the autotrophic community associated with marine snow. Mar. Ecol. Prog. Ser. 87:147-159

Karl DM (1980) Cellular nucleotide measurements and applications in microbial ecology. Microbiol. Rev. 44:739-796

Karl DM, Knauer GA, Martin JH, Ward BB (1984) Bacterial chemolithotrophy in the ocean is associated with sinking particles. Nature 309:54-56

Karl DM, Knauer GA, Martin JH (1988) Downward flux of particulate organic matter in the ocean: a particle decomposition paradox. Nature 332:438-441

Karner MB, DeLong EF, Karl DM (2001) Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature 409:507-510

Kepkay PE (2000) Colloids and the ocean carbon cycle. In: Wangersky P (ed) Handbook of Environmental Chemistry, Vol 5. Springer, Berlin, p 35-56

Kirchman D, K'Ness E, Hodson R (1985) Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. 49:599-607

Kirchman DL, Ducklow HW (1993) Estimating conversion factors for thymidine and leucine methods for measuring bacterial production. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, p 513-517

Kirchman DL, Elifantz H, Dittel AI, Malmstrom RR, Cottrell MT (2007) Standing stock and activity of Archaea and Bacteria in the western Arctic Ocean. Limnol. Oceanogr. 52:495-507

Könneke M, Bernhard AE, Torre JRdl, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437:543-546

Lauro F, Chastain RA, Blankenship LE, Yayanos AA, Bartlett DH (2006) The unique 16S rRNA genes of piezophiles reflect both phylogeny and adaptation. Appl. Environ. Microb 73:838-845

Libes SM (1992) An introduction to marine biogeochemistry, Vol. John Wiley & Sons, New York

Lignell R (1990) Excretion of organic carbon by phytoplankton: its relation to algal biomass, primary productivity and bacterial secondary productivity in the Baltic Sea. Mar. Ecol. Prog. Ser. 68:85-99

Longhurst A, Sathyendranath S, Platt T, Caverhill C (1995) An estimate of global primary production in the ocean from satellite radiometer data. J. Plankton Res. 17:1245-1271

López-Garcia P, Rodriguez-Valera F, Pedrós-Alió C, Moreira D (2001) Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature 409:603-606

López-López A, Bartual SG, Stal L, Onyshenko O, Rodriguez-Valera F (2005) Genetic analysis of housekeeping genes reveals a deep-sea ecotype of *Alteromonas macleodii* in the Mediterranean Sea. Environ. Microbiol. 7:649-659

Martin JH, Knauer GA, Karl DM, Broenkow WW (1987) VERTEX: carbon cycling in the northeast Pacific. Deep-Sea Res. 34:267-285

Martín-Cuadrado AB, López-García R, Alba JC, Moreira D, Monticelli L, Strittmatter A, Gottschalk G, Rodriguez-Valera F (2007) Metagenomics of the Deep Mediterranean, a Warm Bathypelagic Habitat. Plos ONE 2:e914. doi: 910.1371/journal.pone.0000914

Moeseneder MM, Winter C, Herndl GJ (2001a) Horizontal and vertical complexity of attached and free-living bacteria of the eastern Mediterranean Sea, determined by 16S rDNA and 16S rRNA fingerprints. Limnol. Oceanogr. 46:95-107

Moeseneder MM, Winter C, Arrieta JM, Herndl GJ (2001b) Terminal restriction fragment length polymorphism (T-RFLP) screening of a marine archaeal library to determine the different phylotypes. J. Microbiol. Meth. 44:159-172

Müller-Niklas G, Schuster S, Kaltenböck E, Herndl GJ (1994) Organic content and bacterial metabolism in amorphous aggregations of the northern Adriatic Sea. Limnol. Oceanogr. 39:58-68

Nagata T (2000) Production mechanisms of dissolved organic matter. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, p 121-152

Nagata T (2008) Organic matter-bacterial interactions in seawater. In: Kirchman DL (ed) Microbial Ecology of the Oceans, 2nd edition. John Wiley and Sons, Hoboken, N. J., p 207-241

Nagata T, Fukuda H, Fukuda R, Koike I (2000) Bacterioplankton distribution and production in the deep Pacific waters: large-scale geographic variations and possible coupling with sinking particle fluxes. Limnol. Oceanogr. 45:426-435

Obernosterer I, Herndl GJ (1995) Phytoplankton extracellular release and bacterial growth: dependence on the inorganic N:P ratio. Mar. Ecol. Prog. Ser. 116:247-257

Passow U (2000) Formation of transparent exopolymer particles, TEP from dissolved precursor material. Mar. Ecol. Prog. Ser. 192:1-11

Pomeroy LR (1974) The ocean's food web, a changing paradigm. BioScience 24:499-504

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25:943-948

Pulido-Villena E, Reche I (2003) Exploring bacterioplankton growth and protein synthesis to determine conversion factors across a gradient of dissolved organic matter. Microb. Ecol. 46:33-42

Pérez MT, Pausz C, Herndl GJ (2003) Major shift in bacterioplankton utilization of enantiomeric amino acids between surface waters and the ocean’s interior. Limnol. Oceanogr. 48:755-763

Reinthaler T, Aken Hv, Veth C, Williams PJl, Arístegui J, Robinson C, Lebaron P, Herndl GJ (2006) Prokaryotic respiration and production in the meso- and bathypelagic realm of the eastern and western North Atlantic basin. Limnol. Oceanogr. 51:1262-1273

Repeta DJ, Gagosian RB (1984) Transformation reactions and recycling of carotenoids and chlorins in the Peru upwelling region (15ºS, 75ºW). Geochim. Cosmochim. Acta. 48:1265-1277

Robinson C (2008) Heterotrophic bacterial respiration. In: Kirchman DL (ed) Microbial Ecology of the Oceans, 2nd edition. John Wiley and Sons, Hoboken, N. J., p 299-334

Robinson C, Williams PJl (2005) Respiration and its measurements in surface marine waters. In: Williams PJleB & del Giorgio PA (eds) Respiration in Aquatic Ecosystems. Oxford University Press, New York

Schneider B, Schlitzer R, Fischer G, Nöthing EM (2003) Depth-dependent elemental compositions of particulate organic matter (POM) in the ocean. Glob. Biogeochem. Cy. 17:doi:10.1029/2002GB001871

Sherry ND, Boyd PW, Sugimoto K, Harrison PJ (1999) Seasonal and spatial patterns of heterotrophic bacterial production, respiration, and biomass in the subarctic NE Pacific. Deep Sea Res. II 46:2557-2578

Sherry ND, Imanian B, Sugimoto K, Boyd PW, Harrison PJ (2002) Seasonal and interannual trends in heterotrophic bacterial processes between 1995 and 1999 in the subarctic NE Pacific. Deep-Sea Res. II 49:5775-5791

Simon M, Grossart HP, Schweitzer B, Ploug H (2002) Microbial ecology of organic aggregates in aquatic ecosystems. Aquat. Microb. Ecol. 28:175-211

Simon M, Rosenstock B, Zwisler W (2004) Coupling of epipelagic and mesopelagic heterotrophic picoplankton production to phytoplankton biomass in the Antarctic polar frontal region. Limnol. Oceanogr. 49:1035-1043

Smith DC, Simon M, Alldredge AL, Azam F (1992) Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. Nature 359:139-142

Sorokin YI (1971) Bacterial populations as components of oceanic ecosystems. Mar. Biol. 11:101-105

Sorokin YI (1973) Data on biological productivity of the western tropical Pacific Ocean. Mar. Biol. 20:177-196

Staley JT, Konopka A (1985) Measurement of in situ activities of nanphotosynthetic microorganisms in aquatic and terrestrial habitats. Annu. Rev. Microbiol. 39:321-346

Steinberg DK, B. A. Van Mooy, K. Buesseler, P. W. Boyd, T. Kobari, Karl DM (2008) Bacterial vs. zooplankton control of sinking particle flux in the ocean's twilight zone. Limnol. Oceanogr. 53:1327-1338

Steinberg DK, Carlson CA, Bates NR, Johnson RJ, Michaels AF, Knap AH (2001) Overview of the US JGOFS Bermuda Atlanric Time-series Study (BATS): a decade-scale look at ocean biology and biogeochemistry. Deep Sea Res. II 48:1405-1447

Stoderegger K, Herndl GJ (1998) Production and release of bacterial capsular material and its subsequent utilization by marine bacterioplankton. Limnol. Oceanogr. 43:877-884

Suzuki N, Kato K (1953) Studies on suspended materials Marine snow in the sea. Part I. Sources of marine snow. Bull. Fac. Fisheries Hokkaido Univ.:132-135

Tamburini C, Garcin J, Ragot M, Bianchi A (2002) Biopolymer hydrolysis and bacterial production under ambient hydrostatic pressure through a 2000 m water column in the NW Mediterranean. Deep Sea Res. II 49:2109-2123

Tanaka T, Rassoulzadegan F (2002) Full-depth profile (0-2000 m) of bacteria, heterotrophic nanoflagellates and ciliates in the NW Mediterranean Sea: vertical partitioning of microbial trophic structures. Deep Sea Res. II 49:2093-2107

Tanaka T, Rassoulzadegan F (2004) Vertical and seasonal variations of bacterial abundance and production in the mesopelagic layer of the NW Mediterranean Sea: bottom-up and top-down controls. Deep Sea Res. I 51:531-544

Teira E, Reinthaler T, Pernthaler A, Pernthaler J, Herndl GJ (2004) Combining catalyzed reporter deposition-fluorescence in situ hybridization and microautoradiography to detect substrate utilization by Bacteria and Archaea in the deep ocean. Appl. Environ. Microbiol. 70:44411-44414

Teira E, Lebaron P, Aken Hv, Herndl GJ (2006) Distribution and activity of Bacteria and Archaea in the deep water masses of the North Atlantic. Limnol. Oceanogr. 51:2131-2144

Thingstad TF (2000) Control of bacterial growth in idealized food webs. In: Kirchman DL (ed) Microbial ecology of the oceans. Wiley-Liss, New York, p 229-260

Thornton DCO (2002) Diatom aggregation in the sea: Mechanisms and ecological implications. Eur. J. Phycol. 37:149-161

Turley CM, Mackie PJ (1994) Biogeochemical significance of attached and free-living bacteria and the flux of particles in the NE Atlantic Ocean. Mar. Ecol. Prog. Ser. 115:191-203

UNESCO (1994) Protocols for the Joint Global Ocean Flux Study (JGOFS) Core Measurement. Intergovernmental Oceanographic Commission, Manual and Guides 29:169

Valderrama J (1981) The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Mar. Chem. 10:109-122

Verdugo P, Alldredge AA, Azam F, Kirchman DL, Passow U, Santschi P (2004) The oceanic gel phase: a bridge in the DOM–POM continuum. Mar. Chem. 92:67-85

Verdugo P, Orellana MV, Chin WC, Petersen TW, Eng Gvd, Benner R, Hedges JI (2008) Marine biopolymer self-assembly: implications for carbon cycling in the ocean. The Royal Society of Chemistry 139:393-398

Vezzi A, Campanaro S, D'Angelo M, Simonato F, Vitulo N, Lauro FM, Cestaro A, Malacrida G, Simionati B, Cannata N, Romualdi C, Bartlett DH, Valle G (2005) Life at depth: *Photobacterium profundum* genome sequence and expression analysis. Science 307:1459-1461

Wakeham S, Canuel EA (1988) Organic geochemistry of particulate matter in the eastern tropical North Pacific Ocean: implications for particle dynamics. J. Mar. Res. 46:183-213

Weiss M, Abele U, Weckesser J, Welte W, Schiltz E, Schulz GE (1991) Molecular architecture and electrostatic properties of bacterial porin. Science 254:1627-1630

Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: the unseen majority. Proc. Natl. Acad. Sci. USA 95:6578-6583

Williams PJL (1981) Incorporation of microheterotrophic processes into the classical paradigm of the plankton food web. 15th European Symposium on Marine Biology Kieler Meeresforsch, Sonderh., Kiel, F.R.G., p 1-18

Wuchter C, Herfort L, Coolen MJL, Abbas B, Bleijswijk Jv, Timmers P, Strous M, Teira E, Herndl GJ, Middelburg JJ, Schouten S, Damsté JSS (2006) Archaeal nitrification in the ocean. Proc. Natl. Acad. Sci. USA 103:12317-12322

ZoBell C (1946) Marine Microbiology. A Monograph on Hydrobacteriology, Vol. Chronica Botanica Co., Waltham

ANNEXES / ANEXOS

Abbreviations index

AABW: Antarctic Bottom Water

AAIW: Antarctic Intermediate Water

AE: anticyclonic eddie

AGase: α-glucosidase

AOU: apparent oxygen utilization

AP: autotrophic production

APase: alkaline-phosphatase

ARISA: automated ribosomal intergenic spacer analysis

BGase: β-glucosidase

BOD: biological oxygen demand

CARD-FISH: fluorescence in situ hybridization and catalyzed reporter deposition

CE: cyclonic eddie

CF: conversion factor

CTD: conductivity, temperature, depth

CTZ: Canary Coastal Transition Zone

DCM: deep chlorophyll maximum

DIC: dissolved inorganic carbon

DNA: deoxyribonucleic acid

DOC: dissolved organic carbon

DOM: dissolved organic matter

DON: dissolved organic nitrogen

DOP: dissolved organic phosphorus

DPM: disintegrations per minute

DSL: deep scattering layer

EE: extracellular enzymes

EEA: extracellular enzymatic activities

ETS: electron transport system

FCM: flow cytometry

FF: far-field stations

FISH: fluorescence in situ hybridization

HMW-DOM: high molecular weight DOM

H-NA: high nucleic acid cells

HRP: horseradish peroxidase

INT: 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride

JGOFS: Joint Global Ocean Flux Study

K_m: Michaelis-Menten half saturation constant

LAPase: leucine aminopeptidase

LDW: Lower Deep Water

LeuCF: leucine to carbon conversion factor

L-NA: low nucleic acid cells

LNEADW: Lower Northeast Atlantic Deep Water

M-A R: Mid-Atlantic Ridge

MCA: methylcoumarinyl

MICRO-CARD-FISH: CARD-FISH combined with micro-autoradiography

MNEADW: Middle Northeast Atlantic Deep Water

MSOW: Mediterranean Sea Outflow Water

MUF: methylumbelliferyl

MW: molecular weight

NACW: North Atlantic Central Water

NADS: nucleic acid double-staining

NADW: North Atlantic Deep Water

NAtl: North Atlantic Ocean

NEADW: North East Atlantic Deep Water

NEqCC: North Equatorial Counter Current region

NOEX: no oxygen exchange

nsPOC: non-sinking particulate organic carbon

nsPOC_Av: potentially available non-sinking particulate organic carbon

nsPOM: non-sinking particulate organic matter

nsPON: non-sinking particulate organic nitrogen

nsPON_Av: potentially available non-sinking particulate organic nitrogen

OM: organic matter

OMZ: oxygen minimum zone

OTU: operational taxonomic unit

OUR: oxygen utilization rates

PA: prokaryotic abundance

PBS: phosphate-buffered saline

PCD: prokaryotic carbon demand

PCR: polymerase chain reaction

PGE: prokaryotic growth efficiency

PHA: prokaryotic heterotrophic activity

PHP: prokaryotic heterotrophic production

POC: particulate organic carbon

POM: particulate organic matter

PON: particulate organic nitrogen

PP: surface primary production

PR: prokaryotic respiration

RFZ: Romanche Fracture Zone

RNA: ribonucleic acid

RQ: respiratory quotient

RV: research vessel

SACW: South Atlantic Central Water

SAIW: Sub-Arctic Intermediate Water

SAtl: South Atlantic Ocean

SD: standard deviation

SE: standard error

SG: subtropical gyre region

SML: surface mixed layer

SSC: side scatter

T: temperature

TA: transatlantic transect

TCA: trichloroacetic acid

TEP: transparent exopolymer particles

TOC: total organic carbon

Trans: transitional waters

T-RFLP: terminal-restriction fragment length polymorphism

T-S: temperature-salinity diagram

Tt: turnover time

UNEADW: Upper Northeast Atlantic Deep Water

V_{max}: maximum rate of hydrolysis

Índice de abreviaciones

A-AN: células con alto contenido en ácidos nucleicos

AEE: actividad enzimática extracelular

AGasa: alfa-glucosidasa

AHP: actividad heterotrófica de los procariotas

AP: abundancia de procariotas

AtlN: Atlántico Norte

AtlS: Atlántico Sur

B-AN: células con bajo contenido en ácidos nucleicos

BGasa: beta-glucosidasa

CARD-FISH: hibridación in situ fluorescentes con deposición catalizada

CID: carbono inorgánico disuelto

COD: carbono orgánico disuelto

COP: carbono orgánico particulado

COPs: COP suspendido

COPs_Dp: COPs potencialmente disponible para una utilización heterotrófica

COT: carbono orgánico total

DCP: demanda de carbono de los procariotas

ECP: eficiencia de crecimiento procariota

Fasa: fosfatasa alcalina

FF: estaciones fuera del campo de influencia de los remolinos

FOD: fósforo orgánico disuelto

K_m: constante de semisaturación de Michaelis-Menten

LAPasa: leucina aminopeptidasa

LeuFC: factor de conversión de leucina a carbono

MCA: metilcoumarinil

MICRO-CARD-FISH: CARD-FISH combinado con micro-autoradiografía

MO: materia orgánica

MOD: materia orgánica disuelta

MOP: materia orgánica particulada

MOPs: MOP suspendidad

MPC: máximo profundo de clorofila

MUF: metilumbelliferil

NOD: nitrógeno orgánico disuelto

NOP: nitrógeno orgánico particulado

NOPs: NOP suspendido

NOPs_Dp: NOPs potencialmente disponible para una utilización heterotrófica

PAP: producción autotrófica de procariotas

PET: partículas exopoliméricas transparentes

PHP: producción heterotrófica procariota

PP: producción primaria

RA: remolino anticiclónico

RC: remolino ciclónico

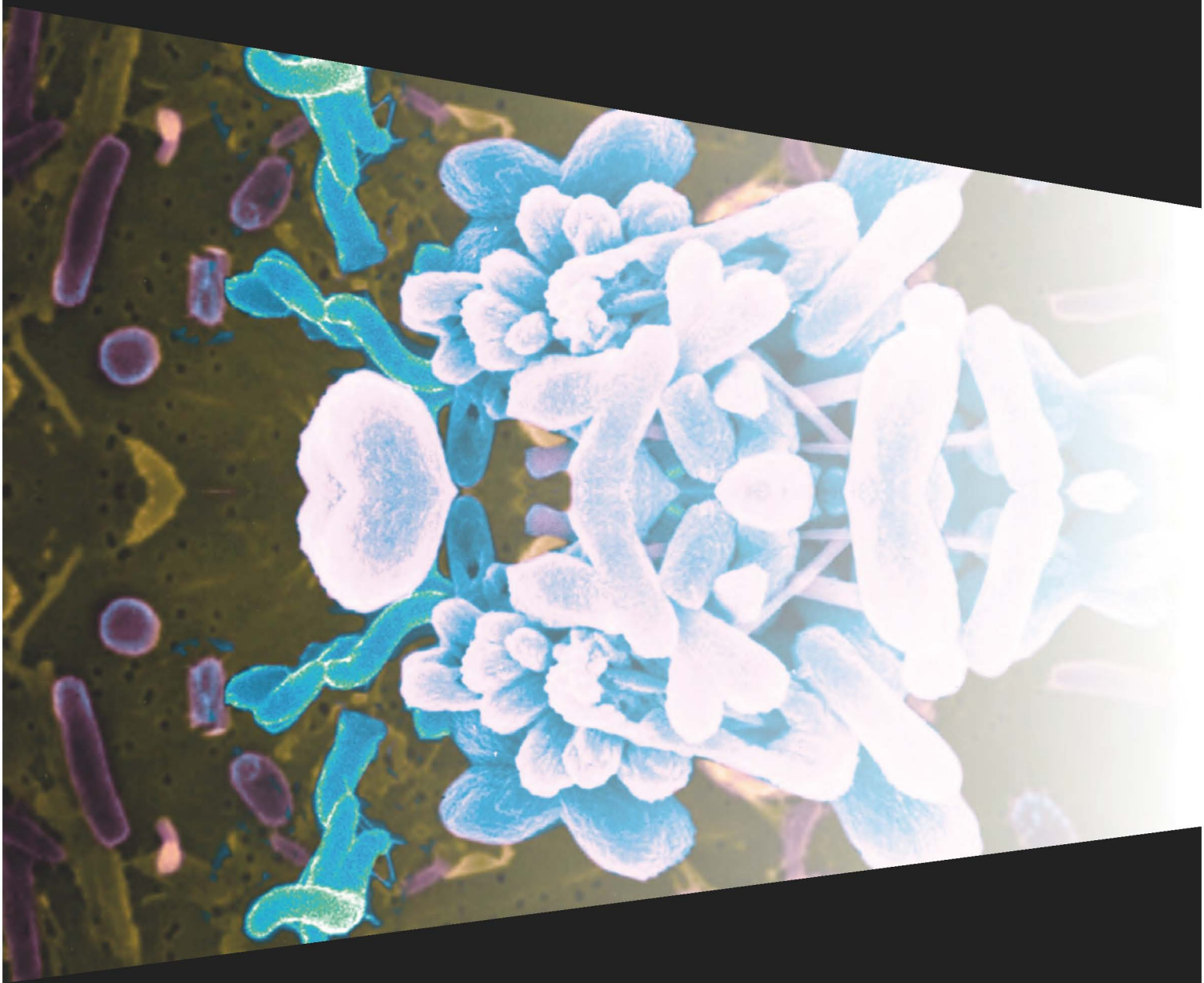
RP: respiración procariota

UAO: utilización aparente de oxígeno

ZTC: Zona de Transición Costera

"If I have ever made any valuable discoveries it has been owing more to patient attention than to any other talent."

Sir Isaac Newton



UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA