The oceans become one of the main protagonists in the current climate change scenario, while zooplankton is found throughout all oceanic environments. Vertical migrations of these organisms daily occur in all marine systems, and it is probably one of the greatest movements of biomass in the ocean. Identified as a major player in the biological pump, accurate estimates of zooplankton carbon fluxes at the large-scale are therefore a requirement. However, methods currently used present many limitations, preventing its use over large study regions. Based on that, through this thesis we developed and tested alternative tools to assess zooplankton biomass and metabolic fluxes at large spatial scales. In this regard, image-based systems may be as reliable as traditional and enzymatic methods, although they result in a faster and inexpensive process.

Zooplankton biomass and metabolism through image analysis systems

DOCTORAL THESIS
JUAN CARLOS GARIJO LÓPEZ

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
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From the development and testing of metabolic equations to the assessment of carbon fluxes

Juan Carlos Garijo López

Las Palmas de Gran Canaria
July 2016

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D. MELCHOR GONZÁLEZ DÁVILA DECANO DE LA FACULTAD DE CIENCIAS DEL MAR DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA,

CERTIFICA,

Que el Consejo de Doctores de la Facultad en sesión permanente tomó el acuerdo de dar el consentimiento para su tramitación a la tesis doctoral titulada "Zooplankton biomass and metabolism through image analysis systems. From the development and testing of metabolic equations to the assessment of carbon fluxes" presentada por el doctorando D. Juan Carlos Garijo López y dirigida por el Doctor D. Santiago Hernández León.

Y para que así conste, y a efectos de lo previsto en el Art nº 6 del Reglamento para la elaboración, defensa, tribunal y evaluación de tesis doctorales de la Universidad de Las Palmas de Gran Canaria, firmo la presente en Las Palmas de Gran Canaria, a 28 de julio de 2016.

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Facultad de Ciencias del Mar
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UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA

DOCTORAL THESIS

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Zooplankton biomass and metabolism through image analysis systems

Abstract

Zooplankton plays an important role in the biogeochemical cycles in the ocean. Due to their central position in the ocean's food web, they recycle and redistribute energy and matter, not only at different levels of the trophic web, but also horizontally and vertically in the water column. Understanding the role of zooplankton in the biological pump and the ocean carbon cycle requires accurate estimates of community biomass and metabolism at large spatial scales. Measurements of the former are relatively affordable. However, traditional methods to estimate metabolism are highly time-consuming processes, preventing its use over large study regions. Similarly, enzymatic methods, although useful, show uncertainties to approach metabolic rates from enzymatic activity. In this thesis we developed and tested alternative tools to assess zooplankton biomass and metabolic fluxes at large spatial and temporal scales. Thus, we examined the suitability of image-based systems (IBS), combined with empirical relationships and metabolic equations, to estimate biomass and metabolism of zooplankton on subtropical regions. We observed that these procedures could be as reliable as traditional and enzymatic methods when equations fitted the conditions of the subtropical region, although the former resulted in a faster and inexpensive process, among other advantages. Nevertheless, enzymatic methods seemed to be a better alternative to assess specific metabolic rates on particular studies, such as those on temporal-series.

The reliability of IBS to study zooplankton was also ascertained along physical structures in a region with a high mesoscale activity near the Canary Islands. Here, we observed a physical-biological coupling, since the

distribution of biomass and metabolism of zooplankton matched the three-dimensional signature of the oceanographic structures. Then, based on the results achieved using the IBS on subtropical waters, we developed specific equations for growth and respiration of zooplankton, fitted to the temperature ranges of the main regions of the global ocean. Thus, comparing equations and methods along the ~40ºN-40ºS latitudinal band, we observed that estimates using these temperature-specific equations were comparable to those from enzymatic methods, and probably more accurate than using existing generalist relationships for the global ocean.

Given their usefulness, we finally applied these temperature-specific metabolic equations to study active fluxes through zooplankton in the tropical and subtropical Atlantic (10ºS-25ºN). We highlight the contribution of zooplankton export to the biological pump, and the importance of addressing studies assessing active fluxes through mortality, but also due to egestion and ammonia excretion processes. Additionally, we illustrate the future possibilities of predicting zooplankton export using remote sensing data.

Resumen

El zooplancton juega un papel fundamental en los ciclos biogeoquímicos oceánicos. Debido a su posición central en la red trófica, lleva a cabo un reciclaje y redistribución de materia y energía, tanto a diferentes niveles de la trama alimenticia como horizontal y verticalmente en la columna de agua. Comprender el papel del zooplancton en la bomba biológica y el ciclo del carbono en el océano requiere estimas precisas de la biomasa y el metabolismo de estas comunidades a gran escala. Estudiar la biomasa es relativamente asequible. Sin embargo, la metodología tradicionalmente usada para determinar el metabolismo consume mucho tiempo, impidiendo su aplicación en estudios sobre vastas regiones. Por su parte, los métodos enzimáticos, aunque útiles, presentan incertidumbres a la hora de aproximar las tasas metabólicas a partir de la actividad enzimática. En esta tesis desarrollamos y probamos herramientas alternativas con las que poder estimar la biomasa y los flujos de carbono mediados por el zooplancton a una escala espacial y temporal grande. Así, examinamos la validez de un sistema basado en el análisis de imágenes (IBS), en combinación con relaciones empíricas y ecuaciones metabólicas, para estimar la biomasa y el metabolismo del zooplancton en regiones subtropicales. Observamos que estos sistemas pueden resultar tan válidos como los métodos tradicionales y enzimáticos cuando las ecuaciones se ajustan a las condiciones de la región subtropical, si bien el empleo del IBS resultó ser un proceso más rápido y económico. En cualquier caso, los métodos enzimáticos parecen ser una mejor alternativa para determinar el metabolismo específico en determinados casos como, por ejemplo, a lo largo de series temporales.

La fiabilidad del IBS para estudiar el zooplancton fue probada además en estructuras físicas en una región con una elevada actividad mesoescalar próxima a las Islas Canarias. Aquí, observamos un acoplamiento físico-biológico, pues la biomasa y el metabolismo del zooplancton se distribuyó siguiendo la señal tridimensional de las estructuras oceanográficas. A continuación, basándonos en los resultados obtenidos usando el IBS en la

región subtropical, desarrollamos ecuaciones específicas de crecimiento y respiración del zooplancton, ajustadas a los rangos de temperatura dados en las principales regiones del océano global. Comparando diferentes ecuaciones y métodos a lo largo de la región ~40ºN-40ºS observamos que las estimas usando estas ecuaciones específicas para la temperatura eran comparables a las obtenidas por medio de métodos enzimáticos, y probablemente más precisas que usando las ecuaciones generalistas existentes para el océano global.

Dada su utilidad, finalmente aplicamos estas ecuaciones específicas en el estudio del flujo activo por parte del zooplancton en el Atlántico tropical y subtropical (10ºS-25ºN). Remarcamos la contribución del zooplancton al funcionamiento de la bomba biológica, y la importancia de estudiar el flujo activo por medio de la mortalidad, pero también de la egestión y la excreción de amonio. Además, se ilustran las posibilidades futuras de predecir el carbono exportado por parte del zooplancton mediante el uso de datos de teledetección.

Agradecimientos

Esta tesis ha sido desarrollada en la Universidad de Las Palmas de Gran Canaria gracias a la financiación proporcionada por el programa de Formación de Profesorado Universitario (FPU) del Ministerio de Educación, Cultura y Deporte del Gobierno de España (AP2010-5080) y el Programa de Formación del Personal Investigador (PFPI) de la Universidad de las Palmas de Gran Canaria. Además, ha contado con el apoyo de los proyectos CONAFRICA (CTM2004-02319/MAR), LUCIFER (CTM2008-03538), MALASPINA (Consolider-Ingenio 2010, CSD2008-00077) y MAFIA (CTM2012-39587) del Gobierno de España, y finalmente la colaboración de la Universidad de Cádiz y la Université Pierre et Marie Curie de Francia.

Y por fin aquí…tras casi treinta años estudiando puedo decir que finalmente he llegado al final del camino. Por fin he conseguido cerrar el círculo: aquí termino mi etapa como estudiante. Si echo la vista atrás me doy cuenta que el camino ha sido largo, demasiado quizá, pero ha sido increíble. En realidad no sé si he aprendido muchos conceptos, teorías, procesos o mecanismos. Creo que no recuerdo tantos como para haber estado treinta años estudiándolos. Tampoco sé si esta tesis ayudará de una u otra forma al desarrollo y felicidad de otras personas. Ojalá. Lo que sí tengo claro es que durante su desarrollo he tenido la suerte de vivir experiencias únicas, inalcanzables de otro modo. Pero sobre todo, he conocido a gente maravillosa. Algunos de ellos serán mis amigos para siempre. Estoy seguro que dentro de unos años seré incapaz de recordar siquiera cuántos capítulos tenía mi tesis. Pero seguro que seguiré conservando parte de lo que he aprendido de muchas personas durante este trayecto. Conocimientos para la vida. Y eso es lo verdaderamente importante.

Gracias Santiago por darme la oportunidad de descubrir el mundo de la oceanografía, por compartir tus conocimientos, pero sobre todo por enseñarme a ser mejor persona.

Aunque es de los compis de laboratorio de los que más he aprendido, por medio de todo tipo de vivencias, dentro y fuera de la facultad. Ellos son los que verdaderamente le dan *vidilla* al día a día de una tesis. Y aquí entran tanto las técnicos: Mine, Lidia, Cory, Ico, como el resto de doctorandos: Nau, Isa, Loreto, Yeray, Alejandro, Inma, Laia, Susana, Adri, Iván, Kai, Gara, Ali,

Walber, Fede, Natalia, Igor...Desarrollar una tesis doctoral consiste en dedicarle muchas horas y todos sabemos que "al final el roce hace el cariño", por lo que finalmente termina pasando lo que tenía que pasar: algunos de tus compis terminan convirtiéndose en verdaderos amigos…

Fede, eres una gran persona. De ti me llevo gran parte de tus valores humanos y tu humildad. "Arrrrrriba Colombia". Sabes que te quiero hermanito. Igor ("hoooola fondo norte…"), creo que con eso te lo digo todo. Y a ti Natalia, gracias por ser una tía tan guay, sin más. Os agradezco que me hayáis transmitido con tanta naturalidad cómo se conforma un gran equipo. Sois una pasada juntos y llegaréis donde queráis. Laia, mi psicóloga personal en Taliarte. Todo ocurre siempre para bien, y en este caso sin duda ha sido para aprender que eres una tía magnífica y que quiero seguir siendo tu amigo. Gracias por escucharme y comprenderme. Me quedo con tu positivismo y tu constante sonrisa.

Yeray, contigo he aprendido de dos de tus grandes dones: la paciencia y la bondad. Lo emanas a kilómetros, no puedes evitarlo. Si pienso en ti, siento que serías incapaz de hacer daño. Ojalá tu cerebro nunca deje de estar ávido de curiosidad y quiera seguir viendo *más allá*, porque eso me encanta. Gracias por hacerme vibrar con nuestras conversaciones trascendentales. Sigue imaginando y soñando porque eso te hace una persona increíble.

Alejandro, en realidad aún me pregunto cómo podemos ser tan buenos amigos siendo tan diferentes. Has sido mi *hermano mayor* en esto de la ciencia. Tus consejos me hicieron aprender rápido y me evitaron sufrimientos innecesarios. Sin ellos, yo no estaría escribiendo estos agradecimientos hoy. Es increíble lo que confío en ti, lo sabes. Desde que te conozco lo hemos compartido casi todo. Somos capaces de llorar de la risa juntos, y eso sólo pasa cuando conectas de verdad con alguien. Eres un amigo increíblemente noble y fiel. Creo que ese es el mayor aprendizaje que me llevo de ti.

Jose, nuestra conexión espiritual es una pasada. Eres sabio y creo que deberías ser el presidente del *Consejo*. Tú me has enseñado muchas cosas en poco tiempo. Por ejemplo, que sabrías ser feliz bajo cualquier condición o en cualquier lugar. Tienes esa capacidad innata. Estás condenado a que la gente te quiera vayas donde vayas, pues sólo sabes transmitir buena energía y generar armonía. Lo tienes fácil en la vida.

Zeben, todavía estoy esperando a que me fundas subiendo un puerto. Aun así te agradezco la batalla presentada. Contigo he compartido los momentos deportivos más épicos dentro de la isla. Gracias a ti he conocido rincones que ni el más canarión de los canariones llegará nunca a descubrir.

Tampoco olvidaré tu humildad Ted, ni tu pasión y valores científicos. Ojalá la ciencia se llenara de gente como tú. Gracias Nacho, Fidel, Andrés, Nerea, Ana, Edu, y el resto de gente de la Universidad de Cádiz por acogerme y por ayudarme a descubrir una tierra que me sedujo para siempre.

Por otro lado, también he aprendido y mucho de todos los marineros con los que he tenido el placer de conversar. Nacieron en *el lado malo* de esto de los embarques y campañas, y sus experiencias en la mar no son tan *guays* como las nuestras. Sin vosotros, nómadas acuáticos que ansían la tierra y los seres que allí esperan, esto no sería posible.

Amigos del Kañamón, con vosotros he aprendido todo aquello que jamás podrá leerse en los libros.

Maintenant, je voudrais dire quelques mots à mes collègues de l'Observatoire de Villefranche sur Mer. Merci Lars de m'avoir montré que faire de la bonne science n'est pas en conflit avec les valeurs humaines. Grâce à vous, je me suis senti un vrai membre de votre laboratoire dès le premier jour. A l'avenir, j'aimerais bien gérer un laboratoire comme vous le faites. Mais aussi un grand merci à Marc, Fabien, Lee, Baptiste, Lionel, Simon, Amanda, pour avoir fait que je garde un si bon souvenir de mon stage chez vous.

Y finalmente llego donde quería llegar. A la parte que me permite daros las gracias a vosotros, familia. Sois lo más importante en mi vida y sin vosotros, nada de esto tendría sentido.

Pisky, tíos Reme y Luis, y Simpsons en general, gracias por quererme tanto. Somos una familia muy guay.

Gracias a los *viejitos* he descubierto una familia que me encanta. Meli, mi representante y *manager*. De tu mano podría llegar a la *Casa Blanca* si te lo propusieras. Gracias por preocuparte por mí durante estos meses, y por quererme (que lo sé), pero sobre todo por confiarme a tu bien más preciado. Gracias *Parguelas* por jugar con mis *sobris,* haciendo lo más difícil de todo: impregnar de vida esta tesis en forma de portada.

Raquel, nadie tiene la suerte de tener dos madres como yo. Por otro lado, es maravilloso tenerte como hermana. Si compartimos la cama de pequeños no fue por casualidad, sino para forjar un amor especial que durará para siempre, no tengo duda. Tú y Jose me enseñáis cada día cómo se forma una familia. Siempre mi referente, ojalá que la mía se parezca un poco a la vuestra.

Nacho y Yago, espero que dentro de unos años me perdonéis por haber estado tanto tiempo en *Canayas*. Pero es que el *Tío Lalos* es un gamberro y no sabe estarse quieto en su casa con su familia. Ya os lo explicaré mejor, pero quiero que sepáis que no pasa un solo día en que no piense en vosotros. Gracias por mostrarme que la vida es mucho más sencilla de lo que creemos los adultos.

Susana, no te das ni cuenta de lo grande que eres. Cuando alguien se cree que "*All is for better*" es imposible que la vida no le parezca maravillosa. Gracias por estar conmigo en mis momentos más bajos. Siempre estás ahí, siempre. Eres mi mejor amiga y nunca dejo de crecer contigo. Qué conexión más guay. Tu pasión por la vida y por lo real te hacen increíble. Estamos aquí para ser felices, así que seguiremos *riéndonos de todo y jugando a vivir*.

Si madre no hay más que una yo lo siento por los demás, porque yo tengo la mejor madre del mundo. Orgullo es lo que siento por ti, mi gran maestra. Por mucho que viaje o estudie, lo verdaderamente importante lo he aprendido de ti. Y a ti te debo la persona que hoy soy. Perdón por el tiempo que nos perdemos juntos. Pero sobre todo, gracias por ser así madre.

Y finalmente tú, Rosalía. A ti no puedo darte las gracias por nada en esta tesis, porque tú eres su coautora. Pero sí puedo admirar cómo diste tantas manos de azules y verdes con las que tapar los grises y negros. Una y otra vez…Desprendes una luz maravillosa, y sin ti hace tiempo que hubiera abandonado el buque. Gracias por enseñarme cómo ser mejor persona cada día, mi espejo. Quiero ser como tú, pues tienes *el bolso de Mary Poppins* de los valores humanos. Gracias por querer soñar conmigo. La vida juntos va a ser una pasada…

Juan Carlos

Abril 2016

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Symbols and Abbreviations

AARS	**A**mino**A**cyl-t**R**NA-**S**ynthetase method
ANOVA	**An**alysis **o**f **Va**riance
ATC	**A**spartate-**T**rans**C**arbamylase method
BW	**B**ody **W**eight
CC	**C**anary **C**urrent
Chla	**Chl**orophyll **a**
CTD	**C**onductivity **T**emperature and **D**epth (Profiler)
CTZ	**C**oastal **T**ransition **Z**one
DCM	**D**eep **C**hlorophyll **M**aximum
DIC	**D**issolved **I**norganic **C**arbon
DOC	**D**issolved **O**rganic **C**arbon
DOM	**D**issolved **O**rganic **M**atter
DVM	**D**iel **V**ertical **M**igration
DW	**D**ry **W**eight
EBUS	**E**astern **B**oundary **U**pwelling **S**ystem
ESD	**E**quivalent **S**pherical **D**iameter
ETS	**E**lectron **T**ransport **S**ystem
POM	**P**articulate **O**rganic **M**atter
PP	**P**rimary **P**roduction
PPi	**P**yrophosphate
SST	**S**ea **S**urface **T**emperature

Introduction

I. Background

I.1 The role of zooplankton in the biological pump and carbon cycle

Zooplankton plays a pivotal role in the biogeochemical cycles in the ocean. It occupies a central position in the ocean's food web, connecting microbial and larger organisms. They consume organic matter to satisfy their energy demands, recycling and redistributing nutrients and material, not only at different levels of the trophic web, but also horizontally and vertically in the water column (Banse, 1995). Therefore, they result a key component of the biological pump in the ocean. This constitutes a complex network of physiological and ecological processes, leading to a transfer of matter in the water column and the transport of atmospheric carbon dioxide (CO_2) into the deep ocean (see Fig. I.1). Briefly, phytoplankton fixes inorganic carbon by photosynthesis, which is ingested by zooplankton. These communities also consume and produce particulate organic matter (POM), and release organic and inorganic nutrients through excretion processes, which may be reutilized by phytoplankton in the upper sunlit layers of the ocean.

In turn, the microbial loop constitutes a pathway of regenerating nutrients, since it also utilizes POM and dissolved organic matter (DOM) to satisfy their demands. The disequilibrium caused by producers in the carbonate system of the atmosphere may be either remineralized back via respiration of epipelagic consumers (Del Giorgio and Duarte, 2002) or transported to the mesopelagic layer through different mechanisms. In this sense, particulate organic matter may conform aggregates that may sink gravitationally to deeper layers, conforming the so-called "passive flux". Additionally, dissolved nutrients and organic carbon may be actively transported to the mesopelagic layer through migrant zooplankton (*e.g.,* Longhurst et al., 1990, Zhang and Dam, 1997, Steinberg et al., 2000) and micronekton (Hidaka et al., 2001; Ariza et al., 2015). Finally, physical processes, such as diffusion and vertical mixing (*e.g.*, Arístegui et al., 2003), may also involve export of dissolved organic and inorganic carbon (DOC and DIC). As a whole, these three processes constitute the carbon pump in the ocean.

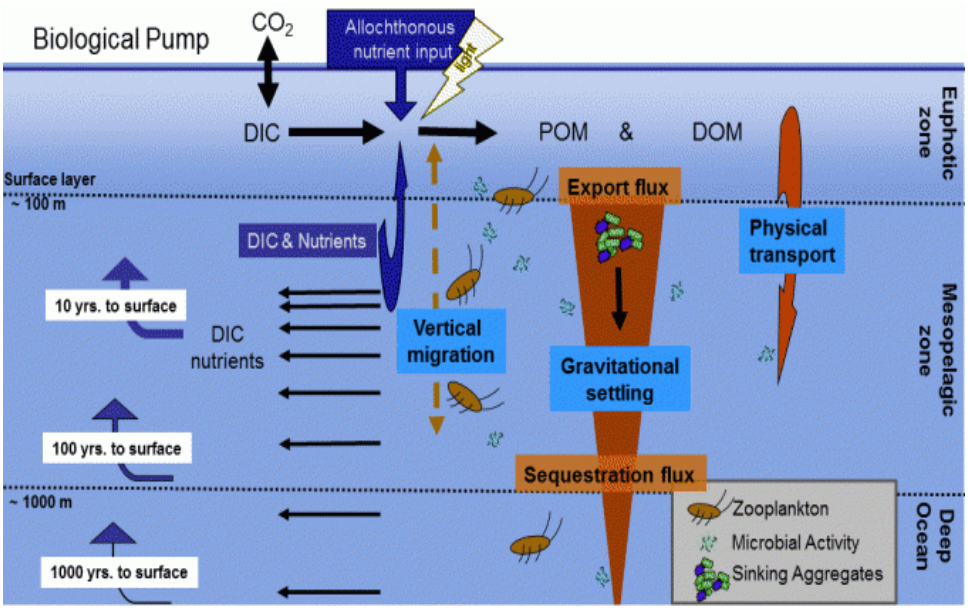

Fig. I.1. Schematic of the carbon pump in the ocean. From Passow and Carlson (2012).

Strikingly, only one-third of the carbon exported to deeper layers is attributed to physical processes (Passow and Carlson, 2012), therefore highlighting the importance of active and passive fluxes. In this regard, although the latter has received much effort (*e.g.,* Buesseler et al., 2007), studies performed during the last decades showed that active fluxes through migrant zooplankton might also account for an important fraction (in the range of the passive flux in some cases) of the total carbon export (*e.g.,* Longhurst et al., 1990; Steinberg et al., 2000, 2008; Kobari et al., 2013).

In this respect, it is known that community production, respiration or egestion fluxes mediated by zooplankton are primarily dependent on community biomass. Huntley and Lopez (1992) observed that the variability of the latter parameter was one to three orders of magnitude greater than that of specific metabolic rates. On the other hand, diel vertical migration of zooplankton occurs in all marine environments, and it is probably one of the largest movements of biomass in the ocean. Organisms migrate to feed at the epipelagic zone at night, returning at dawn to deeper layers (Lampert,

1989), where they excrete nitrogen and release carbon due to mortality and respiratory and defecation processes. Hence, it seems clear the prominent role of these communities on the magnitude of total downward export and the carbon cycle in the ocean.

Respiratory fluxes due to migrant zooplankton (and mortality to a lesser extent) have been addressed at many different environments during the last decades (*e.g.,* Zhang and Dam, 1997; Al-Mutairy and Landry, 2001; Hidaka et al., 2001; Takahashi et al., 2009; Stuckel et al., 2013), highlighting their importance *vs.* passive flux. However, minor attention has received the so-called "gut flux". Non-assimilated food, ingested in the epipelagic zone, may be transported in the gut of migrant organisms as moving downward to deeper layers, where they process a fraction of the material ingested, releasing POM during daytime (Angel, 1989). Despite it is known that gut fluorescence method (Nemoto, 1968; Mackas and Bohrer, 1976) underestimates gut flux on omnivore zooplankton, some authors pointed out the important contribution of gut flux to total export (Lampitt et al., 1993; Hernández-León et al., 2001; Schnetzer and Steinberg, 2002; Yebra et al., 2005b; Kobari et al., 2008; Putzeys et al. 2011; Kobari et al., 2013). Regarding ammonia excretion fluxes, although several studies remarked their importance (Dam et al., 1995; Steinberg et al., 2002; Stuckel et al., 2013), exceeding in some cases the passive flux, export of nitrogen through migrant zooplankton has been in fact scarcely addressed.

Nevertheless, processes to estimate active flux are not standard, and comparisons among regions are always difficult. Results will primarily depend on how migrant biomass is estimated. Thus, this parameter will be probably overestimated when assessments are based on increments in the euphotic layer during nighttime, as the larger organisms may avoid the nets in the upper sunlit layers during daytime (Ianson et al., 2004). By opposite, migrant biomass will be probably underestimated when calculations are based on daytime increments below the epipelagic layer, as it is known that organisms might detect the presence of nets even at these depth layers (Ianson et al., 2004). In addition, the magnitude of active flux depends on

the zooplankton components included in the estimates (meso- and/or macrozooplankton), the mechanisms involved on export (respiration, mortality, egestion), the depth layer considered to delimit migrations (150, 200, 300 m, etc.) or the methodology used to ascertain the metabolism of zooplankton.

At present, the oceans become a main system in the current climate change scenario. Understanding that CO_2 is the main responsible of the climate warming, it should be considered that the oceans contain about 50 times as much carbon as the atmosphere. Consequently, modelers of carbon budgets will require a better understanding of the carbon pump and carbon cycle in the ocean. As a major player, the role of zooplankton should be profoundly explored in this respect (Usbeck et al., 2003). Global approaches of the biomass distribution and the magnitude of carbon fluxes through these communities are then of paramount importance to understand the ocean carbon cycle. However, studies at this scale are challenging and expensive, resulting on the scarce measurements available and the current uncertainty about the contribution of zooplankton to carbon cycle in the ocean. To present, only Moriarty and O'Brien (2013) were able to approach global biomass of these communities, based on data compilation for more than 50 years, while a couple of studies addressed zooplankton respiration (Del Giorgio and Duarte, 2002; Hernández-León and Ikeda, 2005) and production (Stock et al., 2014) at the global scale. Measurements on these studies suggest the importance of these communities in the ocean carbon cycle.

I.2 Methodological constraints of biomass and metabolism

Estimates of zooplankton biomass are relatively affordable in comparison with assessments of metabolism. However, the standard methodology, based on dry weight measurements (Lovegrove, 1966), has become outdated. Neither taxonomic nor individual biomass may be achieved according to this process, while samples are destroyed, precluding their use for further analysis.

In turn, classical methods to study zooplankton physiology, such as incubations, are highly time-consuming and labor-intensive processes (see Basedow et al., 2014), precluding their application along large spatial scales and resulting impractical to match physical and chemical measurements in oceanography. Moreover, they show other limitations, in relation to the number of individuals incubated to ensure optimal conditions, stress of captured animals, bacterial growth or starvation (Ikeda et al., 2000). The low concentration of organisms below the euphotic region (Yebra et al., 2005b) and the scarce possibilities to obtain healthy organisms for experiments also highlight the difficulty of assessing metabolism at depth. Many other methodologies have been developed during the last decades as, for instance, the egg production method to assess zooplankton growth rates (see Basedow et al., 2014) and, more recently, biochemical methods and enzymatic procedures. The former use the rate of nucleic acids (RNA/DNA), while the latter are based on measurements of cellular activities to approach respiration and growth of zooplankton. In this case, individuals are immediately frozen after sampling, allowing the assessment of the enzymatic activity afterwards in the laboratory, and therefore enabling to cover larger regions than previously through incubation methods. Moreover, the enzyme activity can be measured in organisms inhabiting at deep layers, resulting helpful to study vertical profiles of zooplankton metabolism distribution (see Packard et al., 1985). This is the case of the electron transfer system method (ETS, Packard, 1971) to estimate respiration or aspartate transcarbamylase (ATC, Biegala and Bergeron, 1998) and aminoacyl-tRNA-synthetases methods (AARS, Yebra and Hernández-León, 2004) for assessing growth.

However, despite these *proxies* for metabolism were a breakthrough over standard methods, they still show uncertainties in the relationship between the enzyme activity and the metabolic rate (Hernández-León et al., 1995; Hernández-León and Gómez, 1996). For instance, ETS activities are measured under substrate-saturated conditions, something that is not always observed in nature, probably leading to the overestimation of respiration. In this sense, measurements of ETS activities actually represent the potential

respiration of an organism. In contrast, a ratio between respiration and ETS (R/ETS) of 0.5 could underestimate metabolic rates when substrates are not limited *in vivo* (Hernández-León and Gómez, 1996; Hernández-León and Torres, 1997; Putzeys et al., 2005). Consequently, the search for accurate ratios, fitted to specific environmental conditions, is still a bottleneck for the use of enzymatic methods.

I.3 Image-based analysis systems (IBS)

The conception of the recently developed semi-automated image-based systems (IBS) stems from the need to address (1) the constraints of existing methods, and (2) the demands of current oceanography to understand the contribution of zooplankton in the ocean carbon cycle. These systems enable the estimation of taxonomic and size class abundances according to a semi-automated process, resulting in a faster procedure than using traditional methodology. Consequently, spatial scales may be notably increased (Grosjean et al., 2004; Benfield et al., 2007; Gislason and Silva, 2009; MacLeod et al., 2010; Gorsky et al., 2010; Bachiller et al., 2012). Additionally, using empirical relationships based on data from parameters accurately measured through the IBS, such as the equivalent spherical diameter (ESD) or the body area and the prosome length of individuals (*e.g.,* Nakata et al., 2001; Hernández-León and Montero, 2006; Lehette and Hernández-León, 2009; Viñas et al., 2010), these systems also allow assessing individual body mass in terms of dry weight, biovolume, etc. in a faster and non-destructive process (see Fig. I.2).

In turn, assessments of individual biomass through IBS allow the application of predictive equations to each organism, relating temperature and body weight, and metabolic rates of respiration (Ikeda, 1985), growth (Ikeda and Motoda, 1978; Hirst and Lampitt, 1998; Hirst et al., 2003) and mortality (Peterson and Wroblewski, 1984; Hidaka et al., 2001). Additionally, this kind of equations may also incorporate Chl*a* as a *proxy* of the food availability (Hirst and Bunker, 2003; Zhou et al., 2010), while Ikeda (2014) also considered the habitat depth and the taxonomic group of individuals as

predicting variables to obtain estimates of respiration and ammonia excretion rates. Reasonable assessments have been already ascertained using this kind of equations (Peterson et al., 2002; Basedow et al., 2014; Stock et al., 2014). However, it still remains unclear the effect of food availability on zooplankton metabolism (*e.g.,* Webber and Roff, 1995; Richardson and Verheye, 1999). Concerning the relative contribution of the different variables to the overall variability of metabolic rates, Ikeda (2014) has recently observed that respiration and ammonia excretion rates are primarily determined by body weight and temperature, while depth and taxonomy are suggested as factors of lesser importance.

I.4 Remote sensing and zooplankton

Understanding the contribution of zooplankton to the oceanic carbon budget requires the assessment of fluxes mediated by these communities at the global scale. However, this is something unaffordable in terms of time, resources and effort through the methodology currently employed. Due to several factors (*e.g.,* weather conditions, distance to land, etc.), massive regions of the ocean remain unexplored, while oceanographic cruises usually repeat and concentrate along similar areas (Reid et al., 2003; Isla et al., 2004; San Martin et al., 2006). Alternatively, global-scale ecosystem models could be useful to study vast and remote regions of the ocean, approaching carbon fluxes of the different communities according to macroecological functions, body size or food-web ecology (López-Urrutia et al. 2006, Jennings et al. 2009).

Recent advances on satellite remote sensing could be also valued as alternative tools to provide information of the ocean's status at a planetary scale. Thus, satellites regularly provide data of key parameters of the surface ocean, such as chlorophyll, height anomalies, primary production or temperature. In this regard, many researchers hypothesize that data characterizing the ocean's surface, provided by satellites and autonomous tools (gliders, floats, etc.), could be employed to feed models predicting the magnitude of carbon fluxes in the ocean (*e.g.,* EXPORTS,

http://exports.oceancolor.ucsb.edu, see Fig. I.3). Hence, understanding possible correlations between zooplankton and environmental data could help to develop models, through which continuously ascertain the contribution of these communities to the biological pump, including remote and challenging regions, otherwise too expensive to be directly sampled (Stock et al., 2014).

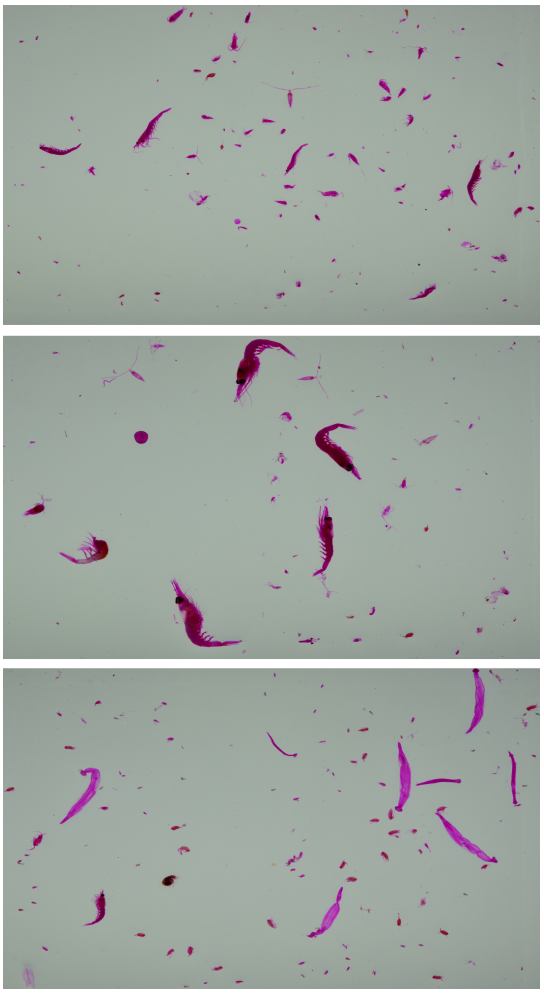

Figure I.2. Digital images of mesozooplankton for further analysis through an image-based system (IBS). In this case, individuals were stained using Rose Bengal and digitized at a resolution of 1850 dpi. We used a Nikon D800 digital camera (36 Megapixel) and a Macro Lens (Micro Nikkor 60mm f/2.8G ED). A white LED illumination provided a uniform background light.

Nevertheless, modelers still need *in situ* measurements of biomass and fluxes mediated by zooplankton over large areas to develop global models. This will require the use of the available technology (*e.g.,* image systems) and reliable equations to predict the metabolism of these organisms. Specific work will be necessary on particular regions demanding special attention, as it is the case of upwelling and coastal waters or oxygen minimum zones (OMZs), where models based on data from the ocean's surface could introduce considerable bias. For instance, organisms inhabiting the OMZs show a reduced metabolism as a consequence of the low oxygen concentration within the water column (Kiko et al., 2015a, 2015b), and therefore influencing the magnitude of zooplankton-mediated fluxes.

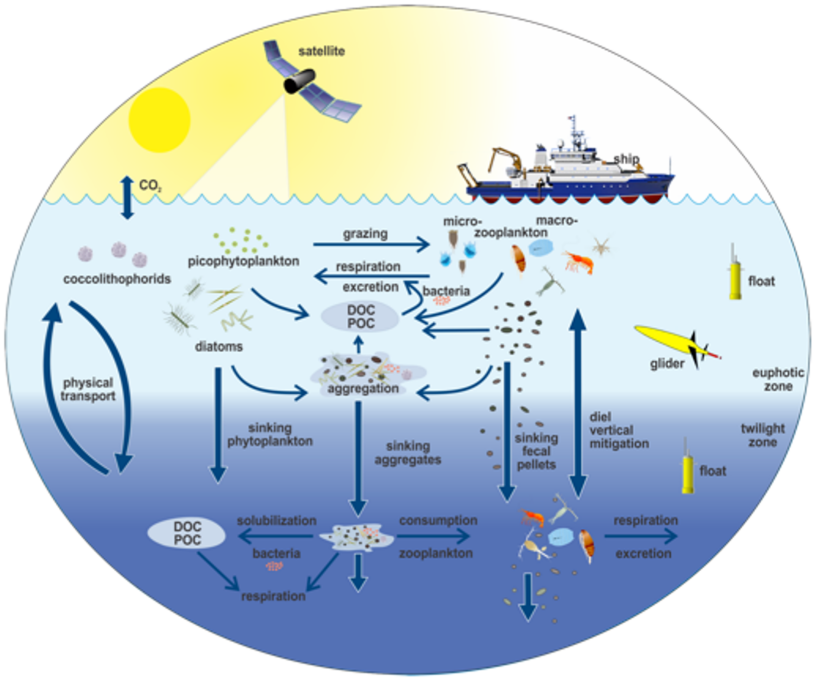

Figure I.3. Schematic of *EXPORTS* scientific program from the *National Aeronautics and Space Administration* (NASA). It hypothesizes that the magnitude of organic matter transferred to deeper layers might be predicted through the state of the surface ecosystem. Models will use data from remote sensing and autonomous tools (gliders, floats, etc.), calibrated through *in situ* data from cruises, to continuously ascertain carbon export.

II. Thesis objectives and outline

Traditional methods employed to estimate metabolism of zooplankton are highly time-consuming processes, preventing its use on large study regions. Likewise, enzymatic methods, although useful, show many uncertainties to approach metabolic rates. In this regard, semi-automated image-based systems (IBS), combined with metabolic equations, could result a suitable alternative to predict zooplankton-mediated fluxes along large spatial scales. On the other hand, relationships between zooplankton and data from environmental variables obtained from remote sensing might eventually help to develop models exploring carbon fluxes mediated by these communities.

Understanding the role of zooplankton in the biological pump in the ocean requires accurate estimates of community biomass and metabolism. The aim of this thesis was to develop and test alternative tools to assess zooplankton biomass and metabolic fluxes at large spatial and temporal scales. The specific objectives of this work are now outlined in order to answer the following questions:

Are image-based systems (IBS) a valid alternative to study zooplankton biomass and metabolism? **Chapter 1**

We addressed this question through a comparative analysis between estimates of biomass and growth, respiration, egestion and ingestion rates using an IBS, and those approached from enzymatic and traditional methods. This study was carried out along a time-series in subtropical waters.

Is there a physical-biological coupling on regions with high mesoscale activity? Are IBS useful to study zooplankton on these particular regions? **Chapter 2**

We answered this double question by applying the IBS and metabolic equations previously developed in this thesis for the subtropical region to the transition zone between the coastal upwelling off NW Africa and the Canary Islands waters. The distribution of biomass and zooplankton

metabolic fluxes was studied along numerous physical structures: filaments, island-induced eddies, jets and fronts.

Does it increase the accuracy of zooplankton metabolic estimates when predictive equations fit to the specific conditions of each region? **Chapter 3**

To answer this question we developed a set of equations predicting growth and respiration of zooplankton, fitted to the temperature ranges found along the main ocean regions and depth layers. Then, estimates using these temperature-specific equations, and those from existing (global) relationships, were compared with measurements from enzymatic methods along the circumnavigation Malaspina-2010 (~40ºN-40ºS, up to 2000 m depth).

What is the magnitude of zooplankton active fluxes in the warm Atlantic? Are they correlated with environmental data available from remote sensing? **Chapter 4**

These questions were addressed by applying the temperature-specific equations to predict metabolism previously developed and tested in this thesis. Using an IBS, we studied the contribution of zooplankton active fluxes to the biological pump through respiration, mortality, egestion and ammonia excretion on a latitudinal transect along the tropical and subtropical Atlantic (10ºS-25ºN). The possible correlation between active flux and environmental data easily available through remote sensing was also investigated.

Results

Chapter 1

The use of an image-based approach for the assessment of zooplankton physiological rates: a comparison with enzymatic methods

The use of an image-based approach for the assessment of zooplankton physiological rates: a comparison with enzymatic methods

Juan Carlos Garijo and Santiago Hernández-León (2015)
Journal of Plankton Research, 37: 923-938

Abstract

Measuring zooplankton biomass and physiological rates is of paramount importance in biological oceanography in order to assess the role of this community in, *e.g.*, carbon fluxes. Classical methods (incubations) are highly time-consuming and impractical to match physical and chemical measurements in oceanography. Attempting to solve this, a variety of methods (*e.g.,* egg production, RNA/DNA ratio or enzyme activities) were developed over the last decades. These methods also show uncertainties and hitherto only incubation methods have been widely accepted. Predictive equations relating physiological processes and body weight and temperature are a rough alternative normally used to ascertain the role of these organisms in the oceanic ecosystem. However, using imaging systems and empirical relationships to determine body weight allow the application of physiological models to each individual, obtaining reliable estimates for taxonomic groups and size classes. In this study, we developed predictive equations suitable for growth and respiration estimations in subtropical regions. In addition, biomass and physiological rates assessed from empirical equations in combination with an image-based system (*ZooImage*) were compared with standard and enzymatic methods respectively. We observed a consistent agreement between methodologies, the former resulting in an inexpensive and faster procedure for the appraisal of biomass and community carbon fluxes at large spatial and temporal scales.

1.1 Introduction

Zooplankton plays a key role in the biogeochemical cycles in the ocean. It occupies a central position in the pelagic realm, connecting the microbial food web with the larger organisms (Hernández-León et al., 2007). They recycle, redistribute and repackage carbon and nutrients, not only at different levels of the trophic web, but also horizontally and vertically in the water column (Banse, 1995). Therefore, they are of importance in the flux of energy and matter in the ocean. Understanding the physiological processes of live zooplankton such as ingestion, growth, respiration and egestion, as well as the precise estimation of their biomass are of importance to assess the rates at which these organisms process material and their contribution to carbon cycle in the ocean.

Estimates of biomass based on dry weight (dw) measurements (Lovegrove, 1966) have not evolved over the last half century, obtaining broad measurements of community biomass without discrimination between taxonomic groups or individuals. Besides, the destruction of samples using this procedure prevents their future analysis. These shortcomings have promoted the development of semi-automated image-based systems (IBS) (Grosjean et al., 2004; Benfield et al., 2007; Gislason and Silva, 2009; MacLeod et al., 2010). These systems use empirical relationships to estimate body weight (bw), allowing taxonomic and size class determination in a faster process, with less effort and increasing the spatial and temporal resolution (Benfield et al., 2007; MacLeod et al., 2010).

In turn, many approaches were developed to estimate physiological rates in marine systems over the last decades. Different methods are found in the literature to estimate growth rates (see Basedow et al., 2014) and respiration. However, these methodologies are highly time-consuming, labor-intensive and impractical to match physical and chemical measurements in oceanography, precluding their application in large spatial and temporal scales. Attempting to solve this, a variety of enzymatic methods have evolved over the last years. Rough estimations of respiration [using *e.g.,*

electron transfer system (ETS), Packard, 1971] and growth [*e.g.,* aminoacyl-tRNA-syntethases (AARS), Yebra and Hernández-León, 2004; aspartate transcarbamylase (ATC), Biegala and Bergeron, 1998] can be inferred from the overall enzymatic activity. However, these methodologies also show many uncertainties and hitherto only incubation methods have been widely accepted. A rough alternative normally used for growth estimates is predictive equations relating physiological processes and temperature (Huntley and Lopez, 1992), temperature and body weight (Ikeda and Motoda, 1978; Hirst and Lampitt, 1998; Hirst et al., 2003) or temperature, body weight and chlorophyll a (Chl*a*) as a food proxy (Hirst and Bunker, 2003; Zhou et al., 2010), obtaining reasonable estimates (Peterson et al., 2002; Basedow et al., 2014; Stock et al., 2014). Nevertheless, it is still unclear how the ambient food quantity and quality accounts for some of the variability observed in growth rates and production when comparing predictions and field measurements (see Calbet et al., 2002; Peterson et al. 2002).

On the one hand, it seems clear that food influences zooplankton growth rates, as it has been shown for copepods in a variety of environments, including freshwater systems (*e.g.,* Ban, 1994), tropical seas (Webber and Roff, 1995), temperate coastal regions (*e.g.,* Bautista et al., 1994), and upwelling systems (*e.g.,* Richardson and Verheye, 1998). On the other hand, some authors have suggested that the degree of food limitation in copepods may be related to their own size, with a progressive limitation at increasing body sizes (Webber and Roff, 1995; Richardson and Verheye, 1999). In any case, two decades ago, Huntley and Lopez (Huntley and Lopez, 1992) already contended that variance in production would be primarily determined by the variance in biomass, since the latter was one to three orders of magnitude greater than the variability observed on individual growth rates. Hence, they claimed for the achievement of more accurate measurements of biomass (*e.g.*, optical techniques) as the key factor for improving production estimates.

Measurements of respiration rates show limitations in both time and space, especially in the vertical distribution. Moreover, the required incubation-based experiments have problems related to factors such as stress, crowding, starvation and bacterial growth (Ikeda et al., 2000). To solve this, Packard (Packard, 1969) proposed alternative methodologies based on the analysis of cellular enzymatic activities as an index of respiration. Thus, electron transfer system (ETS) activity represented a clear advantage compared with classical methods since it could be measured in organisms captured at depth, and solving both horizontal and vertical mapping of plankton metabolism quite precisely (see Packard, 1985). However, the main problem still bearing ETS at present is the uncertainty in the relationship between enzymatic activities and respiration rates (Hernández-León and Gómez, 1996). Adversities finding of a universal R/ETS ratio for the zooplankton in the global ocean have led to considerable resistance on accepting this method to predict metabolic rates. ETS activities are measured under substrate-saturated conditions, while cells might be substrate-limited in nature when determining respiration on incubations. Thus, considerable higher enzyme activities would be measured, resulting in low physiological/enzyme ratios. Values of 0.5-1.0 for the R/ETS ratio (Hernández-León and Gómez, 1996) were observed in many studies determining respiration from enzyme activities (Hernández-León et al., 2001, 2002, 2004 and 2007), despite it is well known that values close to 0.5 tend to underestimate physiological rates when the substrates are not limited *in vivo*. Therefore, better estimates are achieved in cases where the enzyme is not substrate-limited (Hernández-León and Gómez, 1996; Hernández-León and Torres, 1997). Alternatively, Ikeda (Ikeda, 1985) developed an empirical relationship between metabolic rates and body mass as a function of temperature according to Ikeda (1974) and literature therein. It consisted in a general predictive equation for mesozooplankton from a vast collection of data-sets all over the world.

Zooplankton carbon fluxes from ingestion are the result of growth, respiration and egestion. A rough estimation of ingestion rates has traditionally assumed that about two-thirds of the ingested food is assimilated and one-half of the assimilated portion is respired, and the other

half used for growth (Kiørboe et al., 1985; Lenz et al., 1993; Båmstedt et al., 1999; Hernández-León et al., 2001, 2002, 2004). Likewise, another extended procedure assumes respiration to be 40% of ingestion (Ikeda and Motoda, 1978). Nevertheless, both approaches require the determination of the physiological parameters, at least respiration, with the constraints listed above. Moreover, when using respiration from ETS to estimate ingestion rates, results may be widely influenced by the R/ETS ratio adopted.

Since the first estimations of the gut fluorescence (GF) in zooplankton (Nemoto, 1968), grazing upon phytoplankton from measurements of this parameter and gut evacuation rates (*r*) has become a methodology widely accepted in marine zooplankton over the last decades (*e.g.*, Irigoien, 1998). However, despite GF results a relatively simple method, the determination of *r* requires to analyze a higher number of samples and many time-consuming experiments. In any case, this methodology was a breakthrough compared with standard procedures, since it resulted in a faster method suitable for organisms captured at depth. To solve the problem of estimating *r*, Irigoien (Irigoien, 1998) made a review to demonstrate, as previously done by Kiørboe et al. (Kiørboe et al., 1982), that *r* was temperature-dependent and related both parameters in one equation. Such relationships have been commonly accepted (*e.g.*, Landry et al., 2009). Nevertheless, there is a controversy about the variability of *r* in relation to food concentration (Pasternak, 1994) and initial gut content (GC) (*e.g.,* Perissinotto and Pakhomov, 1996). Besides, GF also suffers from the uncertainty of the pigment degradation in the gut (*e.g.,* Conover et al., 1986). Thus, alternative *in vivo* methods, following a non-destructive process, such as pulse amplitude modulated (PAM) fluorometry or laser imaging techniques were proposed to improve grazing assessments (Karaköylü et al., 2009, 2012; Sastri et al., 2011).

The development of imaging systems to obtain body weight (Hernández-León and Montero, 2006) allows the application of physiological models to each individual, obtaining reliable estimates for taxonomic groups and size classes when the habitat temperature is known. Thus, in this study, a total of

92 samples from the subtropical waters off the Canary Islands region were analyzed to compare assessments of zooplankton biomass, physiological rates and carbon fluxes. The objectives were (i) to validate IBS using empirical relationships to estimate biomass comparing with the standard methodology, (ii) to compare physiological rates assessed from predictive equations in combination with an IBS respect to enzymatic methodologies, (iii) to develop a suitable set of predictive equations to estimate growth and respiration of zooplankton in subtropical waters, (iv) to adjust a relationship between bw and GC in zooplankton living in subtropical regions, and (v) to test the potential of the IBS for the appraisal of community carbon fluxes of zooplankton at large spatial and temporal scales.

1.2 Methods

1.2.1 Sampling

Samples used in this study were obtained from the Lucifer II sampling program. We sampled a single transect of four stations, 10 nautical miles apart, north of Gran Canaria Island (Fig. 1.1) in an oligotrophic region considered undisturbed by the high mesoscale activity of the islands (Barton et al., 1998). This sampling was carried out on board the R/V "Atlantic Explorer" from 22nd November 2010 to 2nd June 2011 completing a time-series of 23 weekly samplings. Zooplankton was sampled in vertical hauls from 200 m to the surface using a double WP-2 net (UNESCO, 1968) with a 100 μm mesh. One of the zooplankton samples from the WP-2 net was size fractionated (100-200, 200-500, 500-1000 and >1000 μm) and immediately frozen in liquid nitrogen on board for subsequent analysis of AARS, ETS and GF.

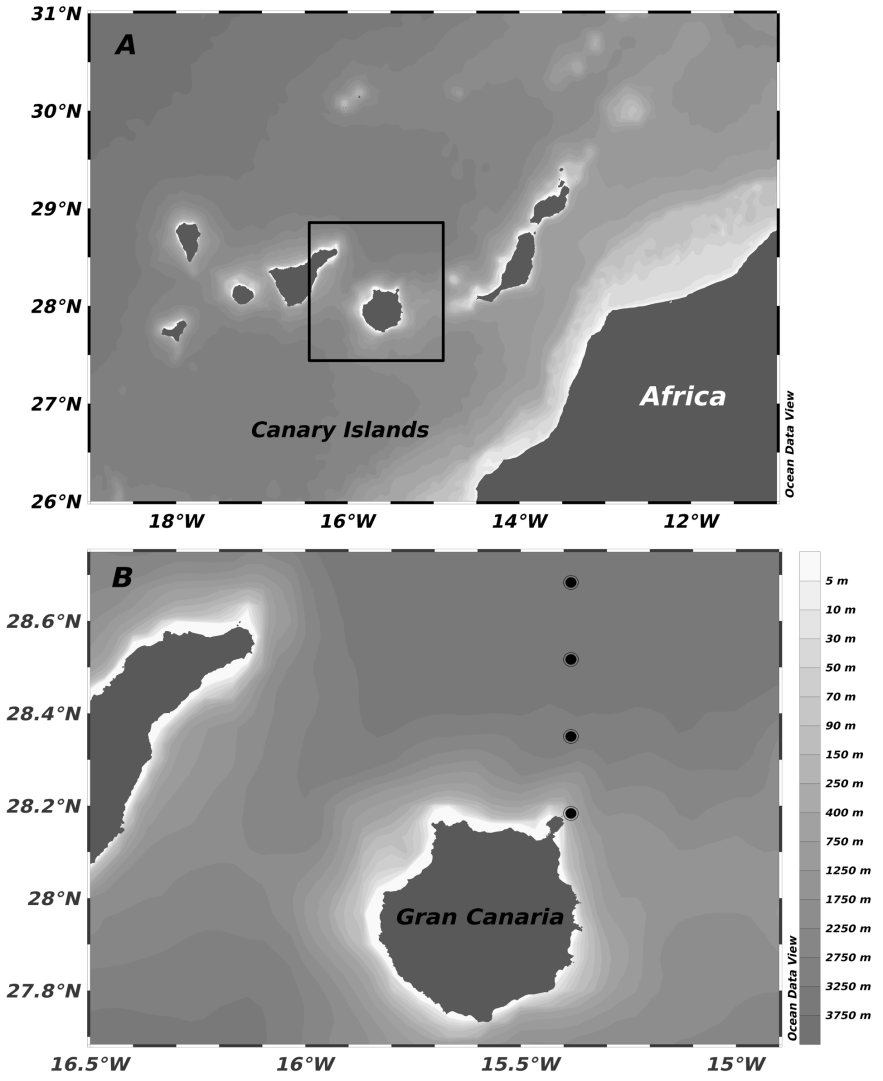

Figure 1.1. Map showing the Canary Islands in the Atlantic Ocean and the study region inside the black box (A). Location of the four stations north of Gran Canaria Island (B).

The other sample was preserved in formaldehyde (1%) and splitted into two parts in the laboratory the next day, using one-half for dry weight measurements (Lovegrove, 1966) and preserving the other half in formaldehyde (4%) for the estimation of biomass and abundance using an IBS. Temperature and conductivity were recorded down to 300 m depth using a *SeaBird SBE 25 plus* CTD mounted on a *General Oceanics* rosette

sampler, equipped with Niskin bottles. Phytoplankton chlorophyll was derived from depth profiles of *in situ* fluorescence measured with a *Turner Scufa* fluorometer, calibrated with samples collected within the upper 200 m of the water column in accordance with the JGOFS recommendations (UNESCO, 1994).

1.2.2 Biomass estimates

To compare biomass using the dry weight method (B_{DW}) and an IBS (B_{IBS}), samples were analyzed in two different ways. In the first case, samples were size fractionated into 100-200, 200-500, 500-1000 and >1000 μm size classes and organisms were dried at 60ºC for 24 h according to Lovegrove (1966), allowing the samples to reach room temperature, avoiding humidity, and then weighed on a microbalance. In the case of B_{IBS} samples were, first of all, sieved into two size fractions using a 1000 μm mesh to analyze separately large and small zooplankton. These size-separated fractions were introduced in a flask where organisms were homogeneously distributed and, depending on the zooplankton density, aliquots of 5 mL were taken with a Hensen pipette and poured onto polystyrene plates (90 x 130 mm). This procedure ensures the best representation of the zooplankton diversity of each sample with minimum overlap of the animals. Subsamples were then digitized using an *Epson Perfection 4990 PHOTO* scanner (with *VueScan Professional Edition 8.4.77* software) at a resolution of 1200 dpi and processed afterwards with the software *ZooImage 1 version 1.2-1* (http://www.sciviews.org/zooimage) according to Grosjean and Denis (2007). Organisms were enumerated, measured, weighed and classified into five taxonomic groups: copepods, chaetognaths, euphausiid-like, gelatinous and other zooplankton.

A manual training set from 24 samples chosen to represent the whole diversity of the studied area was set up to help the software to establish classification patterns. A total of 1685 digitized organisms were manually sorted into the above mentioned categories. The Random Forest algorithm was selected to build the classifier according to Grosjean et al. (2004) as we

checked that it provided the best results. To minimize the global error in this step, mysids, euphausiids and decapods larvae (brachyura) were included into the euphausiid-like group, as well as thaliaceans, siphonophores and ctenophores into gelatinous. The other zooplankton group embraced some scarce organisms such as amphipods, cladocerans, appendicularians, ostracods, polychaets, isopods, pteropods, other larvae or some unidentified organisms. Marine snow, fibers, bubbles, shadows and many other inorganic components of the samples were assembled into an extra group in order to be discarded when determining biomass or abundances. With these conditions the final global error achieved in the automated classification was estimated to be as low as 4.7%.

Estimates of B_{IBS} were based on relationships between body area and dry weight. The software estimated the area of the individuals from their silhouette and then automatically transformed it into an ellipse of equivalent area. Then, we applied the empirical relationships between body area and dw given by Hernández-León and Montero (2006) and improved by Lehette and Hernández-León (2009), using a different equation for each taxonomic group (Table 1.1). Measurements of biomass were expressed in terms of carbon content according to Kiørboe (2013) stressing the different body composition of individuals according to their taxonomic group.

Table 1.1. Empirical relationships between body area and dw given by Lehette and Hernández-León (2009) to estimate biomass with the IBS. Conversion factors were extracted from Kiørboe (2013).

Categories	Dry weight (dw)	Conversion factor (C:dw)
Copepods	$dw = 43.97 \cdot S^{1.52}$ r = 0.972 n =315	0.480
Chaetognaths	$dw = 23.45 \cdot S^{1.19}$ r = 0.840 n =33	0.361
Euphausiid-like	$dw = 49.58 \cdot S^{1.48}$ r = 0.987 n =88	0.419
Gelatinous	$dw = 43.17 \cdot S^{1.02}$ r = 0.916 n =9	0.051
Other zooplankton	$dw = 43.38 \cdot S^{1.54}$ r = 0.947 n =227	0.435

1.2.3 Growth and AARS activity

Growth rates from measurements of AARS activity (G_{AARS}) were compared with the results of four different sets of predictive equations (G_{IBS}) to test their reliability determining growth rates and production: Hirst et al. (Hirst et al., 2003), Hirst and Bunker (Hirst and Bunker, 2003), Zhou et al. (Zhou et al., 2010) and an additional set of equations developed in this study (see below). AARS activity was also measured during the sampling program and it is reported elsewhere (M. L. Torreblanca et al., in revision). Briefly, frozen samples were homogenized in Tris-HCl buffer (20 mM, pH 7.8) and centrifuged (10 min, 0°C) before the AARS enzymes specific activity was assayed following the method of Yebra and Hernández-León (Yebra and Hernández-León, 2004), slightly modified by Herrera et al. (2012). AARS activity was then corrected for the *in situ* temperature by applying an activation energy of 8.57 kcal mol^{-1} (Yebra et al., 2005a) to the Arrhenius equation in order to obtain the *in situ* activity normalized to protein content of samples. A fraction of the initial homogenate was analyzed following the Lowry et al. (Lowry et al., 1951) method, adapted for micro-assay by Rutter (1967), using *Bovin Serum Albumin* as standard (A-4503, from Sigma). Specific activity (spAARS) was then converted to specific growth (G_{AARS}) using the equation given by Hernández-León et al. (in preparation):

$$G_{AARS}\ (d^{-1}) = -0.0117 + 0.0038 \cdot \text{spAARS} \quad (r^2 = 0.738;\ p<0.001) \quad (1.1)$$

where spAARS was expressed in terms of nmol PPi mg $prot^{-1}$ h^{-1}, and PPi is pyrophosphate.

Regarding predictive equations, the purely empirical estimates by Hirst and Bunker (2003) and the combined theoretical-empirical estimates by Zhou et al. (2010) used body weight, temperature and Chl*a* as a food quantity proxy to determine the growth of the community through a single equation (Table 1.2). Zhou et al. (2010) derived a semi-empirical relationship by combining the empirical equations of Hirst and Bunker (Hirst and Bunker, 2003) with the theoretical definitions of growth by Huntley and Boyd (1984), and with theoretical and empirical considerations in relation to clearance rates. In the

case of Zhou et al. (2010) we adopted a food saturation concentration of 38 mg C m^{-3} at 19ºC (as originally given in their manuscript, equation 17) as well as ratios C:Chl*a* of 50 (*e.g.*, Reigstad et al., 2008) and 100 (Almeda et al., 2010). Additional food saturation concentrations of 28.5 and 19 mg C m^{-3} at 19ºC (25% and 50% lower) were also tested. In turn, Hirst et al. (2003) developed a set of predictive equations for epipelagic metazoan zooplankton from a vast compilation of experiments from polar to equatorial waters and from upwelling to more oligotrophic regions. They considered growth rates as a function of temperature and body weight and provided different equations according to taxonomic groups (Table 1.3). In contrast, the set of equations developed in this study was fitted to the physical conditions given in the subtropical waters of the Canary Islands region (16-26ºC) (Table 1.3), and handling the data contained in Hirst et al. (2003) (their Appendices 1 and 2). Regressions were developed using a linear regression program in *SYSTAT version 13*. All individual estimates of growth from predictive equations used body weight from the IBS.

We obtained the production of the community (mg C m^{-2} d^{-1}) from enzymatic measurements (P_{AARS}) using estimates of specific G_{AARS} and the community biomass obtained with the traditional method (Lovegrove, 1966). On the other hand, we used the community biomass from the IBS and estimates of specific G_{IBS} to obtain production using predictive equations (P_{IBS}).

1.2.4 Respiration and ETS activity

Respiration from measurements of ETS activity (R_{ETS}) were compared with estimates using the generalist equation for the global ocean given by Ikeda (Ikeda, 1985) as well as an equation developed in this study according to the physical conditions of our subtropical waters (Table 1.4). It was calculated using a linear regression program in *SYSTAT version 13* with data compiled by Hernández-León and Ikeda (2005). An aliquot of the homogenate used

Table 1.2. Predictive equations used to estimate growth of zooplankton according to Hirst and Bunker (Hirst and Bunker, 2003) and Zhou et al. (Zhou et al., 2010).

Copepods						
	Multiple linear regression	a	b	c	d	r^2
Hirst & Bunker (2003)	$\log_{10} G\ (d^{-1}) = a[T] + b[\log_{10} bw] + c[\log_{10} Ca] + d$	0.0186	-0.288	0.417	-1.209	0.289
Zhou et al. (2010)	$G\ (d^{-1}) = 0.033 \cdot [Ca / (Ca + 205 \cdot e^{aT}] \cdot e^{b\,T} \cdot bw^{-c}$	-0.125	0.090	-0.060	-	-

for AARS activity measurements was also analyzed for ETS activity based on the method of Packard (Packard, 1971) for zooplankton. Details of the procedure are given in Hernández-León and Gómez (1996). ETS activity was corrected for *in situ* temperature using the Arrhenius equation and an activation energy of 15 Kcal mol^{-1} (Packard et al., 1975) and was finally normalized to the protein content of samples (µl O_2 mg $prot^{-1}$ h^{-1}). ETS activity was then approximated to potential respiration rates (d^{-1}) of the community assuming a ratio C:dw = 0.48 (Kiørboe, 2013) and using the relationship between dry weight (dw) and proteins given by Hernández-León et al. (2001) for the Canary Islands waters:

$$dw = 1.445 + 4.283 \cdot prot \qquad (r^2 = 0.900;\ n = 306;\ p<0.001) \qquad (1.2)$$

In both, our equation and the one given by Ikeda (Ikeda, 1985), individual respiration rates (µl O_2 ind^{-1} h^{-1}) were assessed using the body weight (dw content) estimated with the IBS. In the case of Ikeda (Ikeda, 1985), dry weight was converted into N according to Kiørboe (2013), as this equation provided the best results. As occurred with growth, specific respiration from the enzymatic method was converted into community R_{ETS} using the biomass of the community obtained from the traditional method (Lovegrove 1966), and assuming R/ETS ratios of 0.5 and 1.0 (Hernández-León and Gómez, 1996). Likewise, we used the biomass obtained from the IBS to estimate community respiration from predictive equations. In all cases the oxygen consumption was converted into C units using a respiratory quotient of 0.97 (Omori and Ikeda, 1984).

Table 1.3. Log-transformed zooplankton growth equations given by Hirst et al. (Hirst et al., 2003) for the global ocean and the equations developed in this study for subtropical regions as a function of body weight (bw, μg C ind-1) and temperature (T, ºC). We used the crustaceans equations to estimate growth of our defined euphausiid-like and other zooplankton groups. Source: Hirst et al. (2003).

	Copepods		**Chaetognaths**		**Crustaceans**		**Gelatinous**	
	Hirst et al. (2003)	This study	Hirst et al. (2003)	This study	Hirst et al. (2003)	This study	Hirst et al. (2003)	This study
Multiple linear regression ---------- **log_{10} g (d^{-1})**	a[T]+b[log_{10}bw]+c	a+b[log_{10}T]+c[log_{10}bw]	a+b[T]	a+b[log_{10}T]	a[T]+b[log_{10}bw]+c	a+b[log_{10}T]	a[T]+b[log_{10}bw]+c	a+b[log_{10}T]+c[log_{10}bw]
Variables	bw, T	bw, T	T	T	bw, T	T	bw, T	bw, T
Data points	2232	503	87	32	253	24	88	91
T range (ºC)	(-)2.3-29.5	16-26	1.8-31	16-26	0.5-25.6	17-25.6	11-26.5	16.26
bw range (μg C ind^{-1})	0.006-3620	0.5-240	0.9-650.2	0.9-238.4	2.54-64172	3.3-1639.5	4-34868.4	4-34868.4
a	0.035	-2.102	-1.851	-6.078	0.026	-14.840	0.065	-4.299
b	-0.128	1.020	0.037	3.920	-0.327	10.192	0.138	2.489
c	-1.529	-0.227	-	-	-0.919	-	-2.070	0.153
r^2	0.297	0.338	0.323	0.389	0.447	0.732	0.280	0.494

Table 1.4. Log-transformed zooplankton equation of respiration given by Ikeda (Ikeda, 1985) for the global ocean and the equation developed in this study for subtropical regions. Source: Hernández-León and Ikeda (2005).

	Respiration (µl O_2 ind^{-1} h^{-1})	
	Ikeda (1985)	This study
Multiple linear regression	ln R=a+b[ln bw]+c · T	$\log_{10}$ R=a+b[$\log_{10}$ bw]+c[$\log_{10}$ T]
Variables	bw (mg N), T (ºC)	bw (mg dw), T (ºC)
Data points	721	155
T range (ºC)	(-)1.4-30	16-26
a	1.741	-0.133
b	0.851	0.764
c	0.064	0.272
r^2	0.951	0.844

1.2.5 Egestion

Estimates of egestion rates (pigmented food) from GF (E_{GF}) were compared with total egestion rates (pigmented plus non-pigmented food) (E_{IBS}) derived from empirical relationships between bw and GC for organisms living in subtropical waters, and temperature and gut evacuation rates. An aliquot of the same homogenate used for the AARS, ETS and protein analysis was placed in a test tube with 10 mL of 90% acetone and stored at -20ºC for 24 hours in darkness. Fluorescence of samples was measured before and after acidification with three drops of 10% HCl in a Turner Desing fluorometer (model 10-AU-005-CE), previously calibrated with pure Chl*a* as described by Yentsch and Menzel (1963). Pigments were calculated from Strickland and Parsons (1972) and modified by Hernández-León et al. (2001):

$$\text{chlorophyll a} = k \cdot (Fo - Fa) \cdot \text{mg prot}^{-1} \quad (1.3)$$

$$\text{pheopigments} = k \cdot (R \cdot Fo - Fa) \cdot \text{mg prot}^{-1} \quad (1.4)$$

where k is the instrument calibration constant, Fo and Fa are the fluorescence measurements before and after acidification and R is the

acidification coefficient. Gut pigment concentration refers to the addition of chlorophyll a and pheopigments. It was normalized to the protein content of the samples (μg pigment·mg $prot^{-1}$) and then specific phytoplankton E_{GF} (d^{-1}) was obtained assuming a C:Chl*a* ratio of 50 (*e.g.*, Reigstad et al., 2008) and a C:dw ratio of 0.48 (Kiørboe, 2013). Besides, we used the relationship between dw and proteins specified in equation above, and a gut evacuation rate constant determined for the area of 0.056 min^{-1} (Hernández-León et al., 2002).

In turn, estimates of individual biomass and GF from the Lucifer II sampling, as well as data contained in Hernández-León et al. (2004), were averaged into 100-200, 200-500, 500-1000 and >1000 μm size fractions to develop a relationship between bw (μg C ind^{-1}) and GC (ng Chl*a* eq ind^{-1}) for subtropical waters (see Section 1.3). Although we measured pigments through GF they served uniquely as tracers, which represented the whole range of total GC of individuals in terms of carbon (using a C:Chl*a* ratio of 50) according to their body weight. In this sense, lowest values of GF in omnivore organisms did not necessarily imply empty guts since individuals also ingested non-pigmented food as well. However, pigment content at these levels may represent the minimum gut content of an individual for a given body weight. Similarly, highest values of GF represent the maximum GC for each body size class. Zooplankton in subtropical waters show periods of 100% pigmented food in the gut (*e.g.*, during the late winter bloom), as well as periods with low values but indicating a large proportion of non-pigmented food (see omnivory index in Hernández-León et al., 2004). Therefore, based on our relationship between bw and GC we obtained an average of total carbon content in the gut (pigmented plus non-pigmented) as a function of bw. Average values of carbon content could be a valid option since gut fullness in nature is not constant over time and varies throughout the day (see Simard et al., 1985). Besides, Irigoien (Irigoien, 1998) made a review from the literature relating gut evacuation rates (e, min^{-1}) and temperature (T, ºC), developing the equation

$$e = 0.0026 \cdot T + 0.012 \ (r^2 = 0.940; n = 19) \quad (1.5)$$

According to both equations we assessed egestion rates of individuals (ng C ind^{-1} min^{-1}). Finally, community E_{IBS} was estimated using the biomass of the community obtained from the IBS. However, we used the biomass obtained from the traditional method (Lovegrove, 1966) to estimate community E_{GF}.

1.2.6 Ingestion

Specific ingestion rates were derived from previous estimations of growth (G), respiration (R) and egestion (E) rates. Thus, we compared ingestion exclusively from biochemical and standard procedures as

$$I_{biochem} = G_{AARS} + R_{ETS} + E_{GF} \quad (1.6)$$

with the results obtained using the IBS in combination with different equations:

$$I_{IBS} = G_{IBS} + R_{IBS} + E_{IBS} \quad (1.7)$$

where G_{IBS}, R_{IBS} and E_{IBS} were estimated using the equations developed in this study for subtropical waters. We also compared $I_{biochem}$ and I_{IBS} with $I_{I\&M}$ as $2.5 \times R_{IBS}$ (Ikeda and Motoda, 1978) and estimates of ingestion using the Saiz and Calbet (Saiz and Calbet, 2007) equation ($I_{S\&C}$) as

$$\log_{10}(I_{S\&C}) = -1.751 + 0.355 \cdot \log(bw) + 0.893 \cdot \log(F)$$
$$(r^2_{adj} = 0.810;\ p<0.001) \quad (1.8)$$

where bw was measured in μg C and F referred to the Chl*a* concentration (mg C m^{-3}) used as a food proxy, assuming a C:Chl*a* ratio of 50.

Specific ingestion rates from $I_{biochem}$ were converted into ingestion fluxes using the community biomass obtained from the traditional method (Lovegrove, 1966), while the biomass from the IBS was used to estimate community I_{IBS}, $I_{I\&M}$ and $I_{S\&C}$.

1.3 Results

Average temperature in the water column (0-200 m depth) during sampling ranged from 18.3ºC to 19.4ºC, following the general pattern in the region (Fig. 1.2a). Chlorophyll a values were low and typical of oligotrophic areas, with slightly higher values during February and March. Individuals in the 100-500 μm size fraction accounted for more than 90% of the total abundance, while copepods represented around 80-85% of the total (not shown), determining the average body weight of the community (Fig. 1.2b).

The relationship between community B_{DW} estimated according to Lovegrove (1966) and B_{IBS} showed a high correlation ($r^2 = 0.859$; $n = 92$; $p<0.05$) along the time-series ($B_{IBS} = 0.996 \cdot B_{DW} + 4.938$, Fig. 1.3). Values from B_{IBS} seemed to be slightly higher during the first half of the time-series while the opposite occurred from mid-March. However, no pattern according to the amount of biomass on the samples or to the size distribution of organisms (Fig. 1.2b) could be observed, and no significant differences were found between both methodologies (ANOVA, $p>0.05$).

Regarding growth measurements, our set of equations presented better correlations (r^2) than those of Hirst et al. (Hirst et al., 2003) for all categories (Table 1.3), mainly for crustaceans and gelatinous organisms. Although correlation was still low in our equation for copepods ($r^2 = 0.338$) it was around 15% higher than that of Hirst et al. (Hirst et al., 2003). Temperature in our set of equations ranged from 16 to 26ºC, while body weight varied from 0.1 to 1600 μg C ind^{-1} (Table 1.3). Thereby, specific growth and community production from AARS presented greater concordance with estimates from our equations than using those of Hirst et al. (Hirst et al., 2003) (Fig. 1.4a, b and Table 1.5). In fact, average values using our equations and the enzymatic method showed no significant differences (ANOVA, $p>0.05$) in terms of specific growth rates and production. In any case, specific G_{AARS} showed higher variability along the sampling period (Fig. 1.4a), while both methods highly matched in terms of production (Fig. 1.4b). In contrast, average estimates from the Hirst et al. (Hirst et al., 2003), Hirst

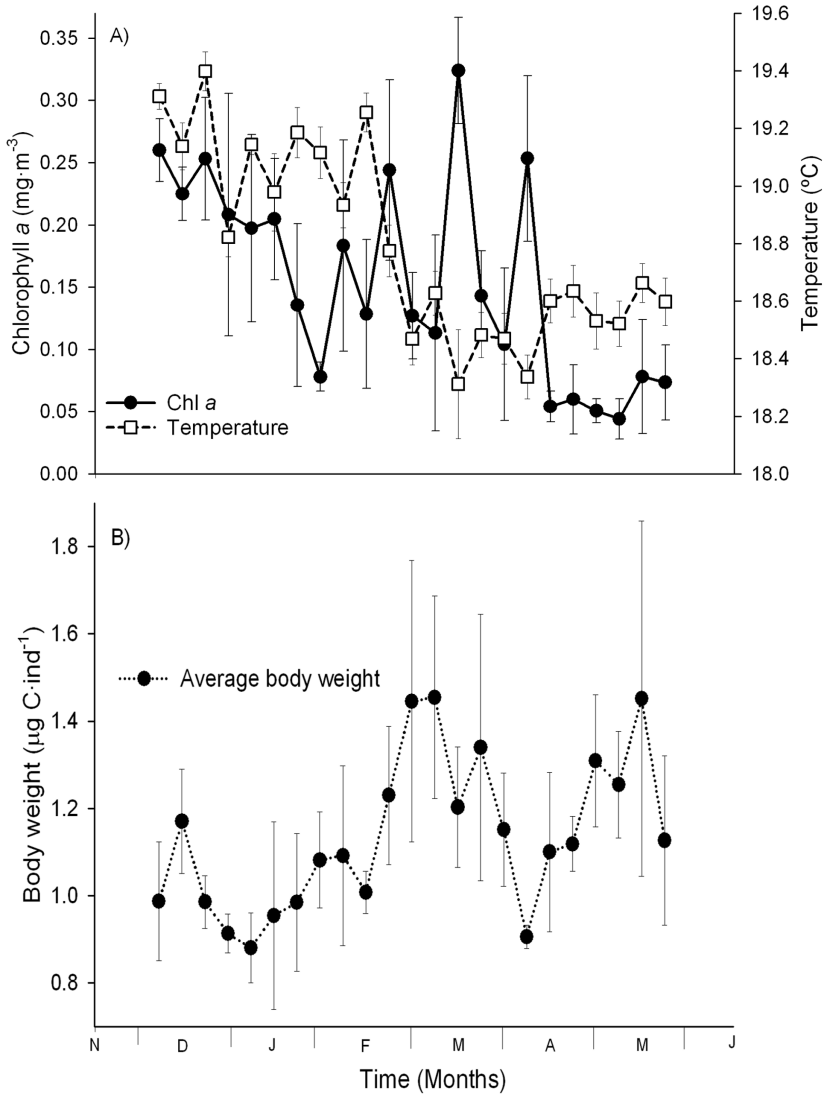

Figure 1.2. Average temperature and Chl*a* (A), as well as body weight of zooplankton (B) during the period of study.

and Bunker (Hirst and Bunker, 2003) and Zhou et al. (Zhou et al, 2010) equations presented significantly lower specific growth and community production (ANOVA, $p<0.05$) than estimates derived from AARS, although differences reduced when production was calculated (Fig. 1.4b and Table 1.5). Estimates from Hirst and Bunker (Hirst and Bunker, 2003) and Zhou

et al. (Zhou et al., 2010) equations presented a similar pattern (Fig. 1.4a, b) and matched the distribution of chlorophyll a (not shown). Additional estimates from the latter using a C:Chl*a* ratio of 100 (Almeda et al., 2010) and food saturation concentrations 25-50% lower than the standard (not shown), were also significantly lower (ANOVA, $p<0.05$) than G_{AARS}.

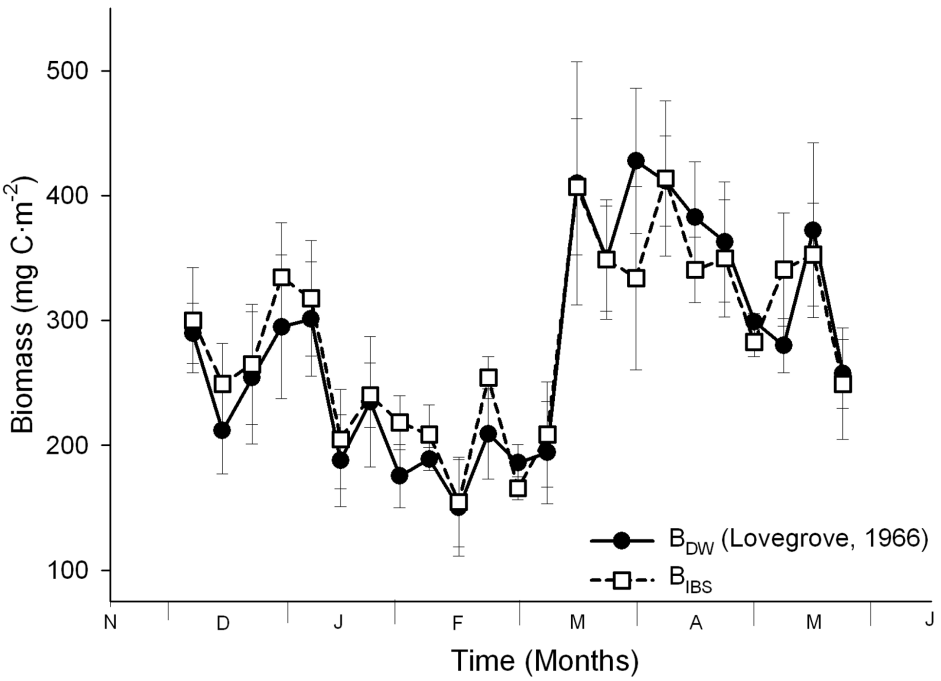

Figure 1.3. Comparison of community biomass between estimates using the image-based system (B_{IBS}) and the Lovegrove (Lovegrove, 1966) method (B_{DW}). Vertical bars denote standard deviations.

Concerning respiration, in the equation given by Ikeda (Ikeda, 1985) temperatures ranged from -1.4 to 30ºC, covering all the ocean regions, while our equation fitted to the specific conditions of our subtropical waters (16-26ºC); both showed high coefficients of determination (r^2) (Table 1.4). Specific respiration rates from the former were, in most cases, higher than potential respiration (ETS) (Fig. 1.5a), showing an average R/ETS ratio of 1.2. However, this ratio reduced to 0.8 using our equation, showing in this

case lower estimates than ETS along the time-series (Fig. 1.5a). Nevertheless, ETS measurements showed higher variability than specific respiration from both equations along the sampling period. On the other hand, community R_{ETS} and both equations followed a similar pattern, although differences on the magnitudes were observed (Fig. 1.5b). Thus, community respiration using the Ikeda (Ikeda, 1985) equation and R_{ETS} assuming a R/ETS ratio of 1 showed no significant differences (ANOVA, $p>0.05$), while the former was around two-fold higher when this ratio was 0.5 (Fig. 1.5b). However, community respiration from our equation ranged between estimates of R_{ETS} assuming ratios of 0.5 and 1.0.

The relationship between bw and GC for organisms living in subtropical waters showed a rather high coefficient of determination ($\log_{10}$ GC = 0.852 · $\log_{10}$ bw – 1.160; r^2 = 0.769; n = 208; $p<0.05$). It was, indeed, close to the one observed when literature data from other ocean regions were added (Fig. 1.6). As expected, specific and community estimates of E_{GF} were significantly lower (ANOVA, $p<0.05$) than those of E_{IBS} (Fig. 1.7a,b). As observed for growth and respiration, specific E_{GF} showed higher variability than E_{IBS} within the sampling period (Fig. 1.7a).

Specific ingestion from $I_{biochem}$ (and also from $I_{S\&C}$) showed the highest variability within the sampling period (Fig. 1.8a). Nevertheless, average community ingestion from I_{IBS} showed no significant differences (ANOVA, $p>0.05$) respect to $I_{biochem}$ and $I_{S\&C}$, although estimates from I_{IBS} were slightly higher (Fig. 1.8b and Table 1.6). However, specific and community ingestion from $I_{I\&M}$ were significantly lower (ANOVA, $p<0.05$) than estimates from $I_{biochem}$ (Fig. 1.8a, b and Table 1.6).

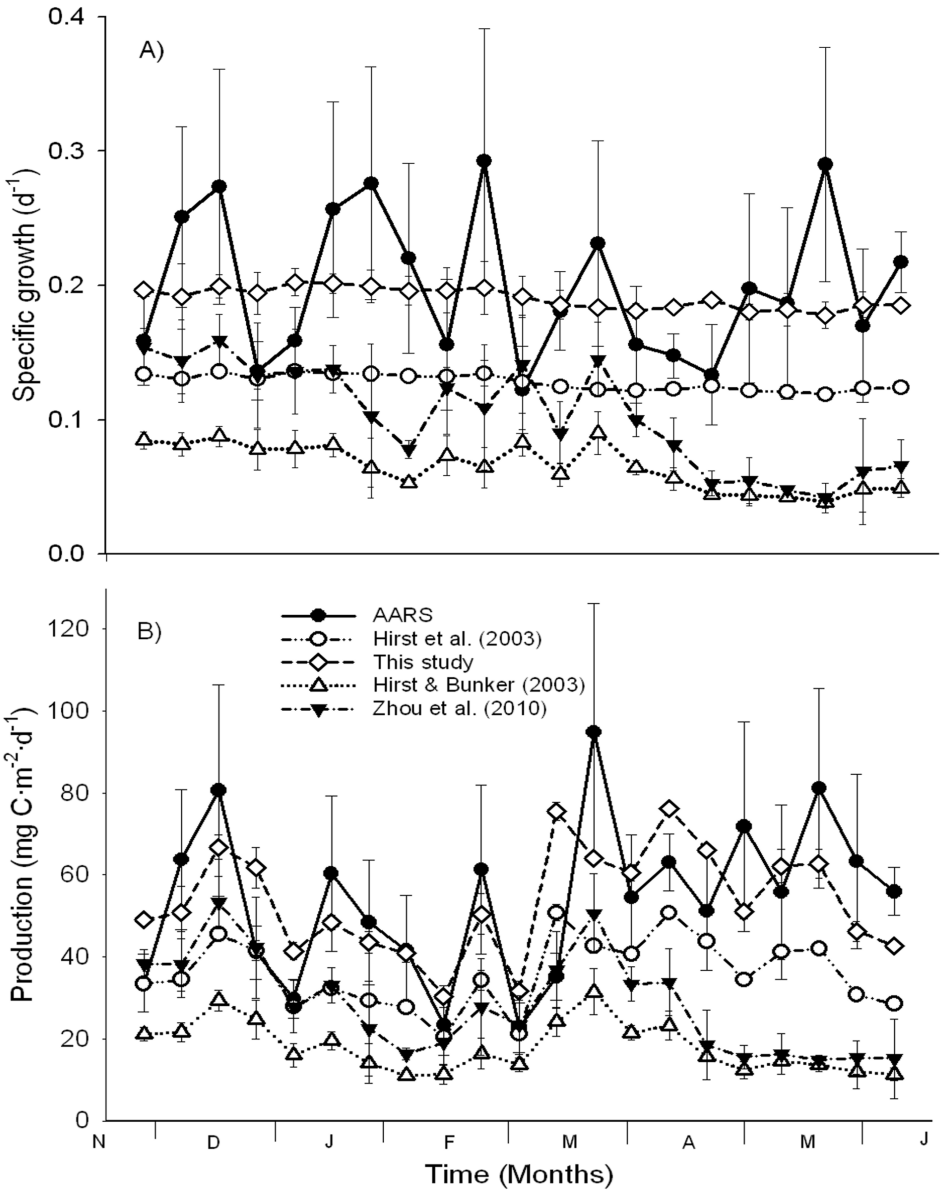

Figure 1.4. Specific growth (A) and community production (B) estimates according to the AARS method and using the IBS in combination with the equations by Hirst et al. (Hirst et al., 2003), Hirst and Bunker (Hirst and Bunker, 2003), Zhou et al. (Zhou et al., 2010), and the one developed in this study. Vertical bars denote standard deviations.

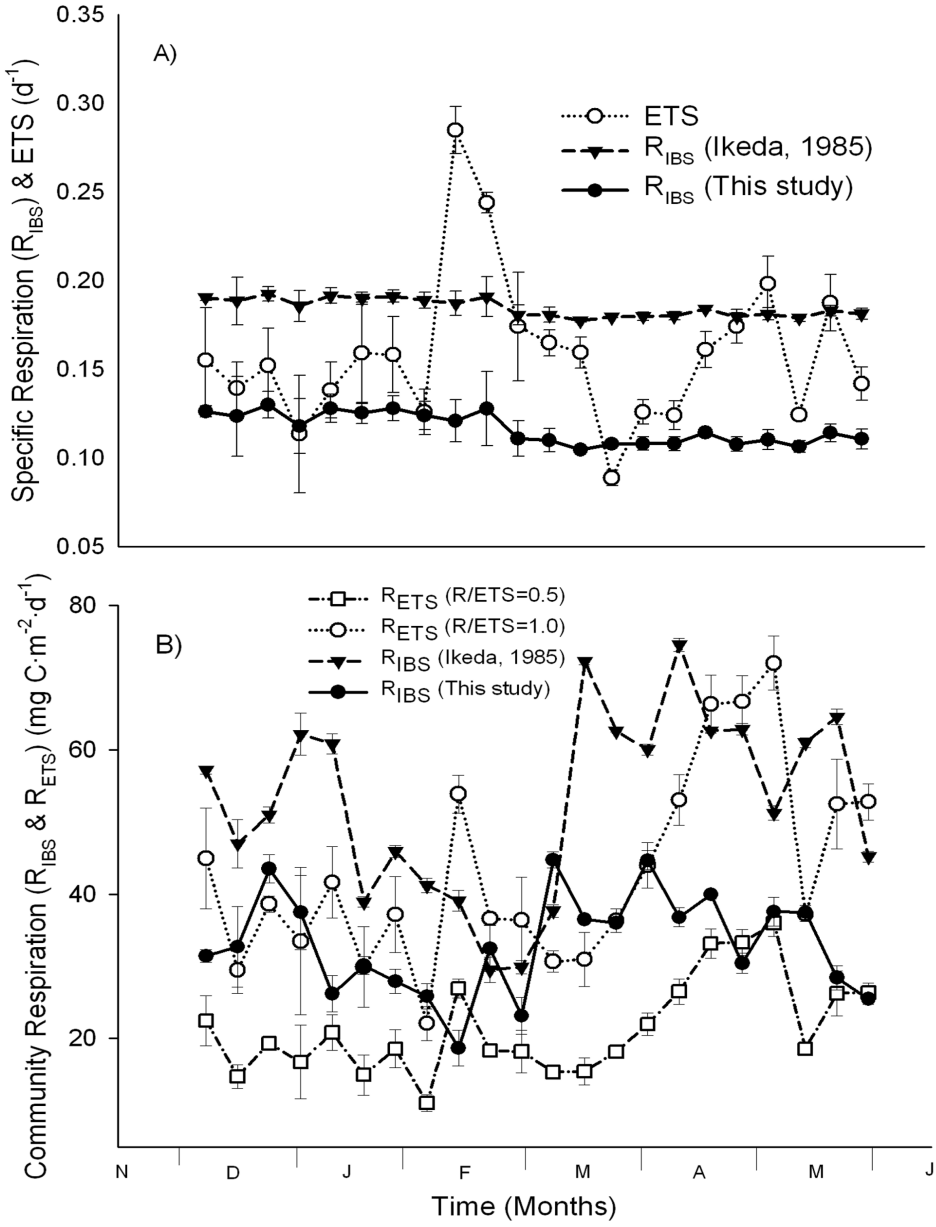

Figure 1.5. Specific respiration estimates from ETS and IBS in combination with the Ikeda (Ikeda, 1985) and the equation developed in this study for subtropical waters (A), and community respiration fluxes from these two equations and from ETS, assuming in this case R/ETS ratios of 0.5 and 1.0 (B). Vertical bars denote standard deviation.

1.4 Discussion

Our results confirm the usefulness and reliability of IBS in combination with adequate empirical relationships (Lehette and Hernández-León, 2009) to determine zooplankton biomass, since estimates using this method showed no significant differences (ANOVA, $p>0.05$) to those from the standard, well-accepted dry weight methodology (Lovegrove, 1966) (Fig. 1.3). In addition to the bias introduced by scarce remnants of molts, which could be retained by the mesh and measured by the latter methodology, slight differences between both methods could be mainly explained by physical effects of the preservative on the organisms. Early studies suggested that preservation with formaldehyde results in a weight loss of planktonic organisms (*e.g.*, Omori, 1970; Durbin and Durbin, 1978; Landry, 1978). However, results regarding possible shrinkage effects in Daphnia (Black and Dodoson, 2003) and crustaceans (Landry, 1978; Durbin and Durbin, 1978; Viitasalo et al., 1995) caused by formaldehyde indicated that this effect, if present, is minor. Moreover, Pollupüü (2007) observed no significant change in the body length of living and preserved organisms either in the case of adults or nauplii. The relationships we managed (Lehette and Hernández-León, 2009) were free of these formaldehyde-effects, since they only preserved their organisms at -20ºC before drying. Therefore, assuming no shrinkage effect, we can confirm the validity and, perhaps, the greater accuracy of the estimates from the IBS in comparison with standard procedures.

The accuracy (r^2) of the equations developed in this study to estimate growth in subtropical regions was significantly greater compared with those of Hirst et al. (Hirst et al., 2003) (Table 1.3). Besides, specific growth and production from the enzymatic method and our equations showed no significant differences (ANOVA, $p>0.05$) and were in agreement with Hernández-León et al. (2002) in the Canary Island waters (their Table 3, transects 5 and 6). However, estimates using the Hirst et al. (Hirst et al., 2003), Hirst and Bunker (Hirst and Bunker, 2003) and the Zhou et al. (Zhou et al, 2010) equations were significantly lower (ANOVA, $p<0.05$) (Fig. 1.4a, b and Table

1.5). In this sense, we consider these equations could underestimate growth and production in subtropical waters. As growth decreases with body weight and the opposite for temperature (*e.g.*, Hirst and Lampitt, 1998; Hirst et al., 2003; Hirst and Bunker, 2003), the equations given by Hirst et al. (Hirst et al., 2003) could be influenced by data from polar waters (-2.3ºC) and individuals up to two orders of magnitude larger than currently found in subtropical regions (Table 1.3). Likewise, Hirst and Bunker (Hirst and Bunker, 2003) and Zhou et al. (Zhou et al., 2010) managed an average temperature 4-5ºC lower than usually given in our region (Fig. 1.2).

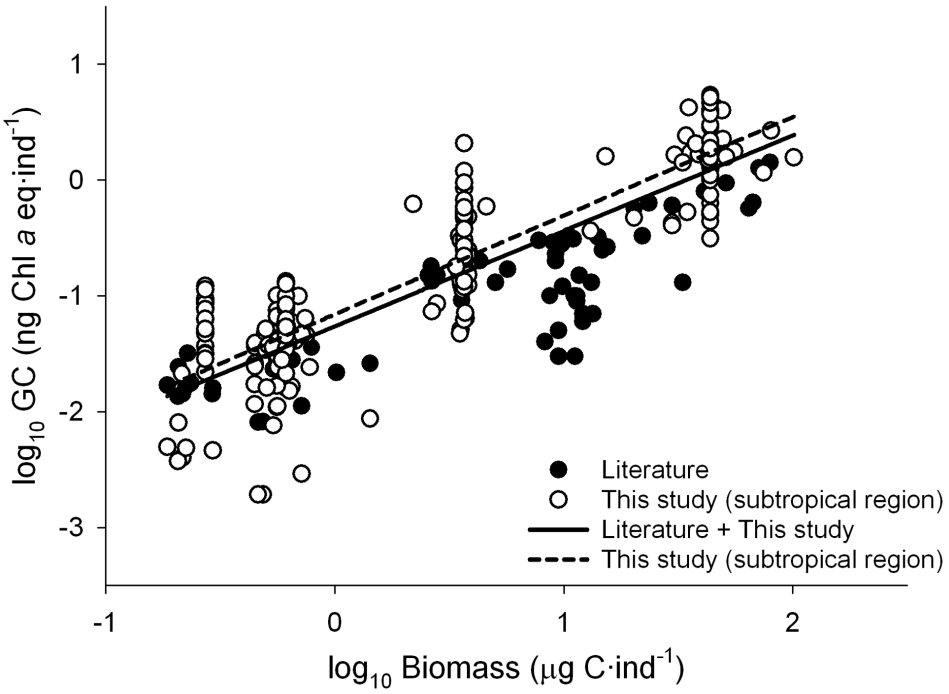

Figure 1.6. Log-transformed relationship between body weight and gut content for mesozooplankton living in subtropical waters (dashed line, n = 208, source: Hernández-León et al. (2004); this study), and the same relationship including also data from other regions (solid line, n = 296, source: Morales et al., 1991; Huskin et al., 2001; López and Anadón, 2008; Lee et al., 2011; Hernández-León et al, 2004; this study).

Moreover, these two equations could be also more appropriated for more productive waters, since the values of chlorophyll a in Hirst and Bunker (2003) (their Table 5) were up to two orders of magnitude higher than usually found in oligotrophic regions. Even more, chlorophyll a was the main variable influencing growth in this case (Fig. 1.2). Therefore, global models to estimate production using the Hirst and Bunker (Hirst and Bunker, 2003) or the Zhou et al. (Zhou et al., 2010) equations (*e.g.*, Stock et al., 2014) should be cautious since they could underestimate production in oligotrophic regions, where the importance of pigmented food in the diet of zooplankton is normally reduced (*e.g.*, Hernández-León et al., 2004). For all that, we claim for the need to set up more taxonomically-specified equations, adapted to each particular region in order to increase accuracy.

The differences between community production from AARS and the four equations analyzed here were lower than those observed for specific growth (Fig. 1.4b). This was in agreement with Huntley and Lopez (1992), who suggested biomass as the main factor influencing production estimates, since the variability of biomass measurements greatly exceeds that of growth rates (one to three orders of magnitude). Consequently, efforts should also focus on accurate determinations of biomass. The analysis of variance in specific growth rates from the AARS measurements revealed that more than 80% of the total variance was due to intrinsic variability of the method. This fact was also reflected by the standard deviations of the AARS measurements within each weekly sampling (Fig. 1.4a). In this sense, despite the enzymatic method has uncertainties, it seemed more sensitive to the variability occurred along the sampling period. Therefore, this method could represent a better alternative when assessing specific growth along a time-series, while the IBS could result more suitable for production and flux estimates when combined with adequate equations (Fig. 1.4b).

The average R/ETS ratio of 1.2 observed using the equation of Ikeda (Ikeda, 1985) was out of the range given by Hernández-León and Gómez (1996), who observed that R/ETS varied from 0.5 to 1.0, as indeed occurred using our equation (R/ETS = 0.8). Moreover, specific respiration from the

latter equation was very close to averaged values obtained by Hernández-León et al. (2001, 2002) and Yebra et al. (2005b) in the same region, as well as to the results of Hernández-León and Ikeda (2005) for this latitude. However, specific respiration using the equation of Ikeda (Ikeda, 1985) was considerably higher. This could be due to several reasons. First, the results could be influenced by the large amount of data from high and low latitudes and the wide range of temperature used to configure his equation. Moreover, the fact that oligotrophic waters are normally dominated by small zooplankton, with higher weight-specific respiration rates (*e.g.*, Ikeda and Mitchell, 1982), could introduce some bias when the size of organisms is not properly considered. In this case, specific respiration rates of the 100-500 μm individuals, which were 90% of total abundance, would determine the averaged rates for the community.

Table 1.5. Linear regressions (type II) between estimates of specific growth (G) and community production (P) from enzymatic measurements (AARS) and using the image-based system (IBS) in combination with empirical equations (n = 84).

	Sp growth (G) (d^{-1})	**Community production (P) (mg C m^{-2} d^{-1})**
Hirst et al. (2003)	$G_{IBS} = 0.636 \cdot G_{AARS} + 0.009$	$P_{IBS} = 0.617 \cdot P_{AARS} + 2.583$
Hirst and Bunker (2003)	$G_{IBS} = 0.324 \cdot G_{AARS} + 0.004$	$P_{IBS} = 0.311 \cdot P_{AARS} + 1.267$
Zhou et al. (2010)	$G_{IBS} = 0.636 \cdot G_{AARS} + 0.006$	$P_{IBS} = 0.482 \cdot P_{AARS} + 2.190$
This study	$G_{IBS} = 0.946 \cdot G_{AARS} + 0.013$	$P_{IBS} = 0.919 \cdot P_{AARS} + 3.837$

Concerning community respiration, our equation seemed more suitable for subtropical and oligotrophic regions since our results were in the range of Hernández-León et al. (2002) in the same region and agreed with Hernández-León and Gómez (1996), were they observed that R/ETS was close to 1 only during the Late Winter Bloom in the Canary Island waters. Therefore, specific equations fitted to each region could be more suitable to estimate respiration. Nevertheless, we suggest the ETS method as the main alternative to estimate the effect of physical structures or time-series (Fig.

1.5a), while IBS may result equally accurate to determine community respiration (Fig. 1.5b).

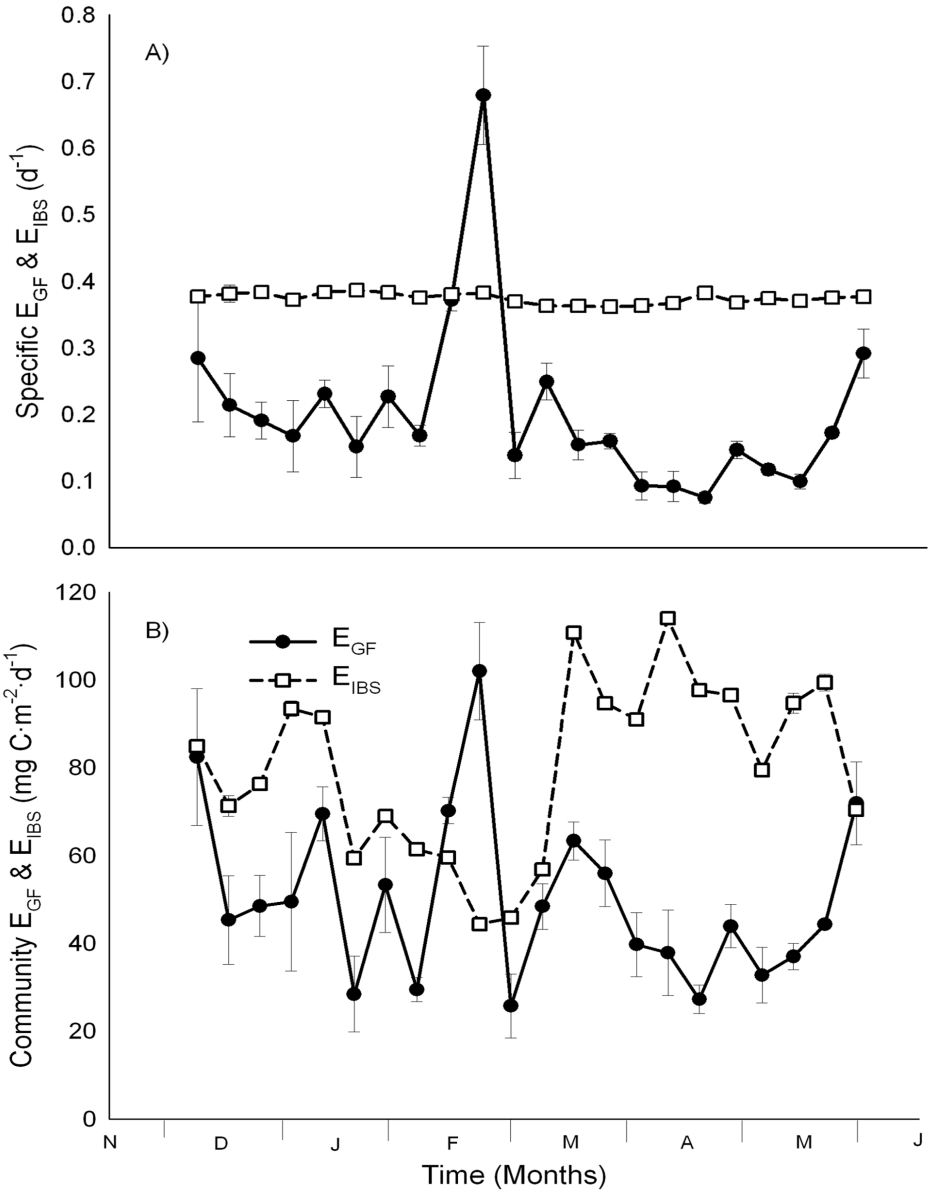

Figure 1.7. Specific egestion rates (A) and community egestion fluxes (B) from gut fluorescence (E_{GF}; pigmented food) and using the IBS (E_{IBS}; pigmented plus non-pigmented food). Vertical bars denote standard deviations.

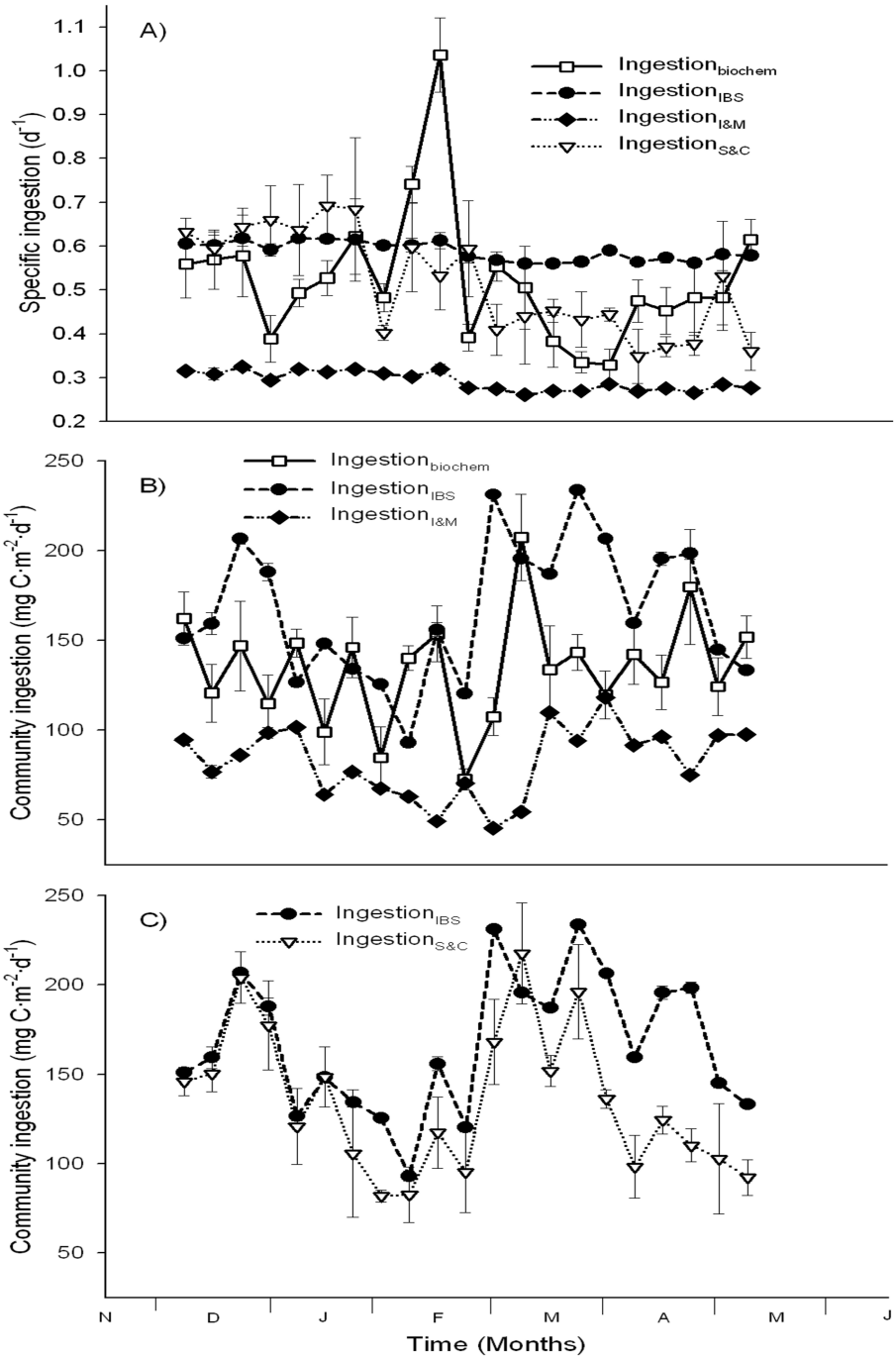

Figure 1.8. Specific ingestion rates (A) and community ingestion fluxes (B) estimated from *proxies* of physiological rates ($I_{biochem}$), using the IBS with equations developed in this study for subtropical waters (I_{IBS}), and also according to respiration estimates from the IBS and Ikeda and Motoda ($I_{I\&M}$; Ikeda and Motoda, 1978). Besides, community ingestion from I_{IBS} was compared with estimates using the equation given by Saiz and Calbet (Saiz and Calbet, 2007) ($I_{S\&C}$) (see Methods section) (C). Vertical bars denote standard deviations.

Table 1.6. Linear regressions (type II) between specific and community ingestion estimated from proxies of physiological rates ($I_{biochem}$), from the IBS in combination with our developed equations for subtropical regions (I_{IBS}) and using the equation given by Saiz and Calbet (Saiz and Calbet, 2007) ($I_{S\&C}$), and from respiration estimates ($I_{I\&M}$, Ikeda and Motoda 1978) (see Methods section, n = 84).

	Sp Ingestion (d^{-1})	**Community Ingestion (mg C m^{-2} d^{-1})**
$Ingestion_{IBS}$	$I_{IBS} = 1.042 \cdot I_{biochem} + 0.042$	$I_{IBS} = 1.117 \cdot I_{biochem} + 6.792$
$Ingestion_{I\&M}$	$I_{I\&M} = 0.520 \cdot I_{biochem} + 0.020$	$I_{I\&M} = 0.551 \cdot I_{biochem} + 3.502$
$Ingestion_{S\&C}$	$I_{S\&C} = 0.979 \cdot I_{biochem} + 0.019$	$I_{S\&C} = 0.960 \cdot I_{biochem} + 5.694$

Our developed relationship between body weight and gut content for organisms in subtropical waters used to assess specific and community E_{IBS} showed a rather high coefficient of determination. Besides, this relationship was also close to the one observed when literature data from other ocean regions was added (see Fig. 1.6). Moreover, our estimated gut evacuation rates derived from the equation given by Irigoien (1998) agreed with the value of 0.056 min^{-1} observed by Hernández-León et al. (2002) in the Canary Island waters. Average values of gut content (converted to carbon) in relation to body mass could be a valid option since gut fullness in nature is not constant over the time (see Simard et al, 1985). Both, community E_{GF} and E_{IBS} were within the range observed by Hernández-León et al. (2004, 2007) in the same region. However, as expected, specific and community estimates from E_{IBS} were significantly higher (ANOVA, $p<0.05$) than E_{GF} since the latter only referred to pigmented material. In this regard, mesozooplankton in subtropical waters are mainly omnivores, with a reduced importance of pigmented food in the diet (*e.g.*, Saiz et al., 1999; Hernández-León et al., 2001, 2002). As observed in estimates of growth and respiration, biomass was the main factor influencing community egestion as well.

$I_{biochem}$ and $I_{S\&C}$ are suggested as the main alternative to measure the variability of specific ingestion rates along a sampling period (Fig. 1.8b). On the other hand, $I_{biochem}$ and I_{IBS} could be used indistinctly to estimate

community ingestion fluxes in zooplankton (Fig. 1.8b and Table 1.6). The strong correlation observed between community I_{IBS} and $I_{S\&C}$ definitely suggests the reliability of ingestion estimates from I_{IBS}, as well as community production, respiration and egestion estimated using the IBS. In fact, community ingestion in all these cases was within the range of values observed by Hernández-León et al. (2004, 2007) in the same region, although somewhat lower than those of Hernández-León et al. (2002). The fact that egestion in $I_{biochem}$ was only referred to pigmented food could explain the slightly higher rates estimated from I_{IBS} (Fig. 1.8b and Table 1.6).

Moreover, community ingestion from the latter agreed with Kiørboe et al. (1985), Lenz et al. (1993), Båmstedt et al. (1999) and Hernández-León et al. (2001, 2002, 2004), where they observed that community ingestion fluxes were roughly the result of about one-third of each component: growth, respiration and egestion. Accordingly, community grazing estimated from gut fluorescence (E_{GF}) was around 35% of community ingestion from $I_{biochem}$, I_{IBS} and $I_{S\&C}$. As stated above, it is well known that mesozooplankton in oligotrophic waters feeds upon an important portion of non-pigmented food, such as microzooplankton, to fulfill their physiological demands.

Community ingestion from $I_{biochem}$ using a R/ETS ratio of 0.5 (Hernández-León and Gómez, 1996) should be considered as conservative because this ratio tends to underestimate physiological rates when the substrates are not limited in vivo (*e.g.*, Hernández-León et al., 2001, 2002; Putzeys and Hernández-León, 2005). Therefore, higher estimates for $I_{biochem}$ could be expected. Specific rates and community ingestion fluxes from $I_{I\&M}$ were definitely too low to be suggested as a valid option. However, considering respiration to be about one-third of ingestion, as stated above, instead of 40% as suggested by Ikeda and Motoda (1978), community ingestion from $I_{I\&M}$ would significantly match (ANOVA, $p>0.05$) estimates from $I_{biochem}$, I_{IBS} and $I_{S\&C}$. Therefore, further work should be made on this issue to accurately estimate community ingestion fluxes through respiration.

In summary, our results confirm that IBS in combination with adequate empirical relationships can be as reliable as standard methodologies to estimate biomass, with countless advantages such as individual, taxonomic or size class estimates in a semi-automated process. Regarding physiological estimates, enzymatic methods, although presenting many uncertainties, could be an alternative to measure variability of specific rates within time-series or physical structures. However, the IBS combined with appropriate empirical equations resulted in an inexpensive and faster procedure than enzymatic analysis, providing non-significant differences among both methodologies to estimate community carbon fluxes in large spatial and temporal scales. The growth and respiration equations developed in this study increased the accuracy of estimates in subtropical regions respect to those from existing generalist equations for the global ocean. Likewise, the proposed relationship between body weight and gut content for subtropical waters could be helpful to estimate egestion rates with the IBS as a rapid procedure. However, we claim here for the necessity to set up more taxonomically-specified equations, suitable for each region instead of generalist proposals, in order to more accurately estimate physiological rates and community carbon fluxes through IBS.

Acknowledgements

This study would have been impossible without the hard work of our colleagues on board the R/V "Atlantic Explorer" during the Lucifer sampling, especially Alejandro Ariza, but also Lidia Nieves, Laia Armengol and Gara Franchy. We are also grateful to Inma Herrera and Loreto Torreblanca for providing invaluable data of enzymatic analysis. Loreto Torreblanca also provided her knowledge about taxonomic identification. Thanks to two anonymous reviewers for critical comments and suggestions that significantly improved the quality of this paper. This study was funded by projects Lucifer (CTM2008- 03538/MAR) and Mafia (CTM2012-39587-C04) from the Spanish Ministry of Science and Innovation. Juan Carlos Garijo was supported by a postgraduate grant (FPU-AP2010-5080) from the Spanish Ministry of Education.

Chapter 2

The influence of mesoscale structures on the biomass and metabolism of zooplankton in the Coastal Transition Zone off Northwest Africa during the weak upwelling season

The influence of mesoscale structures on the biomass and metabolism of zooplankton in the Coastal Transition Zone off Northwest Africa during the weak upwelling season

Juan Carlos Garijo and Santiago Hernández-León

Abstract

The influence of recurrent mesoscale structures (fronts, filaments, eddies) on zooplankton biomass, size distribution and physiological rates and fluxes was studied in the Coastal Transition Zone off Northwest Africa during the weak upwelling season. Biomass and specific rates and community fluxes of respiration, production and ammonia excretion of zooplankton were estimated using an image-based system (IBS) in combination with predictive equations. We observed that, although there was a gradient of decreasing biomass towards the ocean, upwelling filaments transported rather high values of chlorophyll and zooplankton biomass offshore, while their interaction with island-induced eddies increased biomass in localized areas of the oligotrophic region. Moreover, filaments and eddies may also influence the vertical distribution of organisms, since zooplankton highly matched the physical signature of mesoscale structures. The transition zone between the upwelling and the oligotrophic region was characterized by the existence of a frontal system, acting as a natural barrier, retaining Chl*a* and zooplankton. We also emphasize the increase of zooplankton-mediated fluxes along mesoscale structures, due to their capacity to transport and concentrate zooplankton biomass.

2.1 Introduction

The Canary Current System (CCS) is one of the major Eastern Boundary Upwelling Systems in the ocean. Like all these systems, and mainly during the strong upwelling season (summer), it is characterized by the generation of a transition zone between the rather cold, nutrient-rich upwelled waters near the African coast, and the warmer and oligotrophic offshore waters around the Canary Islands (Fig. 2.1). This Coastal Transition Zone (CTZ) off NW Africa has an intense mesoscale oceanographic activity, promoting the development of long and narrow coastal filaments flowing offshore, exporting nutrients and organic matter to oligotrophic areas (Barton et al., 1998; Navarro-Pérez and Barton, 1998; García-Muñoz, 2004; Benítez-Barrios et al., 2011). They influence the distribution of chlorophyll a (Chl*a*) (Hernández-Guerra et al., 1993; Arístegui et al., 1997; Basterretxea and Arístegui, 2000), mesozooplankton (Hernández-León et al., 2002) and neritic fish larvae (*i.e.,* Rodríguez et al., 1999; Rodríguez et al., 2004; Moyano et al., 2014) in this region.

Remote sensing images of sea surface temperature (SST) and Chl*a* distribution indicate that filaments are recurrent structures between Cape Juby and Bojador (Fig. 2.1) (Van Camp et al., 1991; Hernández-Guerra et al., 1993; Arístegui et al., 1997, Barton et al., 1998). They are shed mainly due to the interaction of the Canary Current (CC) and topographic features of the African coast, as well as to the presence of the upwelling frontal system (Benítez-Barrios et al., 2011). These upwelling filaments normally interact with island-induced eddies generated southward of the Canary Archipelago (Arístegui et al., 1994; Barton et al., 1998, 2004; Sangrá et al., 2005), as the Canary Islands represent a natural barrier of about 600 km to the general flow of the CC. This interaction has been associated with slight enrichments southward of the islands due to transport and accumulation of nutrient and Chl*a* from upwelling waters (Van Camp, 1991; Arístegui et al. 1997, 2004).

Ageostrophic cyclonic eddies promote localized upwelling, increasing primary production in their cores due to nutrient pumping into the euphotic

zone (Arístegui et al., 1997). In turn, anticyclonic eddies show a convergent effect, entraining adjacent surface waters and carrying phyto- and bacterioplankton into deeper layers (Arístegui et al., 1997). Eddies in the region south of the Canary Islands are suggested to propagate westward for periods over six months while traveling more than 2000 km (Sangrá et al., 2009). In this sense, these mesoscale structures might influence the plankton distribution in oligotrophic regions beyond the Archipelago. However, the overall significance and mechanisms of upwelling filaments and island-induced eddies to export zooplankton into oligotrophic waters is still poorly known. The process depends on the interaction between the different structures, and also on the strength and seasonality of the upwelling events (Álvarez-Salgado et al., 2001). Studies addressing this topic are very scarce, with most of the work carried out in relation to density, retention and transport of fish larvae on filaments (*i.e.*, Rodríguez et al., 2004; Bécognée et al., 2006, 2009; Moyano et al., 2009, 2014). Moreover, the vertical distribution of zooplankton along mesoscale structures still remains unexplored.

The influence of eddies and filaments on trophic interactions and zooplankton energy fluxes is still unclear due to the limited number of studies performed on this subject. However, there is interest to examine if individuals are simply advected offshore by filaments or, by contrast, production is increased along these structures. Smith and Lane (1991) showed increased egg production of the copepod *Eucalanus californicus* in a filament in the CTZ off the California Current. In contrast, studies carried out in the CTZ off NW Africa (Hernández-León et al., 2001, 2002) showed no clear patterns of zooplankton respiration and growth, with results primarily dependent on size classes. For instance, indices of respiration gradually decreased to typical values of the oceanic region along a filament shed from the upwelling area, while growth proxies increased in the frontal zone between filaments and island-induced eddies. Within the latter, respiration clearly depended on the kind of structure (cyclonic or anticyclonic). Physiological rates in these studies were derived from enzyme activity proxies: aspartate transcarbamylase (ATC, Bergeron and Buestel,

1979; Biegala and Bergeron, 1998) as a proxy for growth, and electron transport system (ETS, Packard, 1971) for respiration. These methods constituted, at that moment, the main alternative to map the different components of the energy budget of the zooplankton community at the mesoscale level. However, these methods suffer from the uncertainty of the relation between the enzyme activity and growth (Hernández-León et al., 1995) and respiration (Hernández-León and Gómez, 1996).

Nevertheless, biomass has been suggested as the main factor influencing zooplankton-mediated fluxes (Huntley and Lopez, 1992), with a rather low importance of specific metabolism in the overall results of community production and respiration. In this sense, semi-automated image-based systems (IBS), using empirical relationships and predictive equations, although showing uncertainties as well, produced comparable estimates of zooplankton community biomass and carbon fluxes to those obtained through standard and enzymatic procedures (Garijo and Hernández-León, 2015), although in a faster and inexpensive process.

In this paper, we explored the CTZ off NW Africa during the weak upwelling season (winter-spring). Our objective was to better understand the influence of persistently-generated hydrodynamic structures (fronts, filaments and eddies) on the biomass, size distribution and energy fluxes of the zooplanktonic community in this oligotrophic region. We used an IBS to estimate community biomass according to empirical relationships between body area and body weight (*bw*). Likewise, specific rates and community fluxes of respiration, ammonia excretion, growth and production, as well as ingestion of zooplankton were assessed using predictive equations, relating physiological rates, *bw* and temperature. This study aimed to continue the previous work carried out by Hernández-León et al. (2002) in the same region during the strong upwelling season, providing contrast data from a rather different scenario. We based on the previous studies carried out by Benítez-Barrios et al. (2011) and Moyano et al. (2014) addressing, respectively, the physics and the larval fish distribution in the region along the same cruise.

2.2 Material and methods

2.2.1 Hydrographic surveys

During the ConAfrica cruise (22 March-7 April 2006) on board the R/V "Hespérides", 78 stations were sampled continuously during day and night from the coastal upwelling waters off NW Africa to the offshore waters of the Canary Islands (Fig. 2.1a). This grid covered different mesoscale structures (coastal upwelling, filaments, jets and eddies) observed after processing SST and Chl*a* images (4 km resolution) derived from the *Moderate Resolution Imaging Spectroradiometer* (MODIS) aboard the *Aqua* (EOS PM) satellite (http://oceancolor.gsfc.nasa.gov/cms/data/aqua) from the NASA (Fig. 2.1). Vertical profiles of temperature, conductivity and fluorescence were recorded down to 2000 m depth (when bathymetry permitted) using a *SeaBird 911 plus* CTD system, mounted on a *General Oceanics* rosette sampler, equipped with twenty-four 12 l Niskin bottles. Phytoplankton chlorophyll was derived from depth profiles of *in situ* fluorescence measured with a *Seapoint* Chl*a* fluorometer, calibrated with samples collected within the upper 200 m of the water column, in accordance with the JGOFS recommendations (UNESCO, 1994). Primary production (PP) data was obtained from the *Ocean Productivity* website (http://www.science.oregonstate.edu/ocean.productivity/index.php), according to the Vertically Generalized Production Model (VGPM, Behrenfeld and Falkowski, 1997), which is based on estimates of euphotic zone depth and MODIS Chl*a* and temperature data. Primary production data was averaged every eight days, according to a very detailed coverage (2160 x 4320 grid size; 9.2 km coverage at Equator).

According to the distribution of the physical structures identified by Benítez-Barrios et al. (2011) along the same cruise, we examined the effects of mesoscale structures upon zooplankton according to three sections (Fig. 2.1b). Transect T1 was perpendicular to the African coast, from the upwelling (U) to the southwest of Gran Canaria Island, covering the filament F2, the cyclonic eddy C1, as well as the upwelling jet and a quasi-permanent anticyclonic eddy to the east of Gran Canaria (A2). In turn, T2

helped to examine the three-dimensional of the A2 eddy, while T3 was parallel to the coast (about 80 km distance), sampling the filament F1 and both anticyclonic (A1) and cyclonic (C1) eddies. Moreover, the horizontal exchange and distribution of zooplankton along the region was studied according to the reference isobath of 50 m depth, where we roughly found the deep chlorophyll maximum (DCM) and the horizontal influence of mesoscale structures was more clearly detected.

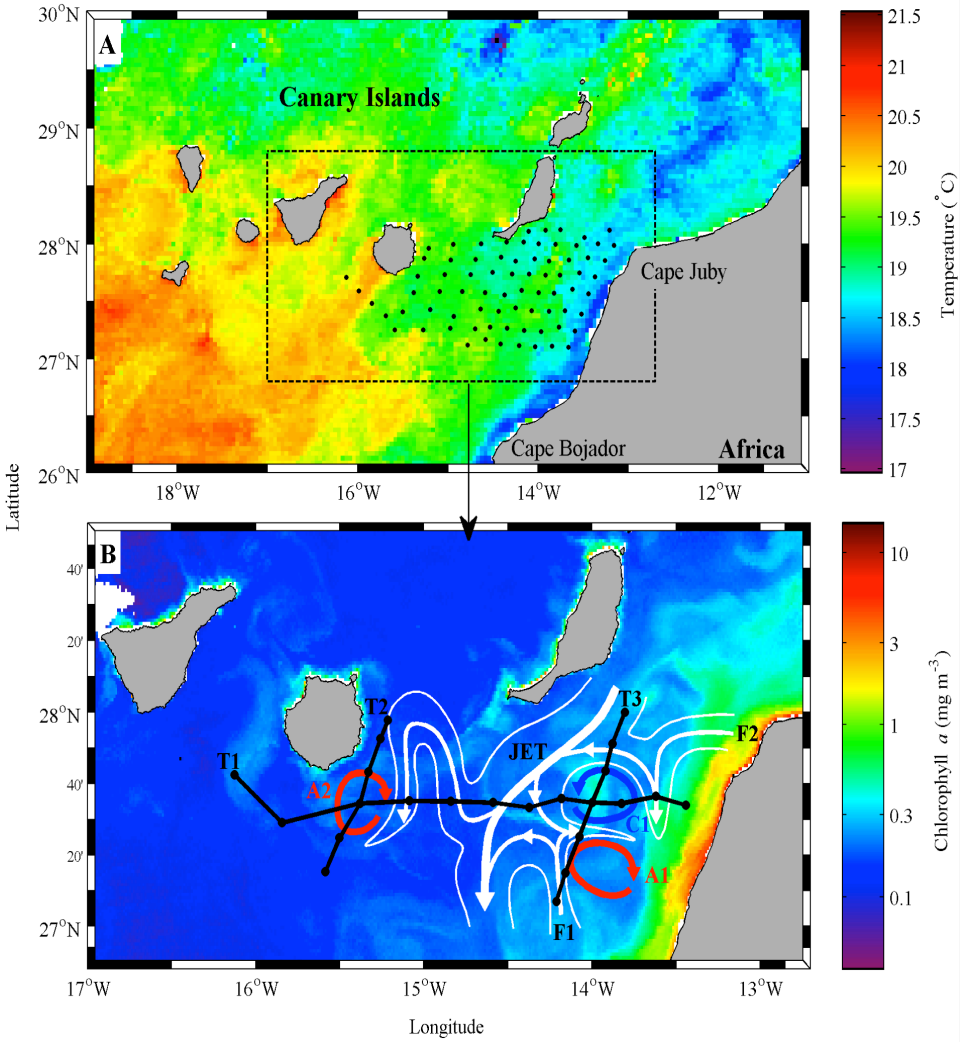

Figure 2.1. Map showing the Canary Islands and the study region (dashed black box) with satellite SST (ºC) for March 29 (A). Coastal transition zone off NW Africa, with satellite Chl*a* (mg m^{-3}) for 7 April, showing the vertical profiles examined (T1, T2 and T3), as well as anticyclonic (A1 and A2) and cyclonic (C1) eddies, the upwelling jet and two upwelling filaments (F1 and F2) (B); black dots within both panels indicate sampling stations.

2.2.2 Zooplankton sampling and biomass assessment

Zooplankton samples were stratified at 10 different depths, within the upper 200 m of the water column, using a *Longhurst-Hardy Plankton Recorder* net (LHPR, Longhurst and Williams, 1976), equipped with a 200 μm mesh size. Oblique trawls were performed at a towing speed of *ca.* 3 knots, measuring the volume of filtered water with a calibrated electronic flowmeter. Samples were immediately preserved on board in 4% buffered formaldehyde.

In the laboratory, larval and fish eggs were removed for further analysis. Thereafter, samples were digitized using an *Epson Perfection 4990 PHOTO* scanner (with *VueScan Professional Edition 8.4.77* software) at a resolution of 1200 dpi. Different aliquots of samples were taken using a Hensen pipette and spilled onto 90x130 mm polystyrene plates. Images of subsamples were processed afterwards with the software *ZooImage 1 version 1.2-1* (http://www.sciviews.org/zooimage), according to Grosjean and Denis (2007). As a result, organisms were automatically counted, measured and individually classified into 5 taxonomic groups: copepods, chaetognaths, euphausiid-like, gelatinous and other zooplankton. Inorganic particles were assembled into an extra non-planktonic group. The software used a manually performed *training set,* containing nearly 5000 images of organisms from the study region to establish patterns for automatic classification. We selected the Random Forest algorithm according to Grosjean et al. (2004), and the global error rate achieved in the classification was estimated below 7%.

Once the organisms were classified, individual biomass was estimated using the empirical relationships given by Hernández-León and Montero (2006), and improved by Lehette and Hernández-León (2009), between body area and body weight, applying a different equation for each taxonomic group, as detailed in Garijo and Hernández-León (2015) (Table 1.1). We also studied the structure of communities along the study region according to three size classes: 200-500, 500-1000 and >1000 μm. Because the sampling grid was covered in a relatively short period and day and night samples needed to be pooled, a day/night ratio was used in the largest size fraction (>1000 μm) to

convert values into a day situation. This correction was important to minimize the effect of the diel variability and, in this case, the ratio was 0.66 due to the vertical migration of the interzonal fauna.

2.2.3 Estimation of metabolic rates and community fluxes

Specific growth rates (G, d^{-1}) were individually estimated according to the predictive equation given by Hirst and Bunker (2003), relating physiological rates and body weight, temperature and Chl*a* (Table 2.1). Given the heterogeneous distribution of the latter along the region we chose this equation, rather than others not including a food *proxy* (*e.g.,* Hirst et al., 2003; Garijo and Hernández-León, 2015), as it could be helpful to understand if food quantity influences growth rates (see Discussion section). Furthermore, specific respiration (R, d^{-1}) was estimated as a function of temperature and body weight according to the equation developed by Garijo and Hernández-León (2015), fitted to the specific temperature ranges of subtropical regions (Table 2.1), and assuming a respiratory quotient of 0.97 (Omori and Ikeda, 1984). Egestion rates of the community (E, ng C ind^{-1} min^{-1}) were in turn derived using two empirical relationships. On the one hand, we approached the gut content of individuals (GC, ng Chla ind^{-1}) (pigmented food) using the relation between this parameter and body weight, developed by Garijo and Hernández-León (2015):

$$(\log_{10} GC = 0.852 \log_{10} bw - 1.160;\ r^2 = 0.769;\ n = 208;\ p<0.05) \quad (2.1)$$

On the other hand, we approximated gut evacuation rates (e, min^{-1}) from habitat temperature (T, ºC), according to the relation developed by Irigoien (1998):

$$e = 0.0026T + 0.012\ (r^2 = 0.940;\ n = 19) \quad (2.2)$$

Then we assumed a C:Chl*a* ratio of 50 (*e.g.*, Reigstad et al., 2008) in order to estimate total gut content of individuals (pigmented + non-pigmented food),

and egestion rates were finally obtained from gut content and estimates of gut evacuation rates.

As a result, specific ingestion rates (I, d^{-1}) were derived from previous estimates of growth, respiration and egestion as

$$I = G + R + E \quad (2.3)$$

Additionally, we assessed specific rates of ammonia excretion using the equation given by Ikeda (2014) for the global ocean, according to body weight, habitat temperature and depth. Corrections made for each taxonomic group are indicated in Table 2.1. Community fluxes (mg C m^{-2} d^{-1}) through production, respiration, egestion, ammonia excretion and ingestion were obtained relating specific rates and community biomass from the IBS. For all calculations, body weight of zooplankton (dry mass) was converted to carbon and nitrogen units using the conversion factors given by Kiørboe (2013), indicated in Table 1.1.

2.3 Results

2.3.1 Hydrography and chlorophyll distribution

Despite some sporadic downwelling-favourable winds occurred before the ConAfrica cruise at the end of February 2006, the sampling was carried out during an upwelling event, as corroborated by the time-series of wind speed and direction over the study region (Benítez-Barrios et al., 2011). In this paper, authors described in detail the hydrography of the area during the ConAfrica cruise. SST and Chl*a* images, derived from MODIS aqua satellite (Fig. 2.1), indicated the existence of a transition zone between the cold and productive waters of the upwelling, and the warm and oligotrophic waters offshore. As a result, Benítez-Barrios et al. (2011) identified a frontal system (their Fig. 3 and 4) as a band of about 30 km width, parallel to the coast, associated to a southwestward baroclinic upwelling jet crossing the study

Table 2.1. Predictive equations used to estimate zooplankton specific growth (d^{-1}), respiration (µl O_2 ind^{-1} h^{-1}) and ammonia excretion (µg N ind^{-1} h^{-1}) according to Hirst and Bunker (Hirst and Bunker, 2003), Garijo and Hernández-León (Garijo and Hernández-León, 2015) and Ikeda (Ikeda, 2014), respectively. *T* is habitat temperature, *bw* is body weight, *Ca* is the concentration of Chl*a* and *D* is the depth where the organisms were captured. Equation to estimate ammonia excretion for *copepods* (referred on table) was corrected for *chaetognaths* and *gelatinous* estimates according to given factors (-0.558 and -1.397, respectively).

	Multiple linear regression	Variables	a	b	c	d	r^2
Specific growth (Hirst and Bunker, 2003)	$\log_{10}$ g (d^{-1})=a[T]+b[$\log_{10}$ bw]+c[$\log_{10}$ Ca]+d	T (ºC) bw (µg C ind^{-1}) Ca (mg m^{-3})	0.0186	-0.288	0.417	-1.209	0.289
Respiration (Garijo and Hernández-León, 2015)	$\log_{10}$ R (µl O_2 ind^{-1} h^{-1})=a+b[$\log_{10}$ bw]+c[$\log_{10}$ T]	bw (mg dw ind^{-1}) T (ºC)	-0.133	0.764	0.272	-	0.844
Ammonia excretion (Ikeda, 2014)	*ln N (µg N ind^{-1} h^{-1})=a+b[ln bw]+c[1000/T]+d[ln D]	bw (mg dw ind^{-1}) T (K) D (m)	15.567	0.796	-5.010	-0.115	0.897

region (Fig. 2.1b and 2.2), which transported approximately 1 Sv (Benítez-Barrios et al., 2011). We detected the front signal in transect T2 as a deepening of isotherms and isopycnals (Fig. 2.3a, b). These figures also show the cold and less salty upwelled waters along the upper 100 m over the African shelf.

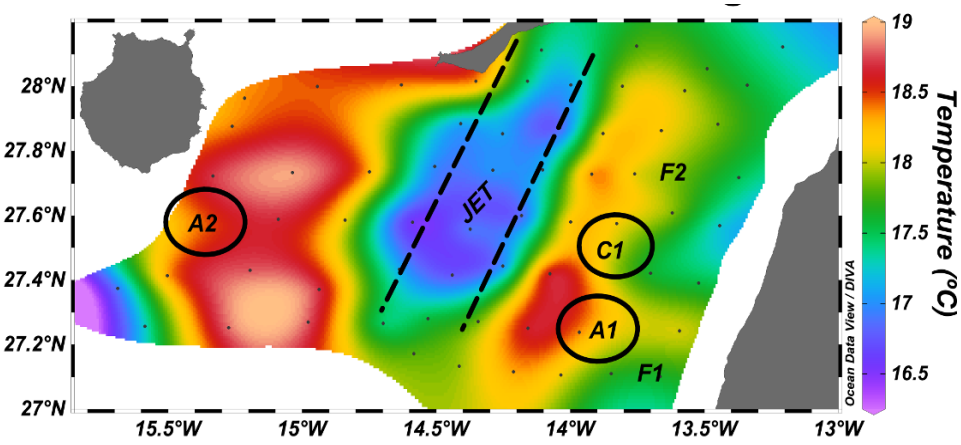

Figure 2.2. Horizontal distribution of temperature (ºC) at 20 m depth. Superimposed is the location of relevant mesoscale structures: anticyclonic (A1 and A2) and cyclonic (C1) eddies, the southwestward upwelling jet and two upwelling filaments (F1 and F2).

Moreover, two upwelling filaments (F1) and (F2) showing cold and high Chl*a* waters flowed offshore due to the interaction between the CC and Capes Bojador and Juby, respectively (Fig. 2.1b). As indicated by Benítez-Barrios et al. (2011), F1 and F2 were likewise associated to respective anticyclonic (A1) and cyclonic (C1) eddies (Fig. 2.1b and 2.2). These eddies were located to the boundaries of the upwelling, conforming a dipole of onshore flow in between. A1 was identified as a deepening of slightly warmer and saltier waters from the surface to deeper layers in transect T1 (Fig. 2.4a, b). By opposite, we observed a slight doming effect (mainly below 100 m depth) in T1 due to the presence of C1 (Fig. 2.4a, b). F2 interacted with the upwelling jet and extended westward as a meandering filament (Fig. 2.1b). It finally entered an anticyclonic eddy (A2) of approximately 70-85 km

diameter generated southeast off Gran Canaria Island. In this regard, sharp gradients of temperature and salinity at the interfaces eddy-filament and eddy-open ocean were observed in Fig. 2.3a and 2.3b, respectively. The eddy entrained warmer and saltier waters from the surface to deeper layers, as observed in the salinity and temperature distribution along T2 and T3 (Fig. 2.3). T2 crossed the meandering westward trajectory of F2, as indicated by the relatively colder and less salty waters observed in the upper 50 m depth (Fig. 2.3a, b).

Chl*a* distribution showed the highest values in the upper 30 m depth near the African shelf (Fig. 2.3c). It extended offshore as a tongue of relatively rich waters, gradually sinking and eventually accumulating around the frontal system and the upwelling jet (Fig. 2.5a). Beyond the front, Chl*a* at 50 m depth sharply decreased to typical oceanic values except for the region south off Fuerteventura Island (Fig. 2.5a), probably due to the influence of the upwelling jet. Both filaments (F1, F2) also transported Chl*a* to the oligotrophic region, through a subsurface layer around 50-75 m depth (Fig. 2.3c and 2.4c), while A1 and C1 eddies also concentrated higher Chl*a* at this depth layer (Fig. 2.4c). Besides, higher values, similar to those found in the upwelling region, were observed at 35-75 m depth of the interface between the A2 eddy and the filament F2 (Fig. 2.3c, 2.5a).

2.3.2 Zooplankton biomass and size distribution

As expected, biomass of zooplankton was highest along the upper layers over the African shelf, coinciding with the upwelling waters (Fig. 2.3d, 2.5b). It gradually decreased towards the ocean, showing a sharp decline beyond the frontal system. However, biomass in the oligotrophic region remained higher along mesoscale structures. Thus, we observed high values in the upper 100 m of both F1 and F2 filaments (Fig. 2.3d, 2.4d). Besides, higher biomass was equally observed in the deeper layers of the eastern flank of the A2 eddy, slightly below the maximum of Chl*a*. In addition, A1 and C1 eddies accumulated similar biomass to the observed in the upwelling zone

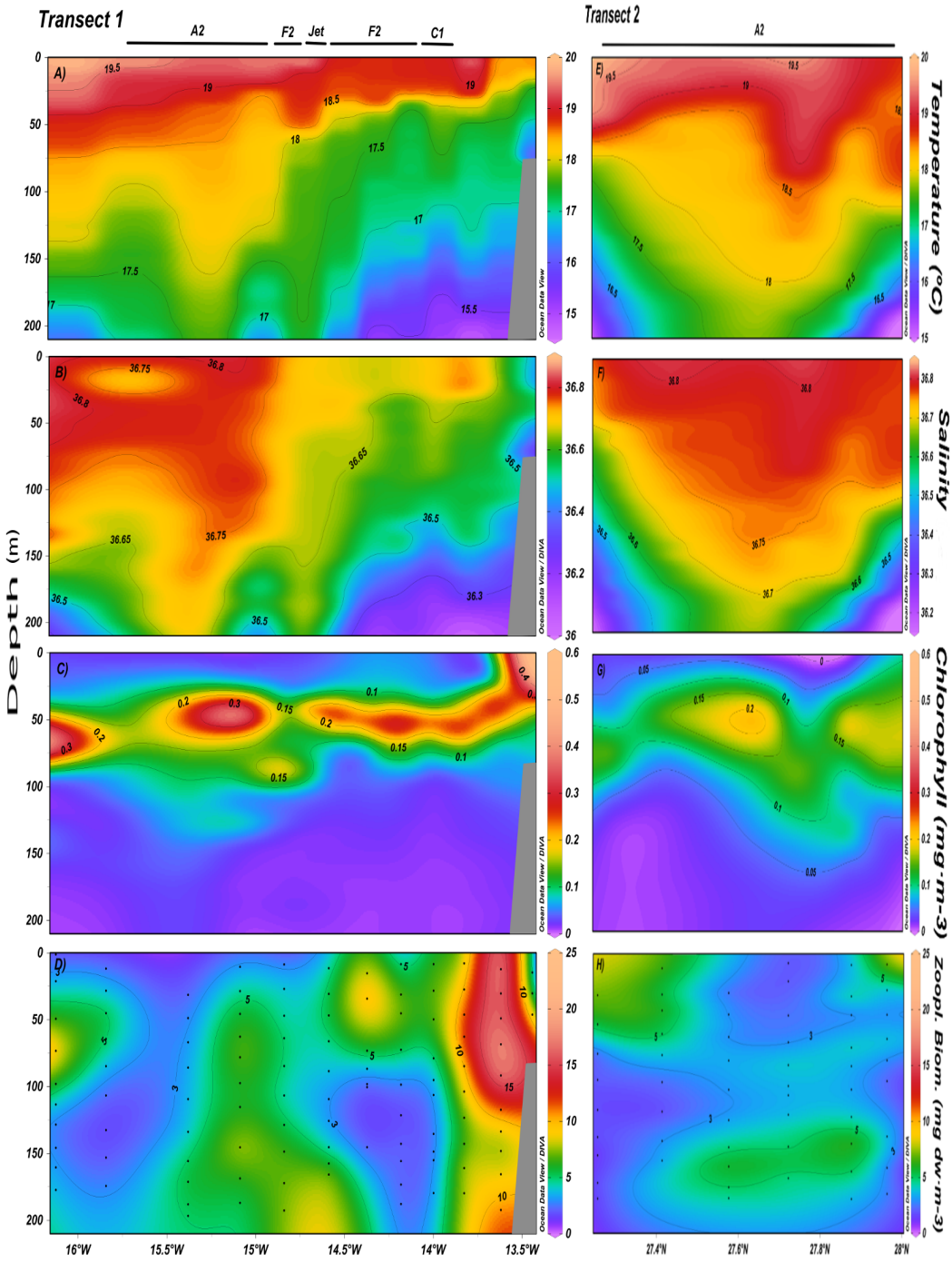

Figure 2.3. Vertical profiles of temperature (ºC) (A, E), salinity (B, F), chlorophyll a (mg m^{-3}) (C, G) and zooplankton community biomass (mg dw m^{-3}) (D, H) along Transects 1 and 2, respectively. A2 and C1 are anticyclonic and cyclonic eddies, while F2 is the upwelling filament from Cape Juby (See Fig. 2.1 and 2.2). The location of the upwelling jet crossing T1 is also indicated.

(Fig. 2.4d), highly matching the physical signature of A1, while mainly concentrating in the upper layers of the cyclonic eddy C1. In turn, total abundance of zooplankton followed the same pattern of biomass distribution, with higher values in the upwelling and decreasing towards the ocean, except within filaments and eddies (Fig. 2.6). In fact, total abundance along the filament F1, and both A1 and C1 (northern edge) eddies was similar to the observed in the upwelling region (Fig. 2.6a, c). Zooplankton also accumulated at the A2-F2 interface (Fig. 2.6a). Intermediate-size organisms were dominant along the study region (Fig. 2.6), while the biggest individuals mainly distributed along the upwelling, the A1 eddy and both filaments (Fig. 2.6a, c). The small fraction, however, was more abundant along C1 and A2 eddies.

2.3.3 Physiological rates and zooplankton-mediated fluxes

Both horizontal (at 50 m depth) and vertical distribution of specific respiration of zooplankton were considerably higher than specific growth rates (Fig. 2.5c, d and Fig. 2.7). Although differences within specific respiration were rather reduced, we observed a generalized horizontal increase (at 50 m depth) towards the ocean, with a maximum around the core of the anticyclonic eddy A2 (Fig. 2.5c). Strikingly, specific respiration clearly matched the vertical structure of this eddy, while higher values were also observed in the surface layers of C1 (Fig. 2.7c). By opposite, concerning the horizontal distribution of specific growth we observed slightly higher values (at 50 m depth) along the upwelling jet and the frontal region, and also within the A2 eddy (Fig. 2.5d). Specific growth mainly matched the vertical distribution of Chl*a*, observing slightly higher values on intermediate layers of both filaments, and cyclonic and anticyclonic eddies (Fig. 2.7d, e, f).

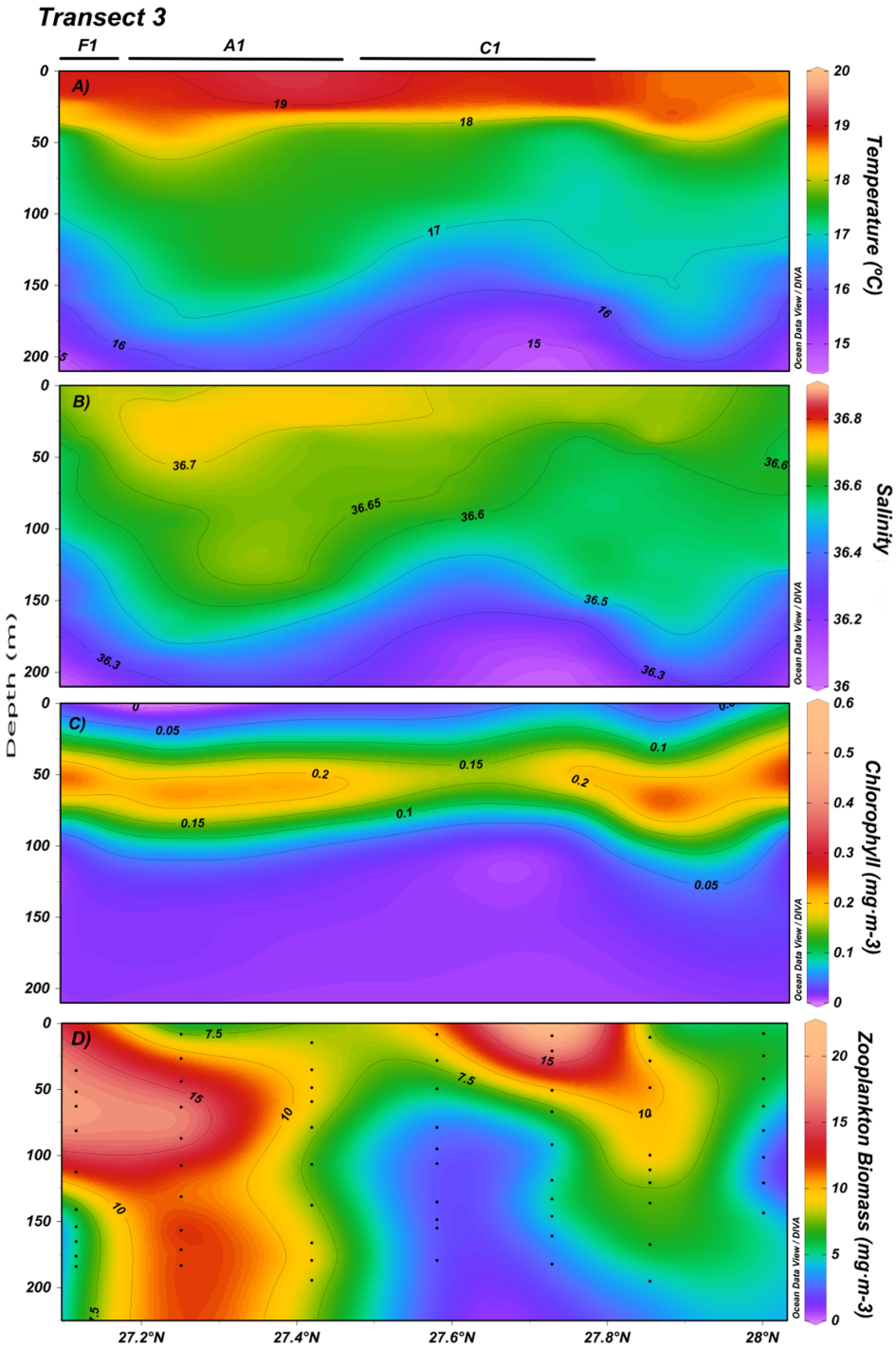

Figure 2.4. Vertical profiles of temperature (ºC) (A), salinity (B), chlorophyll a (mg m^{-3}) (C) and zooplankton community biomass (mg dw m^{-3}) (D) along Transect 3. A1 and C1 are anticyclonic and cyclonic eddies respectively, while F1 is the upwelling filament from Cape Bojador (See Fig. 2.1 and 2.2).

Concerning community fluxes mediated by zooplankton, average estimates of production, respiration and egestion, as well as ammonia excretion and ingestion of these communities followed a similar pattern (Table 2.2). Highest estimates were observed in the upwelling region, and also within F1 and A1 structures, with average values approximately 3-4-fold higher than those assessed in the oligotrophic waters. Despite the lower community fluxes observed within C1 and A2 eddies and the filament F2, estimates were significantly higher (ANOVA, $p<0.05$) than average fluxes assessed along the oligotrophic region. Strikingly, egestion flux accounted on average for about 46-57% of total ingestion flux.

In turn, primary production in the upwelling zone was on average more than 5-fold higher than in the oligotrophic region (Table 2.2). F1 and F2 filaments also transported waters of higher productivity, observing a gradual decrease along F2 as this filament flowed offshore (not shown), while cyclonic and anticyclonic eddies (A1, C1 and A2) also concentrated higher primary production than the surrounding oligotrophic waters (Table 2.2). Finally, average estimates of ingestion flux in the upwelled waters, although clearly higher than assessments in the oligotrophic region, accounted for a lower percentage of primary production (20% *vs.* 28%). In contrast, community ingestion fluxes within the different mesoscale structures represented about 37-53% of primary production (Table 2.2).

2.4 Discussion

Our results show that mesoscale structures may influence the patterns of horizontal and vertical distribution of size and biomass of zooplankton in oligotrophic regions, with a close relationship between biomass and the physical structures. Moreover, the distribution of metabolic rates and zooplankton-mediated fluxes also matched the physical signature of mesoscale structures. As expected, biomass was higher in the productive waters of the coastal upwelling, and remained relatively high (at 50 m depth) until the frontal region, where it sharply decreased (Fig. 2.5b), matching the

distribution of Chl*a* (Fig. 2.5a). Thus, the subsidence of surface colder waters from the upwelling around this front could act as a natural barrier, parallel to the African coast, retaining Chl*a* and zooplankton in the intermediate (Fig. 2.5b) and deeper layers (Fig. 2.3d). Moyano et al. (2014) already observed that fish larvae was highly retained in the same frontal system, and Hernández-León et al. (2002) also reported a peak of zooplankton biomass around this region. In turn, average estimates of biomass in the oligotrophic region were similar to those given by Rodriguez et al. (2001) in the vicinities of Gran Canaria Island (4.5 ± 2.7 mg dw m^{-3}).

Both upwelling filaments (F1, F2) exported Chl*a* and zooplankton offshore, as previously observed in the same region by Hernández-Guerra et al. (1993), Basterretxea (1994), Arístegui et al. (1997) and Basterretxea and Arístegui (2000) for Chl*a*, Baltar et al. (2009) for nano- and picoplankton, Hernández-León et al. (2001, 2002, 2007) for mesozooplankton and Rodriguez et al. (1999), Brochier et al. (2008, 2011), Bécognée et al. (2009) and Moyano et al. (2014) for ichtyoplankton. Thus, biomass of zooplankton along F1 was similar to the values observed in the upwelling region, while slightly lower within F2. The meandering structure of the latter was observed as a shallow patchy distribution of zooplankton in those areas where T2 crossed the filament (Fig. 2.3d). Besides, interactions between both filaments and their associated eddies (A1, C1 and A2) increased Chl*a* concentration in the latter structures, in agreement with Van Camp (1991) and Arístegui et al. (1997, 2004). A similar observation was made for zooplankton biomass, as previously described by Hernández-León et al. (2001, 2002, 2007) in the same zone. The increase of Chl*a* at 50 m depth observed in the region south off Fuerteventura Island could be associated to an "island-mass effect" (Hernández-León, 1991). It was even possible the occurrence of an anticyclonic eddy although, if present, it was partially sampled and was not mentioned by Benítez-Barrios et al. (2011).

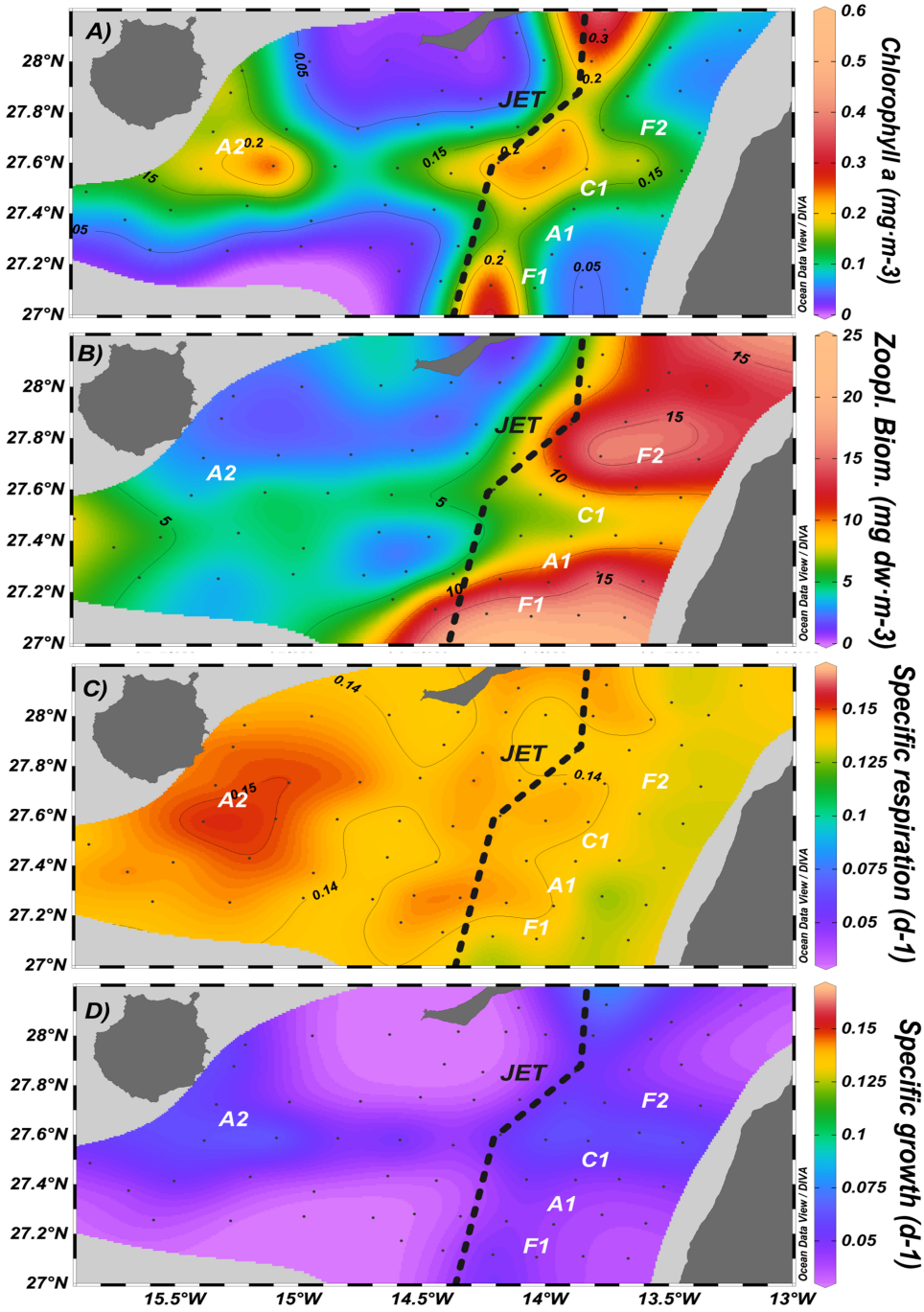

Figure 2.5. Horizontal distribution of chlorophyll a (mg m^{-3}) (A), zooplankton community biomass (mg dw m^{-3}) (B) and specific respiration (C) and growth (D) (d^{-1}) of this community along the study region at 50 m depth. Superimposed is the location of relevant mesoscale structures (filaments F1 and F2, cyclonic eddy C1, and anticyclonic eddies A1 and A2) and the southwestward-crossing upwelling jet.

Remarkably, both Chl*a* and zooplankton biomass mainly accumulated in the region of interaction between eddies (A2, C1) and filaments, giving place to a generalized coupling between Chl*a* and zooplankton along the three-dimensional of both kinds of structures. In this respect, Hernández-León et al. (2002) suggested that higher biomass of zooplankton along filaments and eddies could be explained by the combination of increasing Chl*a* and advection processes. Particularly, the onshore flow produced in between the A1-C1 dipole, might also contribute to the enhancement of biomass observed in this region, as already described by Moyano et al. (2014) for the retention of fish larvae in the same area.

Table 2.2. Average (±SD) primary production and zooplankton community production, respiration, ammonia excretion and ingestion* rates (mg C m^{-2} d^{-1}) along mesoscale structures (detailed in Fig. 1) and the oligotrophic region: U=upwelling, F1-F2=filaments, A1-A2=anticyclonic eddies, C1=cyclonic eddy, O=oligotrophic zone (region offshore not influenced by mesoscale structures). Numbers in brackets represent the percentage (%) of zooplankton community ingestion respect to primary production. *Note the high influence of egestion on total ingestion flux in comparison with that of production and respiration. On average, egestion accounted for 46-57% of ingestion flux.

	Primary production	Production	Respiration	Egestion	Ammonia excretion	Ingestion
U	2746.6±1579.5	57.7±36.8	183.5±124.4	294.2±205.4	113.0±72.2	535.4±366.6 (19.5)
F1	1321.9±352.4	53.2±48.2	166.5±91.2	268.3±189.1	97.1±74.7	488.0±328.5 (36.9)
A1	738.2±75.2	49.4±14.4	161.2±25.0	180.0±55.9	96.7±26.4	390.6±95.3 (52.9)
C1	710.1±38.2	28.3±20.9	101.9±73.1	138.8±89.1	61.7±44.5	269.0±183.1 (37.9)
F2	764.0±89.5	25.0±12.3	83.3±35.7	122.7±56.1	45.5±19.9	231.0±104.1 (30.2)
A2	549.3±41.5	16.3±6.9	54.6±9.7	95.7±19.8	31.0±5.4	166.6±36.4 (30.3)
O	477.9±75.2	13.6±1.9	51.1±17.0	68.7±27.8	27.1±6.8	133.4±46.7 (27.9)

On the contrary, we observed a vertical uncoupling between Chl*a* and zooplankton within the A2 eddy, where higher biomass was observed deeper than the maximum of Chl*a*. This was probably due to the variability occurring in the upper layers of newly formed island-induced eddies (Arístegui et al., 1994). In fact, eddies generated south off the Canary Islands are normally ageostrophic, changing their vertical and horizontal structure during their development, and thus showing elliptical and asymmetric shapes (Sangrá, 1995; Arístegui et al., 1997).

Nevertheless, the distribution of biomass within both anticyclonic eddies (A1, A2) highly matched the signature of the physical structures, showing a higher accumulation of zooplankton along intermediate and deeper layers. This might be attributed to the convergent effect of this kind of eddies, in agreement with Arístegui et al. (1997), who already observed the same process for phyto- and bacterioplankton. Likewise, the accumulation of biomass in the upper layers of C1 (Fig. 2.4d) agreed with general patterns observed for cyclonic eddies. There, isotherms elevation and nutrient pumping into the euphotic zone could enhance biomass in their cores (Arístegui et al., 1997).

Specific growth according to the Hirst and Bunker (2003) equation was probably underestimated (and consequently production fluxes as well) along the oligotrophic region, since Garijo and Hernández-León (2015) observed that this equation, using Chl*a* as unique indicator of the feeding status of organisms, could underestimate growth rates of zooplankton in these regions. In this sense, it is known the rather low contribution of pigmented food to total ingestion of mesozooplankton in oligotrophic waters which, in contrast, feed upon an important portion of microzooplankton to fulfill their physiological demands (*e.g.*, Saiz et al., 1999; Hernández-León et al., 2001, 2002). However, the use of this equation over a region with a highly heterogeneous distribution of Chl*a* seemed to be more appropriated than other relationships only based on temperature and body weight, such as those developed by Hirst et al (2003) or Garijo and Hernández-León (2015). Although it still remains unclear the effect of food on growth rates of

individuals, we observed that the latter generally followed the distribution of Chl*a,* rather than that of temperature or body weight. In this sense, filaments and eddies could enhance growth rates of zooplankton in oligotrophic regions, although more studies are needed to clarify this question.

In contrast, respiration of zooplankton seemed to be primarily controlled by temperature, in agreement with Ikeda (2014) who suggested this parameter as the main factor determining respiration, followed by body weight. In this regard, different authors, such as Ikeda and Motoda (1978), Hirst and Sheader (1997), Hirst and Lampitt (1998) and Hirst et al. (2003), observed a generalized pattern of increasing physiological rates according to higher temperatures and decreasing body size. This trend coincided with the lower respiration rates estimated along the colder waters of the coastal upwelling and the meandering filament F2 where, at the same time, the abundance of largest individuals was the highest along the region (Fig. 2.6). Moreover, Garijo (2011) reported during the same cruise a predominance of smaller individuals towards the ocean, which could also help to explain the higher respiration rates estimated in the warmer waters of the oligotrophic region. Similarly, the accumulation of smaller individuals within the C1 eddy (Fig. 2.6c) probably influenced the increased respiration rates observed within this structure (Fig. 2.7c). Strikingly, respiration matched the physical signature of the anticyclonic A2 eddy, probably due to the deepening of warmer waters from the surface (Fig. 2.7a, b). These increases of respiration rates within eddies agreed with previous observations by Hernández-León et al. (2001) in the same region, who reported enhanced indices of metabolism in the smallest individuals at the core of two cyclonic and anticyclonic eddies.

Nevertheless, the influence of specific metabolic rates in the distribution and magnitude of zooplankton-mediated fluxes was scarce in comparison to community biomass. This was in agreement with Huntley and Lopez (1992) who observed that, estimating production, the variance of community biomass was one to three orders of magnitude greater than that of specific growth rates. In this sense, as occurred with the distribution of community biomass, metabolic fluxes in the upwelling zone were about 3-4-fold higher

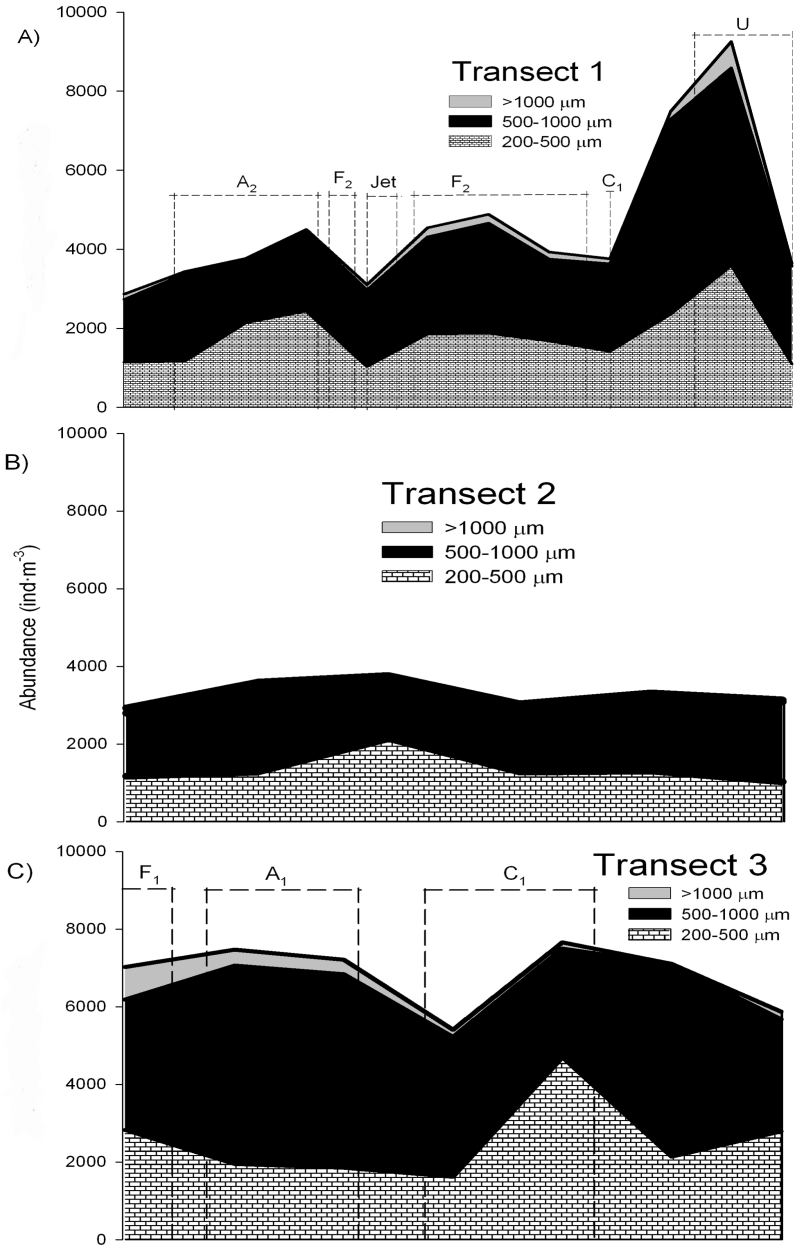

Figure 2.6. Abundance of zooplankton (ind m^{-3}) according to three size classes (200-500, 500-1000 and >1000 μm) along Transect 1 (A), Transect 2 (B) and Transect 3 (C). It is also referred the location of the mesoscale structures studied (according to map in Fig. 2.1): U=upwelling, F1-F2=filaments, A1-A2=anticyclonic eddies, C1=cyclonic eddy and the upwelling jet crossing Transect 1.

than those estimated in the oligotrophic region, showing a gradual decrease towards the ocean. However, as observed in Table 2.2, metabolic fluxes remained considerably high within mesoscale structures, mainly due to the accumulation of biomass. Therefore, special attention should be provided to study these physical structures when assessing carbon fluxes through zooplankton in oligotrophic areas with an intense mesoscale activity. On the other hand, the contribution of egestion to the magnitude of ingestion flux was rather higher than expected (46-57%), since the latter is normally the result of about one-third of each component: growth, respiration and egestion (Kiørboe et al., 1985; Lenz et al., 1993; Båmstedt et al., 1999; Hernández-León et al., 2001, 2002, 2004).

In any case, we suggest seasonality as one of the main factors influencing zooplankton-mediated fluxes in this region. In this respect, our estimates of zooplankton production (during the weak upwelling season) along the African shelf were up to 4-fold lower than those assessed by Hernández-León et al. (2002) in summer (strong upwelling season). The productivity of the upwelling is not constant throughout the year (Barton et al., 1998), possibly leading to a certain variability of community biomass within seasons and, consequently, influencing the magnitude of metabolic fluxes.

As expected, primary production was higher in the upwelling zone and decreased towards the ocean. However, it also remained considerably higher within filaments and eddies in comparison with the oligotrophic zone (Table 2.2), highlighting the importance of mesoscale structures in the export of productive waters from the upwelling to the open ocean. Zooplankton was not able to control primary production, since community ingestion was equivalent to 20-53% of PP along the region (Table 2.2). This range of values is similar to the range of 20-37% given by Hernández-León et al. (2001) in the same area, and included the values given by Hernández-León et al. (1999) in the tropical region (46%), and Dam et al. (1995) in equatorial waters (23%). However, our estimates were lower than those given by Hernández-León et al. (2002) in the same region (47-296%) during the

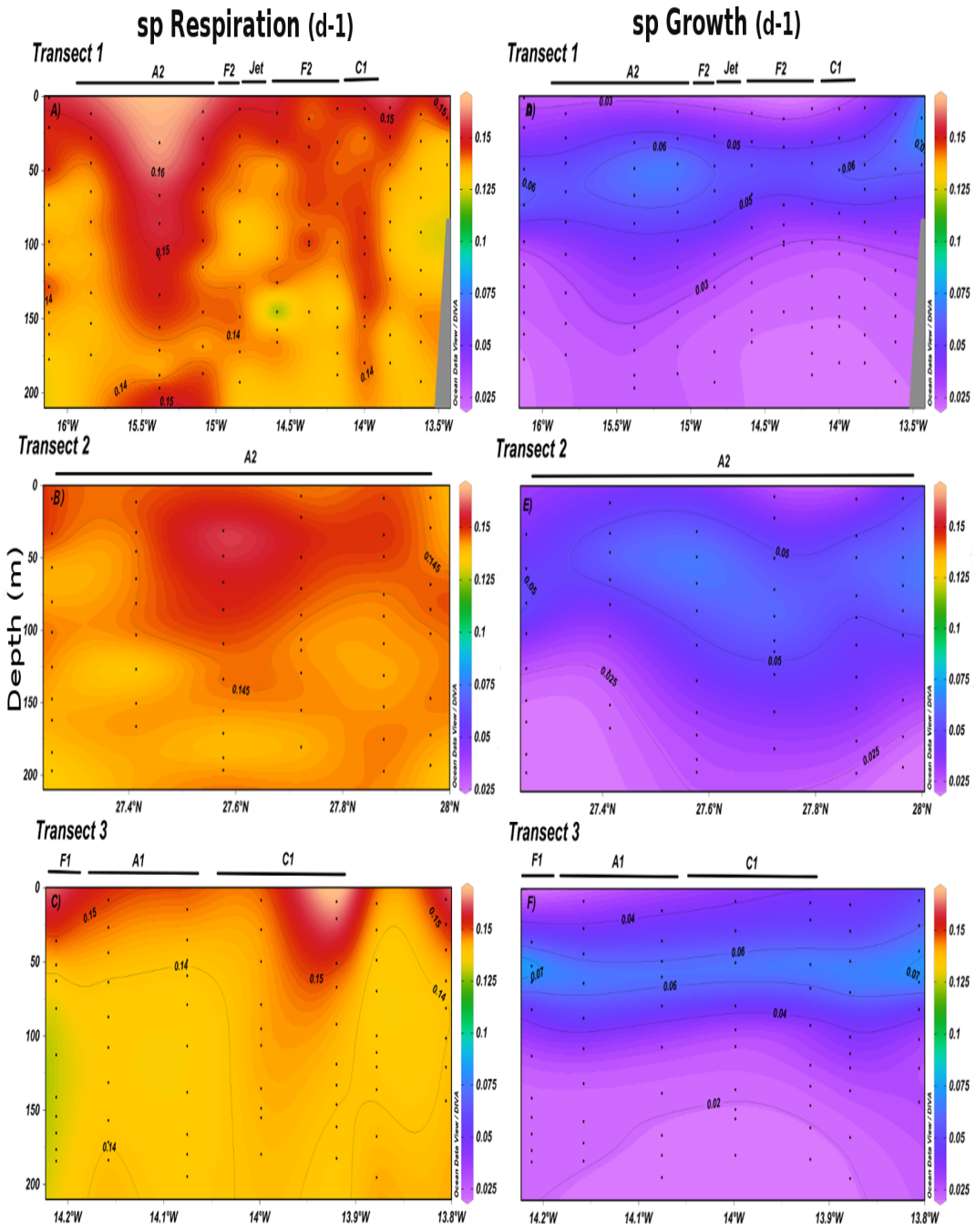

Figure 2.7. Vertical profiles of zooplankton specific respiration (d^{-1}) along Transects 1, 2 and 3 (A, B and C, respectively). Likewise, specific growth (d^{-1}) of this community along Transects 1, 2 and 3 (D, E and F, respectively). A1 and A2, and C1 are two anticyclonic and cyclonic eddies, respectively. F1 and F2 are the two coastal upwelling filaments studied (See Fig. 2.1 and 2.2).

strong upwelling season, evidencing the influence of seasonality in the magnitude of zooplankton fluxes and water productivity. Finally, zooplankton ammonia excretion could support 4-13% of primary production, which agreed with estimates given by Zhang et al. (1993), Dam et al. (1995) and Hernández-León et al (1999, 2001) who assessed a contribution of excretion of this community to primary production in the range 3-17%.

In summary, our results confirm the influence of mesoscale structures on the distribution, biomass, physiology and metabolic fluxes mediated by zooplankton in the CTZ off NW Africa. Thus, we observed that biomass gradually decreased towards the ocean, although upwelling jets and filaments can transport high Chl*a* and zooplankton biomass to the oligotrophic region. In addition, the interaction between upwelling filaments and island-induced eddies may also enhance zooplankton biomass in the latter. In this sense, eddies may also influence the vertical distribution of organisms, since zooplankton mainly distributed following the physical signature of these mesoscale structures. Advection processes and increases in Chl*a* are suggested as a combined mechanism to explain higher biomass of zooplankton within mesoscale structures. The existence of a quasi-permanent frontal system in the transition zone, between the upwelling and the oligotrophic region, acts as a natural barrier retaining Chl*a* and zooplankton, as already observed for fish larvae. The enhancement of zooplankton-mediated fluxes along mesoscale structures in oligotrophic regions is determined by their capacity to transport and concentrate biomass. In any case, this probably depends on the productivity of the upwelling, which changes throughout the year. Therefore, seasonality seems to eventually determine the capacity of physical structures to export zooplankton to the oligotrophic region, close to the Canary Archipelago. Export of these organisms, at the basis of the oceanic food chain and a natural food resource for fish larvae, could finally influence local fisheries, although possible effects in this regard still remain unknown.

Acknowledgements

We would like to thank our colleagues from the ConAfrica project for the hard work on board. Our special gratitude to Walber Melo, Sandra Herms, Lidia Nieves and Acorayda González, who scanned part of the zooplankton samples. We are also grateful to Alejandro Ariza for his invaluable assistance with graphics and satellite data management. Lidia Nieves arranged the available data from the cruise. This study was funded by projects ConAfrica (CTM2004-02319/MAR) and Mafia (CTM2012-39587-C04) from the Spanish Ministry of Science and Innovation. Juan Carlos Garijo was supported by a postgraduate grant (FPU-AP2010-5080) from the Spanish Ministry of Education.

Chapter 3

Large-scale zooplankton metabolism inferred through an image based system: a comparison of methods

Large-scale zooplankton metabolism inferred through an image based system: a comparison of methods

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Abstract

The appraisal of metabolic fluxes mediated by zooplankton at the global scale is essential to understand the role of these communities in the ocean carbon cycle. However, methods normally used during the last decades to estimate both respiration and growth are very time-consuming, precluding their application at large spatial scales. As an alternative, newly-developed image-based systems (IBS), in combination with predictive equations based on temperature and body weight, may be as reliable as enzymatic methods to estimate zooplankton metabolic fluxes, although in a faster and inexpensive way. In this study, we developed specific growth and respiration equations for zooplankton, fitted to the temperature ranges given in the main ocean regions and depth layers. To test their usefulness in a global scenario, we compared our estimates using these temperature-specific equations with those obtained from existing relationships for the global ocean and enzymatic methods along the Malaspina-2010 circumnavigation. The cruise explored the upper 2000 m of the ~40ºN-40ºS region, covering a wide range of habitats in terms of temperature (2.5–30ºC) and productivity. Our results suggest the suitability of temperature-specific equations developed in this study to assess zooplankton metabolism along the main ocean regions (up to 2000 m depth), since estimates were not significantly different than assessments from enzymatic methods. Conversely, measurements using existing generalist equations were significantly lower than the latter. Although still showing many limitations, equations presented here, in combination with current technology, could stand as an alternative to assess zooplankton metabolic fluxes at the large-scale.

3.1 Introduction

Semi-automated image-based analysis systems (IBS), used to enumerate and classify zooplankton, were developed during the last years (Grosjean et al., 2004; Benfield et al., 2007; Gislason and Silva, 2009; Gorsky et al., 2010; Bachiller et al., 2012). These systems assess taxonomic and size class zooplankton abundance with less effort than using traditional procedures. Moreover, they result in an inexpensive and faster process, therefore increasing the spatial and temporal resolution of studies (Benfield et al., 2007; MacLeod et al., 2010; Garijo and Hernández-León, 2015). Using empirical relationships between certain characteristics of organisms, such as the body area or the width and prosome length of individuals, and their body weight (*bw*) or biovolume (*bv*) (Nakata et al., 2001; Hernández-León and Montero, 2006; Lehette and Hernández-León, 2009; Viñas et al., 2010) these systems also enable estimating individual biomass through a non-destructive process.

In this respect, they may constitute an advance respect to traditional methodology (*e.g.,* Lovegrove, 1966), where only broad estimates of the community biomass may be achieved. No information about the contribution of each group to total biomass is provided, while the destruction of samples precludes their future use for other purposes. In turn, because of the growing acceptance of imaging systems to assess zooplankton biomass (*e.g.,* Di Mauro et al., 2011; Vandromme et al., 2012, 2013; Basedow et al., 2014), Garijo and Hernández-León (2015) recently tested the reliability of IBS to assess community biomass according to empirical relationships. They observed that community estimates using these systems were highly correlated to those obtained through the traditional methodology (Lovegrove, 1966), and no significant differences were observed.

Similarly, these authors also showed that IBS could constitute an alternative to estimate zooplankton metabolism. Using empirical equations, relating physiological rates, *bw* and temperature, results using an IBS were at least comparable to measurements of enzymatic activities as proxies of growth

[aminoacyl-tRNA-syntethases (AARS), Yebra and Hernández-León, 2004] and respiration [electron transfer system (ETS), Packard, 1971] in the subtropical waters of the Canary Islands. Enzymatic methods, although solving many problems related to incubations (see Basedow et al., 2014) and are useful to approach the vertical distribution of metabolism, still show some uncertainty when relating enzymatic activities and metabolic rates of both growth (Hernández-León et al., 1995) and respiration (Hernández-León and Gómez, 1996).

In any case, in the light of the results obtained, Garijo and Hernández-León (2015) suggested that enzymatic methods might constitute a reasonable alternative to measure specific metabolism of zooplankton on particular cases. Hence, these methods seemed more accurate than imaging systems to assess the variability of metabolism along a time-series. In contrast, the IBS combined with specific equations, fitted to the conditions of the subtropical region, resulted more suitable to estimate zooplankton community fluxes (through respiration, production and egestion) along large spatial and temporal scales. In this regard, differences between community estimates using both methods were not significant, although the IBS resulted in a faster and inexpensive procedure. As a consequence, ingestion fluxes mediated by these communities (Ingestion = Production + Respiration + Egestion) were also approached according to a relatively simple process.

Unlike previous metabolic equations, which are based on rather large temperature ranges (-2.5–31ºC) and body sizes exceeding in some cases those normally observed within mesozooplankton (Ikeda, 1985; Hirst et al., 2003; Hirst and Bunker, 2003; Zhou et al., 2010), growth and respiration equations developed by Garijo and Hernández-León (2015) fitted to the specific ranges of temperature (16–26ºC) and body weight of the subtropical region. This factor seemed to determine the higher agreement that they observed between assessments using these temperature-specific equations and enzymatic measurements, with respect to existing generalist relationships for the global ocean. As a conclusion, they claimed for the need to develop more specific equations, fitted to the conditions of each

region, in order to increase the accuracy of metabolic estimates elsewhere. In this sense, it was observed that temperature, jointly with body weight, are the main factors determining metabolism of zooplankton, while taxonomy or habitat depth are known to influence physiological rates in a lesser extent (Ikeda, 2014).

There is a growing awareness of the important contribution of mesozooplankton to the carbon cycle and the biological pump in the ocean (*e.g.,* Zhang and Dam, 1997; Steinberg et al., 2000; Del Giorgio and Duarte, 2002; Hernández-León and Ikeda, 2005; Stock et al., 2014). Global approaches of production and respiration of these communities are therefore needed to understand their role in the ocean. However, studies at large spatial scales are challenging and probably only affordable using modern technology. The use of appropriated predictive equations for metabolism, fitted to the specific conditions of each region, might constitute an alternative to estimate zooplankton carbon fluxes in the global ocean.

In this study, we based on the previous results obtained by Garijo and Hernández-León (2015) in the subtropical region of the Canary Islands. Handling data contained in Hirst et al. (2003) and compiled by Hernández-León and Ikeda (2005) we developed mechanistic equations predicting growth and respiration rates of mesozooplankton, according to body weight and habitat temperature. Specific equations were developed for each environment, as they were fitted to the ranges of temperature given in the main ocean regions and depth layers. In order to test their reliability in a global scenario, we compared metabolic estimates using an IBS in combination with these temperature-specific equations and existing generalist relationships, and measurements from enzymatic methods along the Malaspina-2010 expedition. The cruise circumnavigated the globe, exploring the epi- (0-200 m), meso- (200-1000 m) and bathypelagic (1000-2000 m) layers, along the ~40ºN-40ºS latitudinal band. As a result, samples proceeded from highly heterogeneous habitats in terms of productivity (oligo-, meso-, and eutrophic) and temperature (2.5–30ºC). The latter roughly corresponding with the range of temperatures that can be found

somewhere in the ocean. Equations developed in this study aim to be useful to assess specific metabolism and community fluxes of zooplankton on a wide variety of environments, essential to understand the role of these communities in the ocean carbon cycle.

3.2 Materials and methods

3.2.1 Sampling

Comparisons of specific and community growth and respiration rates of zooplankton using an IBS, in combination with predictive equations, and measurements from enzymatic methods were carried out along the Spanish circumnavigation Malaspina-2010. Thus, from December 2010 to July 2011, on board the R/V "Hespérides", a total of 70 samples (34 stations) were collected across the Atlantic, Indian and Pacific Oceans (~40ºN-40ºS, Fig. 3.1a) at four different depth layers (0-200, 200-500, 500-1000 and 1000-2000 m) (Table 3.1) using a Multinet net (0.7x0.7 m mouth, 300 μm mesh). Oblique trawls were performed at a towing speed of *ca.* 3 knots, measuring the incoming water volume through a calibrated flowmeter. Zooplankton samples were split into four fractions on board: one of them was preserved in 4% formaldehyde for subsequent analysis of biomass using the IBS, while the other fractions were preserved for genetics, isotopes and enzymatic activity analysis after the cruise. The latter required the samples to be immediately frozen in liquid nitrogen (-196 ºC).

Average temperature of each depth layer inhabited by the organisms, used to assess metabolic rates through predictive equations, was obtained through vertical profiles from a CTD *911 plus* system mounted on a rosette sampler. Measurements of chlorophyll (Chl*a*) in the epipelagic layer (Fig. 3.1b) were achieved in accordance with the JGOFS recommendations (UNESCO, 1994), while global distribution of primary production (PP) data for 2010 was obtained from the *Ocean Productivity* website (http://www.science.oregonstate.edu/ocean.productivity/index.php),

according to the Vertically Generalized Production Model (VGPM, Behrenfeld and Falkowski, 1997). The latter is based on estimates of the euphotic zone depth and surface Chl*a* and temperature data from the *Moderate Resolution Imaging Spectroradiometer* (MODIS), on board the *Aqua* (EOS PM) satellite (4 km resolution; http://oceancolor.gsfc.nasa.gov/cms/data/aqua), from the NASA. .

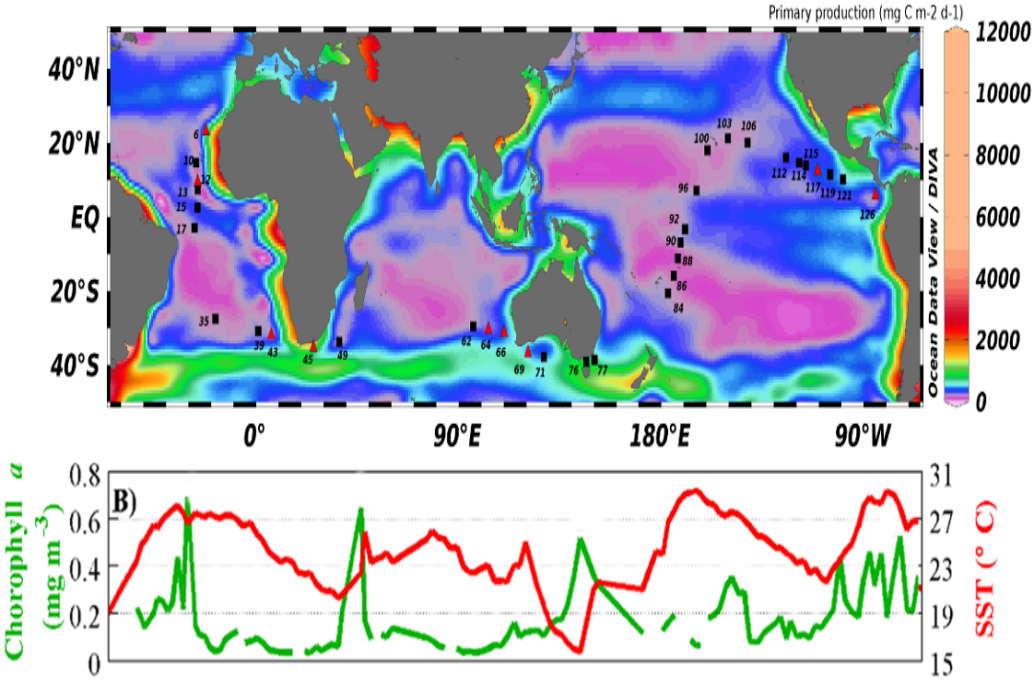

Figure 3.1. Map showing sampling stations used for metabolic assessments (enzymatic methods + IBS using predictive equations) along the Malaspina-2010 circumnavigation cruise, with the average global distribution of primary production (satellite data, mg C m^{-2} d^{-1}) for 2010 (A), as well as *in situ* surface Chl*a* (mg m^{-3}) and temperature (ºC) along the cruise (B). Black squares indicate stations where metabolism was assessed according to both AARS and ETS methods, while red triangles denote stations where only ETS assays were possible.

3.2.2 Biomass assessments

In the laboratory, zooplankton samples were digitized using an EPSON Perfection 4990 PHOTO scanner (with *VueScan Professional Edition 8.4.77*

software) at a resolution of 1200 dpi. Different aliquots of the samples were taken with a Hensen pipette and poured onto polystyrene plates (90x130 mm). Images were then processed with the software *ZooImage 1 version 1.2-1* (http://www.sciviews.org/zooimage), following the same procedure as specified by Garijo and Hernández-León (2015), including the criteria to create and conform the different taxonomic groups. However, unlike the latter, the enormous taxonomic diversity along the main ocean regions in the present study, forced to manage a manually sorted *training set* of nearly 7000 images to establish patterns for automated classification. The global error rate achieved was estimated below 8.5%.

Once the organisms were classified, individual biomass required to assess metabolism was achieved applying empirical relationships, between body area and body weight, for the different taxonomic groups, as specified by Garijo and Hernández-León (2015) (their Table 1). Those equations were developed by Hernández-León and Montero (2006) and improved afterwards by Lehette and Hernández-León (2009). Dry weight estimates obtained from these relationships were then converted into carbon content according to specific conversion factors for taxonomic groups given by Kiørboe (2013), indicated in Table 1.1.

3.2.3 Respiration estimates

Respiration rates derived from measurements of ETS activity (R_{ETS}), according to 65 samples (34 stations) within the 0-200, 200-500, 500-1000 and 1000-2000 m depth layers (24, 17, 17 and 7 samples respectively, Fig. 3.1, Table 3.1), were compared with estimates from the IBS (R_{IBS}) using the generalist equation for the global ocean given by Ikeda (Ikeda, 2014; his Table 4 (carbon units)) and mechanistic equations developed in the present study (Table 3.2). The latter equations were specifically fitted to the temperature ranges found at the main ocean regions and depth layers. To establish those ranges we based on the *in situ* records of temperature found along the Malaspina cruise (Table 3.1). On the other hand, we used the

equation for copepods given by Ikeda (Ikeda, 2014), and then we applied the specified correction factors for chaetognaths (-0.345), euphausiid-like (0.600) and gelatinous (0.547) respiration. We assumed a respiratory quotient of 0.97 (Omori and Ikeda, 1984) to estimate specific respiration (d^{-1}) in all cases.

In turn, ETS activity was measured from frozen samples according to the method of Packard (1971) for zooplankton, with the modifications introduced by Owens and King (1975), Kenner and Ahmed (1975) and Gómez et al. (1996). Details of the procedure are given in Hernández-León and Gómez (1996). ETS activity, normalized to protein content, was approached to potential specific respiration (d^{-1}), assuming a respiratory quotient of 0.97 (Omori and Ikeda, 1984), as well as using a C:dw ratio of 0.48 (Kiørboe, 2013) and the relationship

$$dw = 1.445 + 4.283 \cdot prot \qquad (r^2 = 0.900;\ n = 306;\ p<0.001) \qquad (3.1)$$

given by Hernández-León et al. (2001). Finally, potential respiration was converted to specific rates (spR_{ETS}, d^{-1}) taking into account that the R/ETS ratio normally varies with water productivity (Hernández-León and Gómez, 1996). Hence, we assumed a ratio of 1.0 for samples on eutrophic waters (Agulhas and California Currents, Southern Australia, Equatorial upwelling in the Pacific Ocean), while 0.5 for estimates on the remaining (oligotrophic) regions.

3.2.4 Growth assessments

Growth rates of zooplankton derived from measurements of AARS activity (G_{AARS}) were compared, according to 25 samples from the epipelagic layer (Fig. 3.1a), with estimates from the IBS (G_{IBS}) in combination with mechanistic equations developed in this study (Table 3.3), and those given by Hirst et al. (Hirst et al., 2003) (their Tables 4 and 7) and Hirst and Bunker (Hirst and Bunker, 2003) (their Table 6, all data). The latter proposed a unique equation, using Chl*a* (food proxy) additionally to body weight and temperature, while Hirst et al. (Hirst et al., 2003) developed different

Table 3.1. Average in situ temperature (ºC) of samples used to compare zooplankton growth and respiration rates according to enzymatic methods and predictive equations. It is specified the oceanic region and depth layer where each sample was obtained: a, b, c and d denote each depth layer (see Fig. 3.2 and 3.3), while numbers in brackets detail specific depth layers, different than the others. Moreover, ranges of body weight (µg C ind^{-1}) according to each taxonomic group along the cruise are also indicated.

		Depth (m)			
	Station	**0-200 (a)**	**200-500 (b)**	**500-1000 (c)**	**1000-2000 (d)**
ATLANTIC	**6**		16.3 (200-300)	12.6 (500-600)	
	10	18.7		7.6	
	12		10.3		4.3
	13	17.9			
	15	20.4			
	17	19.8			
	35	18.0	12.4	5.7	
	39	18.4			3.1
	43		12.9	6.6	
INDIAN	**45**	15.9	10.4	5.8	3.0
	49	19.8	14.8		
	62	17.6			
	64		12.0	7.9	
	66	20.2		4.6	
	69		10.6	7.3	3.0
	71	14.7	10.0	7.6	
	76	13.3		6.7	
	77	16.9		7.0	
WEST PACIFIC	**84**	24.5	16.1	6.2	3.2 (1000-1500) 2.5 (1500-2000)
	86	26.8	15.6	5.6	
	88	27.0			
	90	26.8	11.8	6.0	
	92	24.9	10.1	6.0	3.2
	96	24.1	9.2		
	100	23.8	11.4	5.1	
EAST PACIFIC	**103**	21.0			
	106	21.4			
	112	17.9			
	114	18.0	10.4 (300-400) 8.8 (400-500)	6.0	
	115	17.6			
	117	19.1			
	119	16.8			
	121	17.1			
bw range (µg C ind^{-1})	copepods	chaetognaths	euphausiid-like	gelatinous	other zooplankton
	0.8-172.1	4.2-235.4	38.6-1246.1	4.7-20.1	0.2-110.2

equations according to the main taxonomic groups. Unlike our equations, these relationships disregarded the heterogeneity of temperature given on the main oceanic environments, and authors developed generalist equations for the global ocean pooling data from *e.g.,* polar and equatorial waters. In contrast, as occurred in equations for respiration, our mechanistic relationships fitted to specific temperature ranges, using data from Hirst et al. (2003) (their Appendices 1 and 2). We developed different equations for the main taxonomic groups.Regarding AARS measurements, specific enzyme activity was assayed following the method of Yebra and Hernández-León (2004), slightly modified by Herrera et al. (2012). Specific activity was then converted to specific growth rates (d^{-1}) using the equation given by Hernández-León et al. (in preparation):

$$G_{AARS}\ (d^{-1}) = -0.0117 + 0.0038 \cdot spAARS \qquad (r^2 = 0.738,\ p<0.001) \quad (3.2)$$

where spAARS was expressed in terms of nmol PPi mg $prot^{-1}$ h^{-1}, and PPi is pyrophosphate.

Table 3.1. Log-transformed equations to estimate zooplankton specific-respiration (µl O_2 ind^{-1} h^{-1}), and their Q_{10}, developed in this study according to the temperature ranges (ºC) of the main ocean regions and depth layers (epi-, meso- and bathypelagic). Body weight ranges (mg dw ind^{-1}) are also indicated.

T range (ºC)	bw range (mg dw ind^{-1})	Multiple linear regression (log_{10}R (µl O_2 ind^{-1} h^{-1}))	Variables	n	a	b	c	Q_{10}	r^2
1-8	0.007-15.0	$a+b[\log_{10} bw]+c \cdot T$	bw, T	45	-0.734	0.753	0.039	2.45	0.933
8-16	0.003-71.1	$a+b[\log_{10} bw]+c \cdot T$	bw, T	327	-0.461	0.727	0.024	1.72	0.667
16-28.5	0.002-117.2	$a+b[\log_{10} bw]+c \cdot T$	bw, T	236	-0.936	0.651	0.043	2.69	0.713

Community production (P_{AARS} and P_{IBS}) and respiration (R_{ETS} and R_{IBS}) (mg C m^{-2} d^{-1}) of zooplankton were eventually assessed using the community biomass (mg C m^{-2}) obtained from the IBS and specific metabolic rates (d^{-1}) derived from both, predictive equations and enzymatic methods.

3.2.5 Development of predictive equations

For both growth and respiration, we developed mechanistic equations using a linear regression program in *SYSTAT*, *version 13*, as well as the software *SPSS-IBM, version* 22, according to the stepwise multiple linear regression method. The best fit was achieved according to logarithm of metabolism ($\log_{10}$ G, $\log_{10}$ R) and body weight ($\log_{10}$ bw), and temperature (T). The different Q_{10} were obtained from the parameter *c* of equations, according to the following:

$$c = \log_{10} (Q_{10}{}^{\wedge(1/10)}) \tag{3.3}$$

In the case of equations for growth we used data given by Hirst et al. (2003) (their Appendices 1 and 2), available for the main taxonomic groups of zooplankton, while we managed the data compiled by Ikeda and Hernández-León (2005) to develop equations for respiration. In both cases, we only employed data satisfying the established ranges of body weight (mesozooplankton) and temperature (ocean regions and epi-, meso- and bathypelagic layers) (see Table 3.1 and Discussion section below).

3.2.6 Statistics

Linear regressions relating assessments of respiration and growth from the different methods (enzymatic procedures and using the IBS combined with predictive equations) (see Tables 3.4 and 3.5) were estimated using the reduced major axis (RMA) regression model (linear regression type II). It is assumed that the different variables were evenly measured with a certain error (view Smith 2009, and literature therein).

Table 3.2. Log-transformed equations to estimate zooplankton specific-growth, and their Q_{10}, developed in this study according to the main taxonomic groups and the temperature (ºC) ranges of the main ocean regions and depth layers (epi-, meso- and bathypelagic). Body weight (µg C ind^{-1}) ranges are also indicated. We employed the *crustaceans* equations to estimate growth of our defined *euphausiid-like* group, while *other zooplankton* rates were estimated using *copepods* equations.

	T range (ºC)	bw range (µg C ind^{-1})	Multiple linear regression (log_{10} g (d^{-1}))	Variables	n	a	b	c	Q_{10}	r^2
Copepods	1-8	2.7-164.3	$a + b[\log_{10} bw] + c \cdot T$	bw, T	39	-1.742	-0.308	0.048	3.02	0.272
	8-16	0.5-240	$a + b[\log_{10} bw] + c \cdot T$	bw, T	81	-1.586	-0.324	0.043	2.69	0.275
	16-28	0.01-39	$a + b[\log_{10} bw] + c \cdot T$	bw, T	611	-1.473	-0.156	0.041	2.57	0.367
Chaetognaths	1-16	20.8-650.2	$a + b[\log_{10} bw] + c \cdot T$	bw, T	51	-1.672	-0.132	0.041	2.57	0.404
	16-26	0.9-238.4	$a + b[\log_{10} bw] + c \cdot T$	bw, T	28	-1.373	-0.273	0.034	2.19	0.344
Crustaceans	1-8	6.0-64172	$a + b[\log_{10} bw] + c \cdot T$	bw, T	116	-1.046	-0.333	0.044	2.75	0.581
	8-16	38.7-14262	$a + b[\log_{10} bw] + c \cdot T$	bw, T	118	-1.015	-0.400	0.044	2.75	0.609
	16-26	2.8-15955.2	$a + b[\log_{10} bw] + c \cdot T$	bw, T	55	-0.918	-0.372	0.028	1.91	0.569
Gelatinous	8-16	0.1-76581	$a + b[\log_{10} bw] + c \cdot T$	bw, T	50	-1.128	-0.190	0.047	2.92	0.500
	16-28	3.9-34868.4	$a + b[\log_{10} bw] + c \cdot T$	bw, T	84	-0.967	-0.211	0.029	1.93	0.476

3.3 Results

The sampling program of the Malaspina-2010 circumnavigation expedition roughly covered the main ocean regions of the ocean (~40ºN-40ºS, Fig. 3.1a). As a result, sea surface temperature ranged from approximately 15ºC in the Southern Australia to nearly 30ºC in the Equatorial Pacific (Fig. 3.1b). Moreover, temperature dropped to levels as low as 2.5ºC at deeper layers (Table 3.1, 1500-2000 m). Similarly, surface Chl*a* and PP were highly heterogeneously distributed along the cruise: from the lower levels of the vast oligotrophic gyres to the high productivity of mesotrophic and eutrophic regions, such as the Agulhas and California Currents, the region

off Southern Australia or the Equatorial upwelling in the Pacific Ocean (Fig. 3.1a, b). The temperature and body weight ranges of our specific equations (Tables 3.2 and 3.3) were defined according to measurements of both parameters along the main ocean regions during the Malaspina cruise (Fig. 3.1b, Table 3.1).

The overall range of temperature covered by our specific equations for respiration (1.0–28.5ºC) mainly matched that of Ikeda (Ikeda, 2014) (-2.0–30ºC), while similarly high coefficients of determination (r^2) were also observed for all equations (Table 3.2). As also observed on this table, estimates of Q_{10} ranged from 1.72 to 2.69. Using our temperature-specific equations, estimates of specific and community respiration were not significantly different (ANOVA, $p>0.05$) than R_{ETS} (Fig. 3.2 and 3.3). However, estimates using the Ikeda equation (Ikeda, 2014) were significantly lower (ANOVA, $p<0.05$) than the latter, despite measurements from both methods followed a similar pattern of distribution along the cruise (Fig. 3.2 and 3.3). As a result, specific and community respiration using our equations were better correlated to R_{ETS} estimates than using the Ikeda equation (Ikeda, 2014) (Table 3.4). Generally, we observed that R_{ETS} assessments were higher than R_{IBS} using both, Ikeda (Ikeda, 2014) and our equations, on samples from the epipelagic layer of lower latitudes, where temperatures were higher (Fig. 3.2; Table 3.1). In contrast, estimates from the ETS method and equations were comparable in samples along deeper layers (Fig. 3.3). Nevertheless, differences in the epipelagic layer decreased in relation to community respiration, due to the effect of community biomass (Fig. 3.2).

As occurred with respiration, the range of temperatures covered by our specific equations for growth (1–28ºC) roughly matched that of generalist equations given by Hirst et al. (Hirst et al., 2003) and Hirst and Bunker (Hirst and Bunker, 2003) (-2.3–31ºC) for the global ocean. As observed on Table 3.3, Q_{10} values ranged from 1.91 to 3.02, with increasing effect of temperature on equations for colder waters. Furthermore, our equations showed higher coefficients of determination (r^2) than those observed for previous generalist equations for the global ocean (Table 3.3), and mainly in

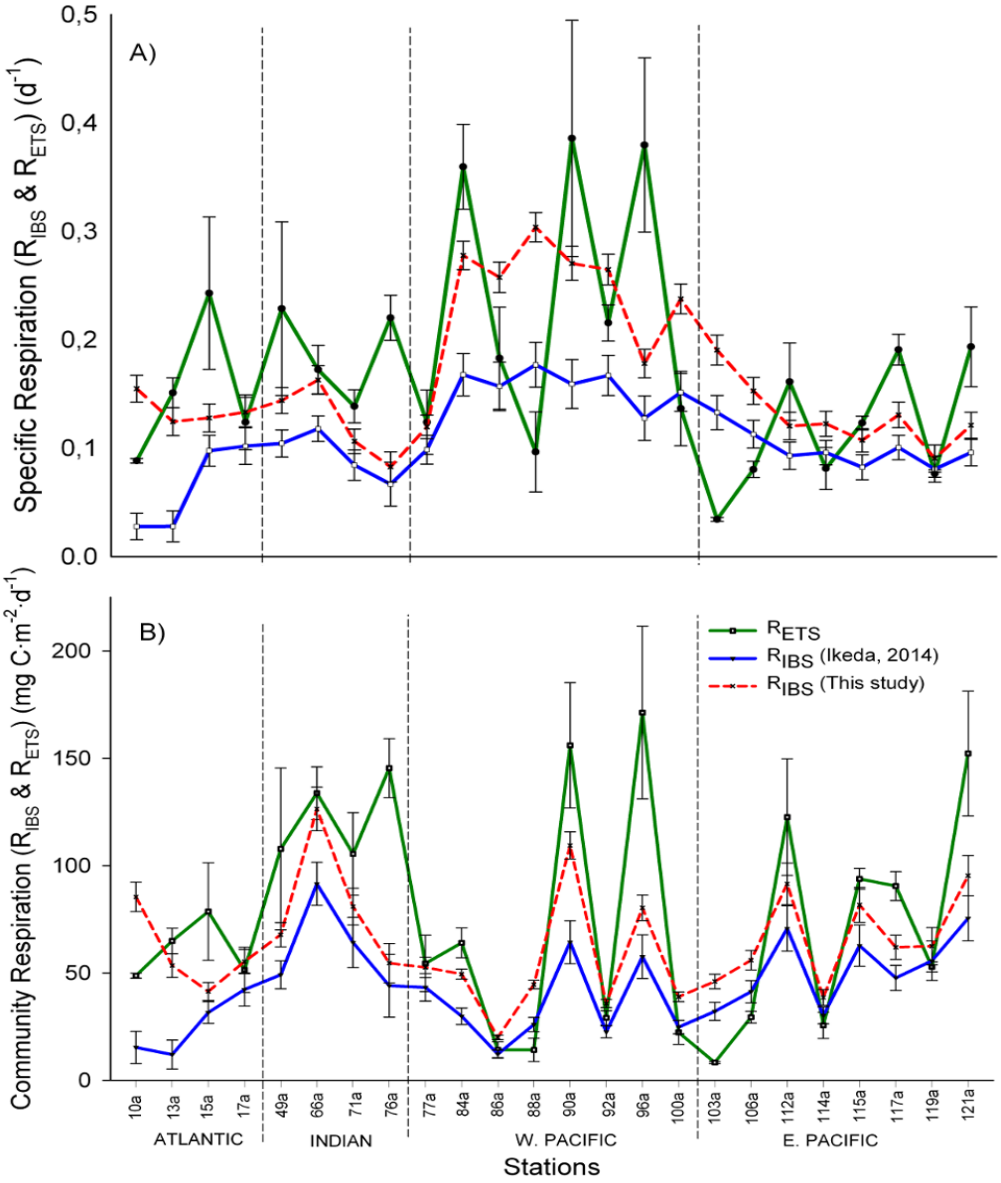

Figure 3.2. Epipelagic zooplankton specific-respiration (A) and community respiration fluxes (B) along the Malaspina cruise, according to estimates from the ETS* method and the IBS, in combination with the equation given by Ikeda (Ikeda, 2014)** and using the temperature-specific equations developed in this study. *a,* next to each sample number indicate 0-200 m depth layer (see Table 3.1). Vertical bars denote standard deviation.
*We assumed a R/ETS ratio of 0.5 for oligotrophic regions, and a ratio of 1.0 for samples from productive zones, in order to adopt suitable ratios for each region according to productivity (Hernández-León and Gómez, 1996) (see Methods section). **We applied correction factors given by Ikeda (Ikeda, 2014) to estimate respiration of *chaetognaths* (-0.345), *euphausiid-like* (0.600) and *gelatinous* (0.547) from the generalized equation for *copepods* (his Table 4).

the case of crustaceans and gelatinous zooplankton. Although the accuracy of our equations for copepods was still rather low, it increased respect to that of generalist equations from Hirst et al. (Hirst et al., 2003) and Hirst and Bunker (Hirst and Bunker, 2003), mainly in the equation for higher temperatures (16–28ºC). Consequently, estimates of specific growth and community production using our temperature-specific equations showed a higher correlation with G_{AARS} and P_{AARS} estimates than those using generalist equations (Fig. 3.4a, b). In fact, estimates using our equations were not significantly different (ANOVA, $p>0.05$) than G_{AARS} and P_{AARS} assessments. As observed for respiration, higher agreement between all methods and equations was observed when community biomass was applied (Fig. 3.4b). However, estimates of community production using the equations given by Hirst et al. (Hirst et al., 2003) and Hirst and Bunker (Hirst and Bunker, 2003) were nevertheless significantly lower (ANOVA, $p<0.05$) than assessments of P_{AARS}.

Similarly to respiration, we also observed a higher agreement between estimates of G_{IBS} using our equations and those of G_{AARS} along colder waters, such as the Southern Australia region (Fig. 3.4a). Conversely, differences increased with increasing rates, as it was on the warmer waters of the West Pacific. Nevertheless, specific growth and community production from the AARS method and estimates using the different equations followed, in general terms, a similar pattern (Fig. 3.4a, b).

3.4 Discussion

The present study is based on the previous results shown by Garijo and Hernández-León (2015) on subtropical waters. These authors observed that IBS might result as reliable as enzymatic methods to estimate zooplankton-mediated fluxes when using temperature-specific equations, fitted to the conditions of the subtropical waters. Hence, we tested the suitability of a new set of metabolic equations for further regions, with the aim to be

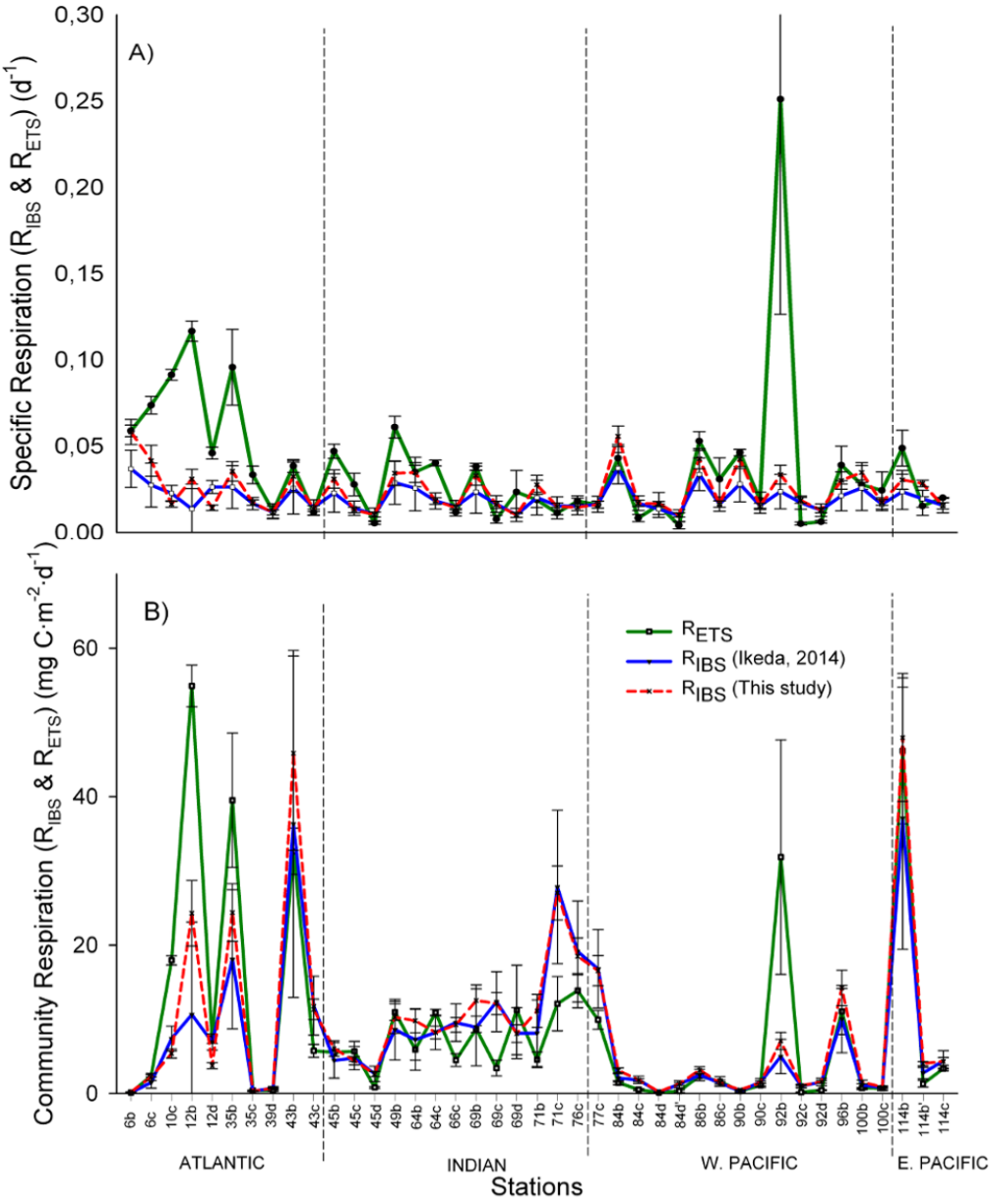

Figure 3.3. Meso- and bathypelagic zooplankton specific-respiration (A) and community fluxes (B) along the Malaspina cruise, according to estimates from the ETS* method and the IBS, in combination with the equation given by Ikeda (Ikeda, 2014)** and using the temperature-specific equations developed in this study. *b, c* and *d*, next to each sample number indicate 200-500, 500-1000 and 1000-2000 m depth layers, respectively (see Table 3.1). Vertical bars denote standard deviation.

*We assumed a R/ETS ratio of 0.5 for oligotrophic regions, and 1.0 for productive zones (Hernández-León and Gómez, 1996) (see Methods section). **We applied correction factors given by Ikeda (Ikeda, 2014) for *chaetognaths* (-0.345), *euphausiid-like* (0.600) and *gelatinous* (0.547) from the generalized equation for *copepods* (his Table 4).

applied globally, and from the surface to bathypelagic layers. These new relationships were developed according to temperature ranges in order to increase the accuracy of metabolic estimates of mesozooplankton using an IBS. Those ranges matched average measurements of this parameter along the main ocean regions (Longhurst, 2007), while body weight ranges for mesozooplankton were established according to estimates of this parameter along the cruise (Table 3.1).

In this respect, Ikeda (2014) recently observed that respiration and ammonia excretion processes in zooplankton are mainly governed by temperature and body weight, while habitat depth or taxonomy are factors of lesser importance. Therefore, it seems that adjusting the ranges of these determinant parameters of metabolism to the environmental conditions could be a reasonable way to increase the accuracy of estimates through predictive equations.

The quasi-global sampling program Malaspina-2010 (~40ºN-40ºS, Fig. 3.1a) roughly covered the main ocean regions (Longhurst, 2007). Apart of subpolar and polar environments, the overall temperature ranges observed along the cruise (2.5–30ºC) roughly covered the values that might be found anywhere in the upper 2000 m of the ocean. Moreover, it also explored highly diverse zones in terms of productivity (eu-, meso- and oligotrophic regions) (Fig. 3.1). In this sense, it seems that zooplankton samples managed in the present study could broadly represent the communities found along the 40ºN-40ºS latitudinal band of the planet. Consequently, these samples seemed to be appropriated to test the validity of our developed equations at a large and heterogeneous scale.

Our results confirmed the usefulness of the IBS in combination with both respiration and growth temperature-specific equations to estimate zooplankton-mediated fluxes in a quasi-global scenario. As previously observed by Garijo and Hernández-León (2015) on the subtropical region, our estimates of metabolic rates using temperature-specific equations were better correlated with enzymatic measurements than using generalist

equations given by Hirst et al. (Hirst et al., 2003) and Hirst and Bunker (Hirst and Bunker, 2003) for growth, and Ikeda (Ikeda, 2014) for respiration.

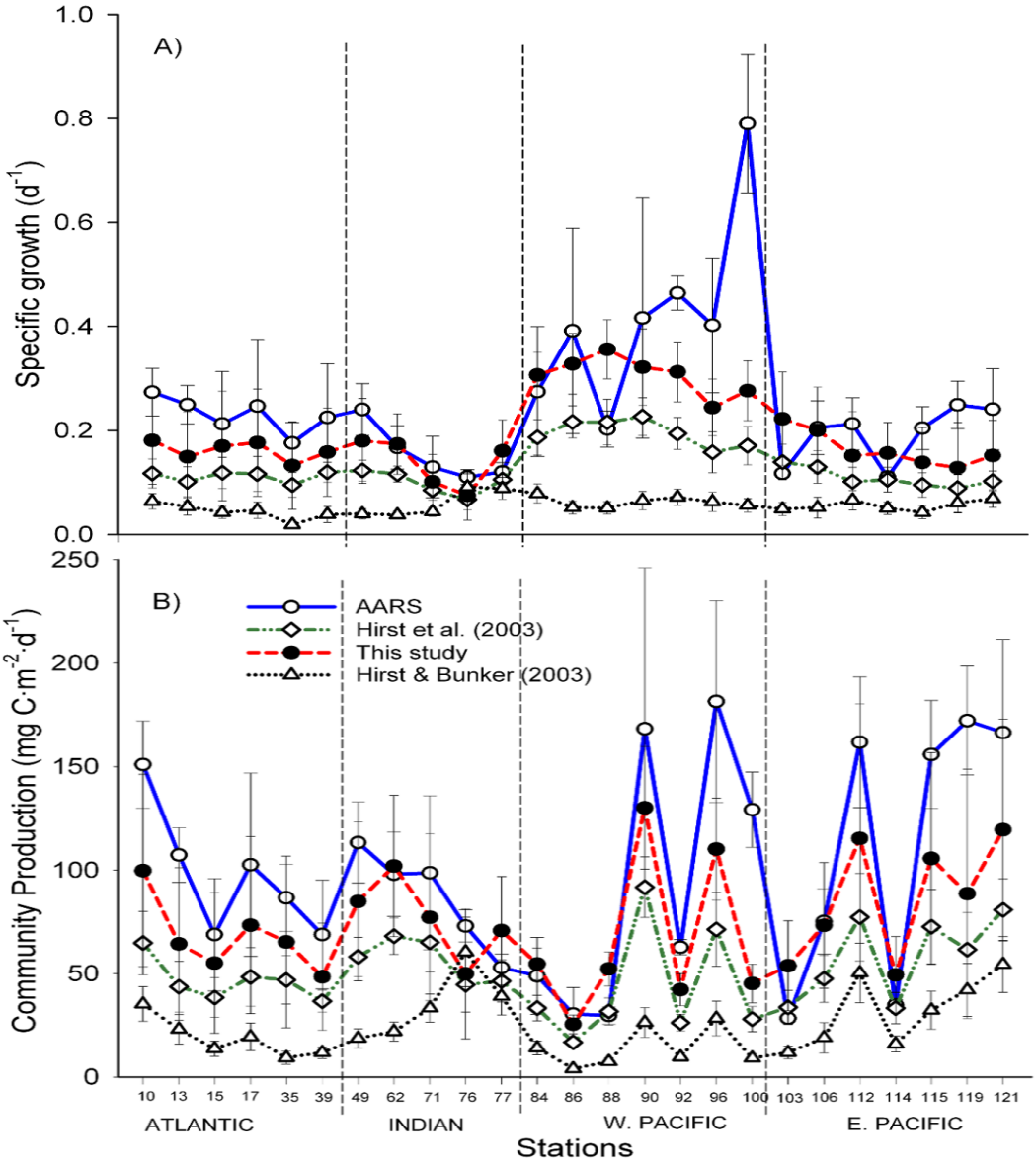

Figure 3.4. Epipelagic (0-200 m) zooplankton specific-growth (A) and community production (B) along the Malaspina cruise, according to the AARS method, and using the IBS in combination with equations given by Hirst et al. (Hirst et al., 2003) and Hirst and Bunker (Hirst and Bunker, 2003), and using temperature-specific equations developed in this study. Vertical bars denote standard deviations.

Attending to average temperatures given on the main latitudinal bands of the planet, equations developed in the present study (Tables 3.2 and 3.3) could be applied to the epipelagic layer of (1) Polar to Temperate regions: equation 1–8ºC, (2) Temperate to Subtropical regions: equation 8–16ºC, (3) Subtropical to Equatorial regions: equation (16~28ºC), and depth layers (4) Meso-bathypelagic (up to 2000 m): equations 1–8ºC and 8–16ºC. Although it is known that enzymatic measurements show some uncertainties (*e.g.,* Hernández-León and Gómez, 1996), estimates from these methods were assumed as a reference, necessary to ascertain a comparative among metabolic equations. One of the main problems resides on the incertitude of the relation between enzymatic and metabolic rates of zooplankton (Hernández-León et al., 1995; Hernández-León et al., 1996). Concerning the ETS method for example, it is known that a R/ETS ratio of 0.5 normally underestimates respiration when substrates are not limited *in vivo* (*e.g.,* Hernández-León et al., 2001; Putzeys and Hernández-León, 2005), and the opposite for a ratio of 1.0 when food resources are limited. In any case, the present study was mainly performed through oligotrophic regions and below 200 m depth, where ambient food is rather scarce. Hence, a ratio of 0.5 was assumed to estimate R_{ETS} for all samples, except for those from the epipelagic layer on eutrophic regions (see Methods section), where we adopted a ratio of 1.0 (Hernández-León and Gómez, 1996).

Coefficients of determination (r^2) of our temperature-specific equations for growth and respiration increased, in some cases, respect to those of previous equations (Tables 3.2 and 3.3). Nevertheless, it seems evident that growth equations should still be improved, and mainly in the case of copepods, due to their ubiquity and abundance in the global ocean. In turn, estimates of Q_{10} were similar for respiration and growth equations, ranging the values given on previous studies dealing with predictive equations for mesozooplankton respiration (*e.g.,* Ikeda, 1985; Ikeda, 2014) and growth (*e.g.,* Hirst and Bunker, 2003; Bunker and Hirst, 2004; Almeda et al., 2010).

Table 3.3. Linear regressions (type II) between estimates of zooplankton specific (spR) and community respiration (R) derived from the ETS method*, and using the image-based system (IBS) in combination with predictive equations (n = 65; $p<0.05$). *We assumed a R/ETS ratio of 0.5 for oligotrophic regions, and a ratio of 1.0 for samples from productive zones, in order to adopt suitable ratios for each region according to productivity (Hernández-León and Gómez, 1996) (see Methods section for further details).

	Specific respiration (spR) (d^{-1})	**Community respiration (R) (mg C m^{-2} d^{-1})**
Ikeda (2014)	$spR_{IBS} = 0.513 \cdot spR_{ETS} + 0.016$ $r^2 = 0.521$	$R_{IBS} = 0.498 \cdot R_{ETS} + 4.013$ $r^2 = 0.763$
This study	$spR_{IBS} = 0.769 \cdot spR_{ETS} + 0.018$ $r^2 = 0.521$	$R_{IBS} = 0.703 \cdot R_{ETS} + 5.326$ $r^2 = 0.782$

Accepting measurements from the ETS method as a reference for respiration, it seems that both the Ikeda equation (Ikeda, 2014) and those developed in this study could be useful to ascertain specific and community respiration in a quasi-global scenario (up to 2000 m depth) (Fig. 3.2 and 3.3). Thus, estimates from the IBS using generalist and specific equations were roughly in the range of values and matched the latitudinal distribution of the global review carried out by Hernández-León and Ikeda (2005). However, it is certain that respiration using the Ikeda equation (Ikeda, 2014) was significantly lower (ANOVA, $p>0.05$) than R_{ETS}, also indicated by the low slope coefficients of the regressions correlating these two methods (Table 3.4), in comparison with those between R_{ETS} and respiration using our specific equations. Although respiration estimates using the Ikeda equation (Ikeda, 2014) were corrected for the main taxonomic groups, the influence of this variable on the results was observed to be minimum in comparison to temperature and body weight (Ikeda, 2014). Nevertheless, it is obvious that the development of more taxonomically-specified equations, also including all significant variables, will eventually reduce our present error when determining respiration of zooplankton through predictive equations.

Differences between estimates from enzymatic methods and predictive equations (all cases) were lower in the case of community fluxes. This was in agreement with Huntley and Lopez (1992), who suggested community biomass as the main factor determining community fluxes through zooplankton. They observed that the variability of community biomass was one to three orders of magnitude greater than that of specific growth rates. In spite of this, significant differences (ANOVA, $p<0.05$) were observed between community production and respiration in the epipelagic layer from enzymatic methods and generalist equations for the global ocean (Fig. 3.2b, 3.4b). Thus, community production using the Hirst et al. (Hirst et al., 2003) and Hirst and Bunker (Hirst and Bunker, 2003) equations was significantly lower than P_{AARS} assessments along the circumnavigation. As suggested by Garijo and Hernández-León (2015), estimates using these equations could be underestimated in some particular cases. As a possible reason, the use of extreme data (*e.g.,* $T < -2.0$ºC) used by Hirst et al. (Hirst et al., 2003) to develop their equations could introduce certain bias on warmer regions, as it is known that growth decreases with temperature (*e.g.,* Hirst and Lampitt, 1998; Hirst et al., 2003).

Similarly, Hirst and Bunker (2003) fitted their equation according to average values of Chl*a* up to two orders of magnitude higher than normally given in oligotrophic waters (Garijo and Hernández-León, 2015). Therefore, it seems that the use of this equation could result more appropriated on more productive systems. In fact, estimates of P_{AARS} on the eutrophic waters off Southern Australia were more highly correlated with P_{IBS} using the Hirst and Bunker equation (Hirst and Bunker, 2003) than respect to any other (Fig. 3.4b). However, zooplankton organisms in oligotrophic regions are known to be mainly omnivores, with a reduced importance of pigmented food in the diet (*e.g.*, Saiz et al., 1999; Hernández-León et al., 2002). Hence, Chl*a* levels could result inappropriate to represent the food availability and the feeding status of organisms in these regions. Oligotrophic waters constitute the major portion of the ocean's surface and therefore special attention should be taken when estimating metabolism through predictive equations along these regions.

Table 3.4. Linear regressions (type II) between estimates of zooplankton specific-growth (G) and community production (P) derived from enzymatic measurements (AARS method), and using the image-based system (IBS) in combination with predictive equations (n = 25; $p<0.05$).

	Sp growth (G) (d^{-1})	**Community production (P) (mg C m^{-2} d^{-1})**
Hirst et al. (2003)	$G_{IBS} = 0.433 \cdot G_{AARS} + 0.021$ $r^2 = 0.338$	$P_{IBS} = 0.475 \cdot P_{AARS} + 3.789$ $r^2 = 0.682$
Hirst and Bunker (2003)	$G_{IBS} = 0.168 \cdot G_{AARS} + 0.013$ $r^2 = 0.011$	$P_{IBS} = 0.232 \cdot P_{AARS} + 1.510$ $r^2 = 0.306$
This study	$G_{IBS} = 0.659 \cdot G_{AARS} + 0.029$ $r^2 = 0.338$	$P_{IBS} = 0.696 \cdot P_{AARS} + 5.620$ $r^2 = 0.695$

In fact, the use of predictive equations still shows limitations in some cases. For instance, Garijo and Hernández-León (2015) suggested that IBS were not as precise as enzymatic methods to represent the variability of specific metabolism of zooplankton (d^{-1}) along a time-series. However, we observed that predictive equations could be also useful to study specific metabolic rates along large study regions (Fig. 3.2a, 3.3a and 3.4a). In any case, special attention should be taken on particular regions, such as coastal and upwelling waters, mesoscale structures or oxygen minimum zones (OMZs), where the use of these equations could be inappropriate. Regarding the latter regions for instance, despite the extreme conditions, organisms are present in the water column. However, the lack of oxygen limits their metabolism as an adaptation mechanism (Kiko et al., 2015a, 2015b). Therefore, specific work should be carried out on these large regions of the ocean.

In summary, our results show that IBS, in combination with the temperature-specific equations developed in this study, may constitute an alternative to estimate zooplankton-mediated fluxes along the upper 2000 m of the main ocean regions. Our equations were developed according to

temperature ranges, and comparisons were ascertained in a quasi-global scenario using samples from the circumnavigation Malaspina-2010, according to a wide range of temperatures (2.5–30ºC) and productivity (oligo-, meso- and eutrophic waters). Our metabolic estimates were comparable to those using enzymatic methods, and no significant differences were observed. By opposite, metabolism from previous equations for the global ocean was significantly lower than measurements from enzymatic methods. According to these results, although both generalist and specific equations could be useful, the latter are expected to increase the accuracy of estimates of zooplankton metabolic fluxes using an IBS. Nevertheless, the use of predictive equations still shows limitations on particular studies and regions, and therefore specific work is still required. In addition, new equations for more taxonomic levels, and including all significant variables, should be developed in order to increase the accuracy of carbon fluxes mediated by zooplankton at the large-scale.

Acknowledgements

This research was conducted by the Malaspina 2010 Expedition project, funded by the Spanish Ministry of Economy and Competitiveness (Consolider-Ingenio 2010, CSD2008-00077). Thanks are due to all those who contributed to the success of the expedition, specially those colleagues who made a successful and hard work on board, coordinating groups and obtaining samples and data. Juan Carlos Garijo was supported by a postgraduate grant (FPU-AP2010-5080) from the Spanish Ministry of Education.

Chapter 4

Zooplankton active flux in the warm ocean: relationship with chlorophyll and temperature

Zooplankton active flux in the warm ocean: relationship with chlorophyll and temperature

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Abstract

Zooplankton migrant biomass and active fluxes of carbon (respiratory, mortality and egestion) and nitrogen (ammonia excretion) were assessed through a latitudinal transect along the tropical and subtropical Atlantic Ocean (10ºS-25ºN). Metabolic estimates were derived from an image-based system in combination to recently developed predictive equations. Our results showed that large copepods (>1000 μm) were by far the major contributors (47% on average) to total active flux. Moreover, we observed a relationship between surface chlorophyll concentration and migrant biomass, which was identified as the main factor determining the magnitude of active fluxes through zooplankton. Sampling was performed along a wide latitudinal gradient, and our estimates covered the entire range of zooplankton active fluxes and migrant biomass found in the literature. Hence, the latter ranged from 35.4 mg C m^{-2} along the oligotrophic region south the Equator to 1649.4 mg C m^{-2} within the rich waters of the Cape Blanc oceanic upwelling, while total active flux through zooplankton followed the same distribution ranging from 1.4 to 81.7 mg C m^{-2} d^{-1}. Mortality flux was higher than respiratory, while gut flux was on average 2–3-fold lower than the former. Simple models were developed to assess zooplankton downward export through data easily available from remote sensing: sea surface temperature and chlorophyll concentration. The strong correlations observed could indicate the possibility of using satellite data to assess active fluxes along vast regions of the ocean in a near future.

4.1 Introduction

The biological pump constitutes one of the major pathways of vertical transport of organic and inorganic matter in the water column (Ducklow et al., 2001; Fasham, 2003). Thus, understanding its functioning is of paramount importance in developing accurate oceanic global models (Usbeck et al., 2003). Much effort has been dedicated to the so-called gravitational flux, which refers to the sinking of particulate organic matter to deeper layers (Fowler and Knauer, 1986; Buesseler et al., 2007). However, a handful of studies performed during the last decades showed that active fluxes of dissolved carbon and nitrogen through migrant zooplankton, may also account for an important fraction (in the range of the passive flux in some cases) of the total export (*e.g.*, Longhurst et al., 1990; Steinberg et al., 2000; Al-Mutairi and Landry, 2001; Hernández-León et al., 2001; Hidaka et al., 2001; Steinberg et al., 2002, 2008; Yebra et al., 2005b; Kobari et al., 2013; Stukel et al., 2013; Isla et al., 2015). Organisms migrate every day to feed in the epipelagic zone at night and return to deeper layers at dawn (Lampert, 1989), where they excrete nitrogen and release carbon by respiration, excretion and egestion.

Community fluxes mediated by zooplankton through respiration or production are primarily dependent on community biomass, since the variability of the latter parameter may be one to three orders of magnitude greater than specific metabolic rates (Huntley and Lopez, 1992). In turn, diel vertical migration (DVM) of zooplankton occurs in all marine regions and it is probably one of the most important movements of biomass in the ocean. Consequently, the amount of matter exported downwards through migrant zooplankton must be forcedly considerable at a global scale, with subsequent implications for the biological pump.

Estimates of zooplankton migrant biomass are relatively affordable. However, metabolic rates at depth are difficult to assess due to the low concentration of organisms in the mesopelagic zone (Yebra et al., 2005b) and constraints related to incubation methods traditionally used, as *e.g.,* the

number of individuals to ensure optimal conditions, stress of animals captured, bacterial growth or starvation (Ikeda et al., 2000). Indirect indices, such as the activity of the electron transfer system (ETS; Packard, 1971) constitute an alternative, although this method suffers from the uncertainty of the relation between the enzyme activity and respiration (Hernández-León and Gómez, 1996). On the other hand, the use of image-based systems (IBS) in combination with empirical models, based on temperature and body weight, may also result helpful to approach metabolic rates of each individual. On this subject, Garijo and Hernández-León (2015) observed that results using an IBS were comparable to those derived from enzymatic methods, although in a faster and inexpensive way. The use of predictive equations, fitted to the specific ranges of temperature given at each depth layer, seem to be a reliable alternative to assess growth and respiration rates of zooplankton at depth (Garijo et al., Chapter 3), and therefore they could be useful to ascertain metabolic profiles in the water column. Similarly, Peterson and Wroblewski (1984) developed a weight-specific mortality model based on the biomass of individuals, which was modified afterwards by Hidaka et al. (2001).

Most of the effort has been dedicated to respiratory flux, and mortality in a lesser extent, while little attention has received the so-called gut flux (egestion flux): carbon exported to the mesopelagic layer as non-assimilated food in the gut of migrants, releasing fecal pellets during daytime (Angel, 1989). In this respect, some authors have pointed out that organisms are able to transport a significant amount of pigments in their guts (Lampitt et al., 1993; Hernández-León et al., 2001; Yebra et al., 2005b; Putzeys et al., 2011). Estimates of gut flux according to the gut fluorescence (GF) method are biased, since authors are forced to assume (1) an herbivorous diet of organisms, (2) the remaining of pigments in the guts of animals during downward migration or (3) that they do not feed on pigmented material at depth. Nevertheless, this kind of estimates should be considered as conservative for omnivorous organisms, and therefore it is expected that gut flux might also contribute to enhance total export through zooplankton.

Similarly, despite some studies showed that excretion of nitrogen through migrant zooplankton ranged or even exceeded the passive flux (Dam et al., 1995; Steinberg et al., 2002; Stuckel et al., 2013), this mechanism has been scarcely addressed. In addition, most of the results are derived from incubations (*e.g.,* Steinberg et al., 2002) with the associated limitations that these methods present at deeper layers (see above). Alternatively, Ikeda et al. (2001) developed a nitrogen excretion model based on temperature and body weight, which seemed to provide reliable results (Stuckel et al., 2013).

On the other hand, Irigoien et al. (2014) recently pointed out that biomass of mesopelagic fish strongly depends on primary production at a global scale. In this regard, it seems that understanding how productivity could influence zooplankton-mediated fluxes might be a key factor to determine the contribution of these communities to the biological pump in the ocean. However, as occurs for biomass and metabolic assessments, vast and remote regions of the ocean still remain unexplored in terms of active fluxes, while most repeated cruises concentrate along established navigation routes (Reid et al., 2003; Isla et al., 2004; San Martin et al., 2006). In this sense, many researchers hypothesize that carbon export and the state of the biological pump might be predicted through characteristics of the ocean's surface (*e.g.,* EXPORTS, http://exports.oceancolor.ucsb.edu). This builds on recent advances in satellite remote sensing and autonomous tools (gliders, floats, etc.), which regularly provide information on oceanic parameters such as chlorophyll a (Chl*a*), primary production (PP), oxygen concentration or sea surface temperature (SST). Zooplankton models based on these parameters could stand as a valuable tool to continuously examine the role of these communities in oceanic carbon budgets, including environments that otherwise could result expensive and challenging to sample directly (López-Urrutia et al., 2006; Jennings et al., 2009; Stock et al., 2014).

In this paper, we examined the vertical distribution of zooplankton biomass and metabolic profiles of respiration, mortality (assessed through growth assuming the community was in steady-state), egestion and ammonia excretion rates, in order to assess carbon and nitrogen active fluxes due to

vertical migration along the tropical and subtropical Atlantic Ocean (10ºS-25ºN). Our estimates of metabolism were derived from an IBS in combination with recently developed predictive equations, based on temperature and body weight, fitted to the temperature ranges given at each depth layer. Our results from this highly heterogeneous region were compared to surface temperature and chlorophyll concentration.

4.2 Materials and methods

4.2.1 Hydrographic surveys

From 3rd to 29th April 2015, 11 stations along a latitudinal transect in the Atlantic Ocean (10ºS-25ºN) were sampled during the Mafia cruise on board the R/V "Hespérides" (Fig. 4.1a). Vertical profiles of temperature, salinity and fluorescence were recorded down to 800 m depth using a *SeaBird 911 plus* CTD system, mounted on a *General Oceanics* rosette sampler, equipped with twenty-four 12 l Niskin bottles. Phytoplankton chlorophyll was derived from depth profiles of *in situ* fluorescence measured with a *Turner Scufa* fluorometer, calibrated with samples collected within the upper 200 m of the water column in accordance with the JGOFS recommendations (UNESCO, 1994). In addition, continuous underway measurements of surface (~2 m) temperature and fluorescence were recorded every minute using a *Seabird* thermosalinometer and fluorometer. Data was represented every nautical mile and checked by comparison with near-surface values from the CTD records.

4.2.2 Zooplankton sampling and biomass assessments

Paired day-night zooplankton samples at 8 discrete depth intervals (0-50, 50-100, 100-200, 200-300, 300-400, 400-500, 500-600 and 600-800 m) were taken at each station with a 1 m^2, 200 μm mesh, *Multiple Opening and Closing Net and Environmental Sensing System* (MOCNESS). Oblique trawls were performed at a towing speed of *ca.* 3 knots, measuring the volume of filtered

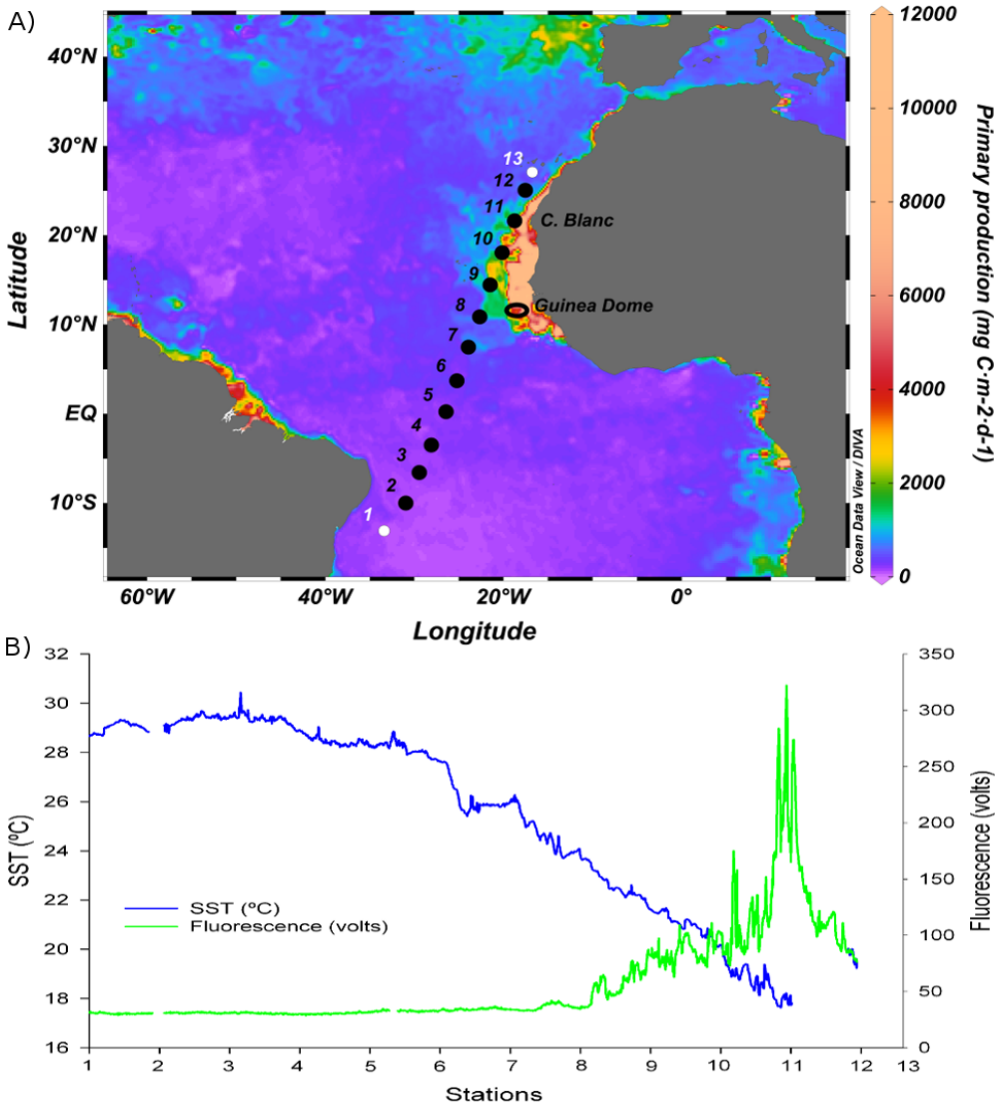

Figure 4.1. Zooplankton sampling stations* with the average primary production (mg C m^{-2} d^{-1}) during April 2015 superimposed (A), and *in situ* sea surface temperature (ºC) and fluorescence (volts) along the cruise (B). The ship's thermosalinometer and fluorometer were fed from the ship's continuous seawater supply and recorded data every minute, although it was represented every nautical mile. *White points indicate stations that were not sampled for zooplankton. Primary production was obtained from the *Ocean Productivity* website (http://www.science.oregonstate.edu/ocean.productivity/index.php), according to the Vertically Generalized Production Model (VGPM, Behrenfeld and Falkowski, 1997) and data from sensor MODIS aboard the *Aqua* satellite (http://oceancolor.gsfc.nasa.gov/cms/data/aqua).

water through a calibrated electronic flowmeter. Zooplankton estimates of biomass were directly obtained on board through image processing using the software *ZooImage 1, version 1.2-1* (http://www.sciviews.org/zooimage). For that, an aliquot of each sample was immediately taken after sampling using a Hensen pipette, and stained with 1 ml of Rose Bengal (5g l^{-1}). Both samples and aliquots were preserved in 4% buffered formaldehyde. After 24 hours, stained aliquots were spilled onto 70x105 mm methacrylate plates in order to obtain different images (5 to 10 replicates depending on the concentration of individuals) of each aliquot.Images were obtained with a *Nikon D800* digital camera (36 Megapixel) using a Macro Lens (*Model Micro Nikkor* 60mm f/2.8G ED). Since a uniform background light is essential for an appropriate identification of particles by the software, a white LED backlight was also used. Digital images at a resolution of 1850 dpi were then processed with *ZooImage* according to Grosjean and Denis (2007). We used the *Scanner color* plugin and modified the *Color threshold* parameter according to the properties of our images (*e.g.,* illumination, contrast between particles and background, shadows, etc.).

The software automatically enumerated, measured and classified organisms into 5 taxonomic groups: copepods, chaetognaths, euphausiid-like, gelatinous and other zooplankton; inorganic particles were assembled into an extra non-planktonic group. See Garijo and Hernández-León (2015) for further details. The software used a manually performed classification (*training set*), containing about 1750 images of organisms from the study region, to establish patterns for automatic classification. We selected the *Random Forest* algorithm, according to Grosjean et al. (2004), and the global error rate achieved was estimated below 7% in the automatic classification. The use of *Rose Bengal*, which only stained lipids, resulted essential to perform a more efficient processing, since it provided the required contrast to identify the individuals and, at the same time, limited the number of inorganic particles detected. Consequently, the number of images required to build a suitable *training set*, as well as the processing time, were considerably lower than in previous studies using image-based analysis systems (*e.g.,* Garijo and Hernández-León, 2015).

Once the organisms were classified, individual biomass was estimated using the empirical relationships given by Hernández-León and Montero (2006), and improved by Lehette and Hernández-León (2009), between body area and dry weight. We applied a different equation for each taxonomic group, as detailed in Garijo and Hernández-León (2015) (Table 1.1). These authors observed that estimates of community biomass using an IBS in combination with these relationships were highly comparable to those from the traditional dry weight method (Lovegrove, 1966). Body weight was then converted to carbon and nitrogen content according to Kiørboe (2013), using a different conversion factor for each taxonomic group.

We studied the contribution of three size classes (200-500, 500-1000 and >1000 μm) to total migrant biomass and active fluxes of the community, while very large organisms (mainly decapods and fish), which occasionally appeared in our samples, were removed because those organisms are thought to normally avoid the inlet of the MOCNESS net. Day and night biomass for each depth interval were subtracted to study migratory patterns, and then we estimated migrant biomass as the difference between day and night biomass values in the euphotic zone (upper 200 m).

4.2.3 Estimation of metabolic rates

Day and night specific respiration and growth rates (d^{-1}) of zooplankton were individually estimated according to predictive equations developed by Garijo et al. (Chapter 3), relating metabolic rates and body weight and temperature (Tables 4.1 and 3.3, respectively). These authors fitted empirical equations to the temperature ranges given at the main ocean regions and depth layers, showing that temperature-specific equations may result as reliable as enzymatic methods, while more accurate than existing relationships for the global ocean (*e.g.,* Hirst et al, 2003; Hirst and Bunker, 2003; Ikeda, 2014). In the case of specific growth, we were able to use a different equation for each taxonomic group. Assuming that zooplankton community was in a steady-state along the study region, we approached

mortality from growth rates (Hirst and Kiørboe, 2002). We also assumed a respiratory quotient of 0.97 to assess specific respiration (Omori and Ikeda, 1984).

In turn, we estimated specific egestion rates (d^{-1}) of each individual combining the empirical equation developed by Garijo and Hernández-León (2015) and that from Irigoien (1998) (Table 4.1). The former related body weight and gut content (in terms of Chl*a*), while the latter approached gut evacuation rates from habitat temperature. We then assumed a C:Chl*a* ratio of 50 (*e.g.*, Reigstad et al, 2008) to obtain specific egestion rates from pigmented plus non-pigmented feeding.

Finally, specific rates of ammonia excretion (d^{-1}) were individually assessed using the generalist equations for the global ocean given by Ikeda (2014), according to body weight, habitat depth and temperature, and taxonomic groups (Table 4.1).

4.2.4 Active flux assessment

Active carbon flux due to respiration (R), mortality (M) and egestion (G) (mg C m^{-2} d^{-1}) of zooplankton, as well as nitrogen export through ammonia excretion processes (N) (mg N m^{-2} d^{-1}) below the euphotic zone (>200 m depth) were estimated as in Zhang and Dam (1997). Thus, we applied the equation:

$$F = B \cdot M \cdot T \quad (4.1)$$

where B is diel-migrating zooplankton biomass (mg C m^{-2} or mg N m^{-2}); M is the average daytime specific metabolic rate (d^{-1}) of the community at 300-500 m depth layer, where the majority of migrant organisms were observed to reside during the day (see the vertical distribution of daytime biomass, Fig. 4.3a); and T is the average number of daylight hours (12 h). In the assessment of gut flux (egestion flux, G), we assumed that food remained in

the gut during downward migration; the residence time was therefore long enough to allow the defecation at mesopelagic layers.

4.3 Results

4.3.1 Hydrography

Temperature in the upper 100 m depth gradually decreased from south to north along the latitudinal transect (Fig. 4.1b and 4.2a). In fact, SST exceeded 30ºC in the region south the Equator, while it approximated to 17ºC in the Cape Blanc oceanic upwelling region (CBU, Fig. 4.1b). In this zone, isotherms and isopycnals in the mesopelagic layer deepened more sharply (Fig. 4.2a, b) than in the 10ºS-20ºN region, where a thermocline was observed in the upper 200 m depth, denoting the typical situation of an oligotrophic environment. However, we also found a surface tongue of less salty water around 7ºN-11ºN, which coincided with a slight increase of chlorophyll at 50-75 m depth (Fig. 4.2b, c). This roughly corresponded to the predicted location of the Inter-Tropical Convergence Zone (ITCZ) for March-April presented by Hastenrath and Lamb (1977). The annual cycle of the surface wind stress determines the latitudinal displacements of the ITCZ and its associated oceanic equatorial upwelling (EU) that, attending to the chlorophyll levels observed, was relatively weak during our sampling period. In contrast, chlorophyll and fluorescence were higher in the surface layers of the CBU (Fig. 4.1b, 4.2c), also matching the maximum of primary production measured from remote sensing along the cruise (Fig. 4.1a). Nevertheless, as observed in this figure, our sampling rather covered the transition zone between the upwelling and the oligotrophic waters, perhaps explaining the moderate values of chlorophyll observed within the upper 50 m of the water column in this region.

Table 4.1. Predictive equations and their Q_{10} used to estimate zooplankton respiration (Garijo et al., Chapter 3), egestion (Garijo and Hernández-León, 2015) and ammonia excretion (Ikeda, 2014) using an image-based system. *T* is habitat temperature, *bw* is body weight and *D* is the depth where the organisms were captured. In the case of respiration we applied a different equation according to the habitat temperature of each sample (ocean regions and depth layers). Equation to estimate ammonia excretion on the table corresponded to copepods, and was also applied to *euphausiid-like* and *other zooplankton* groups. For estimating *chaetognaths* and *gelatinous* rates, this equation was corrected according to given factors (-0.558 and -1.397, respectively).

	T range (ºC)	Variables	Multiple linear regression	n	a	b	c	d	Q_{10}	r^2
Respiration (R) ($\mu l\ O_2\ ind^{-1}\ h^{-1}$)	1-8	bw (mg dw ind^{-1}) T (ºC)	$\log_{10} R=a+b[\log_{10} bw]+c \cdot T$	45	-0.734	0.753	0.039	-	2.45	0.933
	8-16			327	-0.461	0.727	0.024	-	1.72	0.667
	16-28.5			236	-0.936	0.651	0.043	-	2.69	0.713
Egestion ($\mu g\ C\ ind^{-1}\ h^{-1}$)	-	bw ($\mu g\ C\ ind^{-1}$)	$\log_{10} GC=a \cdot \log_{10} bw+b$ GC (ng Chl*a* ind^{-1})	208	0.852	-1.160	-	-	-	0.769
	-	T (ºC)	$e=a \cdot T+b$ e (min^{-1})	19	0.0026	0.012	-	-	-	0.940
Ammonia excretion (N) ($\mu g\ N\ ind^{-1}\ h^{-1}$)	-	bw (mg dw ind^{-1}) T (K) D (m)	$\ln N=a+b[\ln bw]+c[1000/T]+d[\ln D]$	266	15.567	0.796	-5.010	-0.115	1.84	0.897

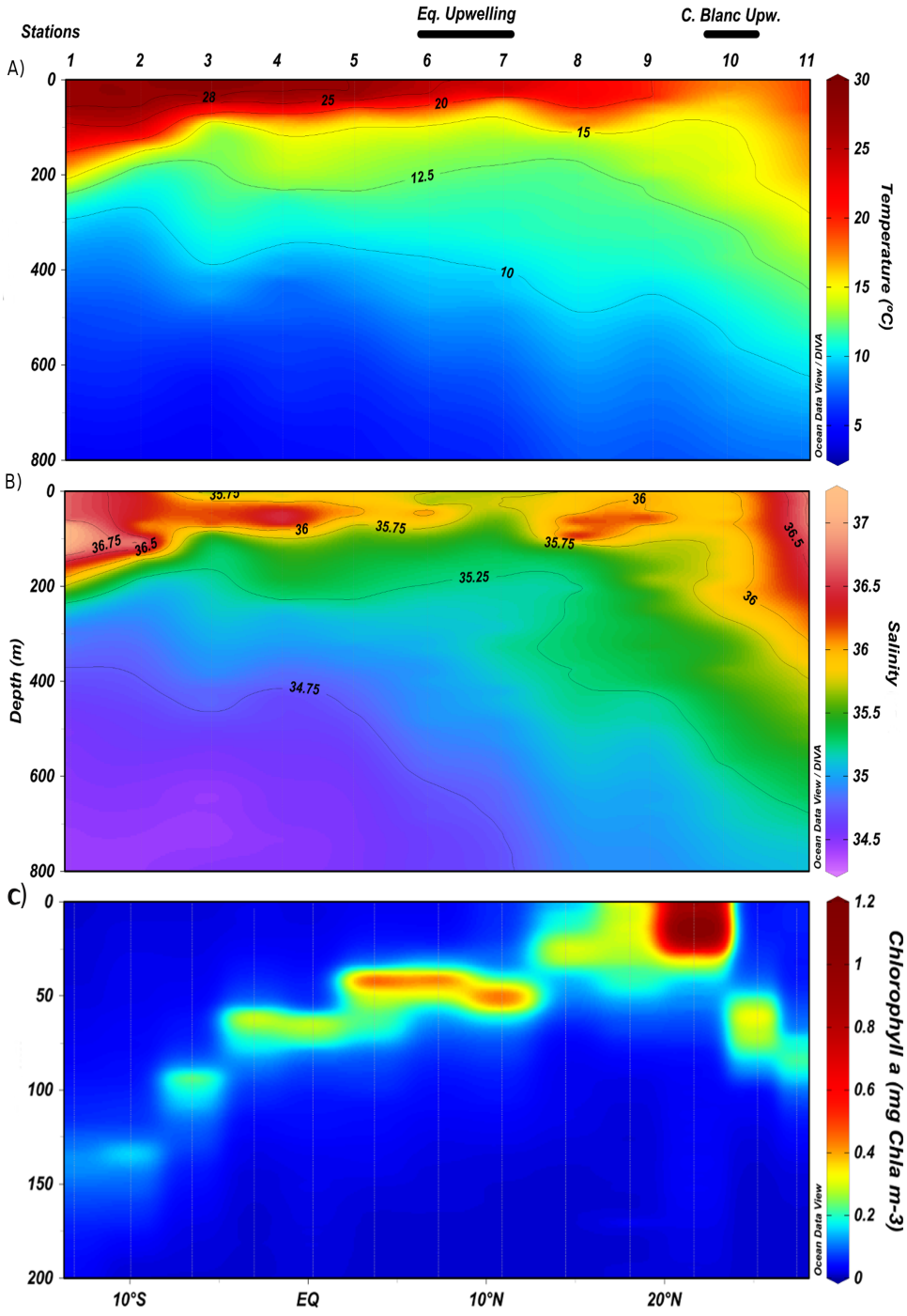

Figure 4.2. Vertical profiles of temperature (A, °C), salinity (B) and chlorophyll a (C, mg Chl *a* m^{-3}) along the study region. It is indicated the approximate location of the Cape Blanc and equatorial upwellings.

4.3.2 Vertical distribution of zooplankton and migrant biomass

Vertical distribution of zooplankton community biomass showed a clear pattern during both day and night samplings (Fig. 4.3a, b), with higher values above 200 m depth. We also found a deeper layer of biomass around 300-500 m depth, which intensified at night in the proximities of the weak EU and mostly in the CBU regions. In these areas, community biomass (day and night) in the water column was much higher than in the oligotrophic zones. Moreover, we observed a general pattern of increasing biomass at night within the epipelagic zone due to the migrating fraction (Fig. 4.3a, b), resulting in negative values in the day-minus-night profiles in the surface layers (Fig. 4.3c). By opposite, slightly positive values were generally observed below 200 m depth, indicating increases of biomass at depth during daytime.

Migrant biomass in the euphotic zone increased at both upwelling regions (Fig. 4.3c), ranging from 513.3 to 682.6 mg C m^{-2} in the weak EU and approximated to 1649.9 mg C m^{-2} in the CBU (Table 4.2). On the other hand, lower and heterogeneous values were observed along the oligotrophic regions (35.4-693.8 mg C m^{-2}). Migrant biomass in terms of nitrogen was directly estimated from migrant values in carbon units and therefore followed the same pattern than the latter parameter (Table 4.3), ranging from 3.6 to 168.3 mg N m^{-2} along the whole transect.

4.3.3 Metabolic rates

Daytime specific metabolic rates (d-1) of largest organisms (>1000 μm) followed a similar pattern, showing higher values in the upper 200 m and decreasing with depth (Fig. 4.4a, b, c, d). Horizontally, specific rates slightly increased in the epipelagic zone south the equator, as a consequence of the higher SST in this region. Nevertheless, none of the metabolic parameters (R, M, G, N) showed significant differences (ANOVA, $p>0.05$) between stations in the mesopelagic layer along the cruise. On the other hand, egestion rates within the 300-500 m layer were significantly lower (ANOVA,

p<0.05) than estimates of respiration, mortality and ammonia excretion. This is the layer where most of the migrant organisms were observed to reside during daytime (Fig. 4.3a), releasing most of the carbon and ammonia exported downwards.

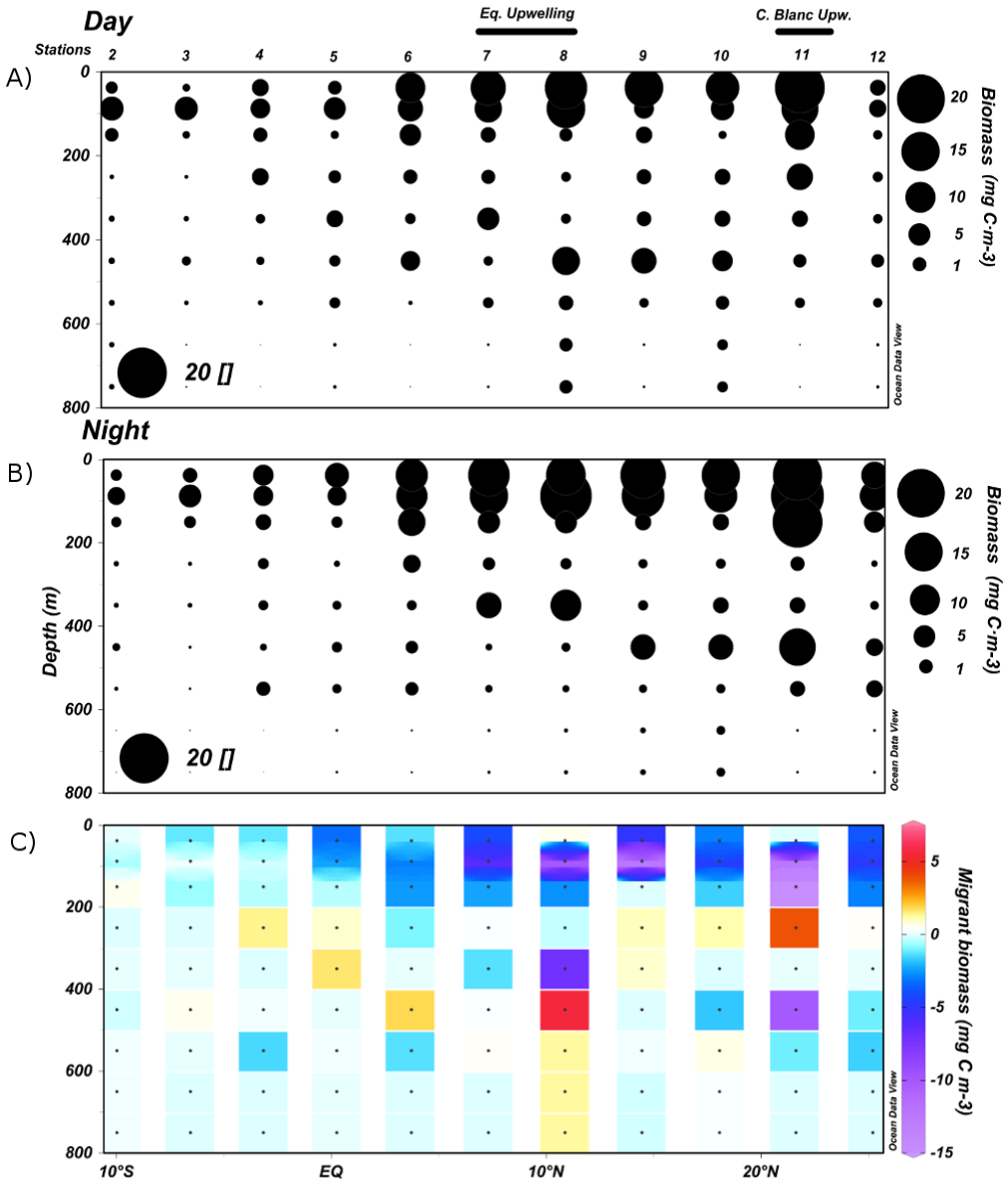

Figure 4.3. Vertical profiles of zooplankton community biomass (mg C m^{-3}) during daytime (A) and nighttime (B), as well as migrant biomass (C, mg C m^{-3}) at the sampling stations. The latter was the result of day-minus-night vertical profiles of biomass.

4.3.4 Active flux

Carbon fluxes through respiration, mortality and egestion of zooplankton followed a similar pattern along the whole transect, matching the distribution of migrant biomass (Fig. 4.5a), since metabolic rates presented a homogeneous distribution along the mesopelagic zone (Fig. 4.4a, b, c). A similar scenario was observed for ammonia excretion flux (Fig. 4.5b). Thus, migrant biomass and active fluxes generally increased from south to north along the cruise (Fig. 4.5a, b), highlighting the intensification around the weak EU, and more sharply in the region of the CBU (see ranges in Tables 4.2 and 4.3). Contribution of mortality flux, derived from differences in the magnitude of specific metabolic rates, was higher than respiratory or egestion fluxes, and differences expanded at regions with higher export (Fig. 4.5a). On average, about 48% and 32% of the total carbon exported corresponded to mortality and respiratory flux respectively, while gut flux accounted for about 20% along the cruise. As their distribution was mainly influenced by migrant biomass (MB), we observed a high correlation (linear regression type II) between the latter parameter and total carbon flux (TF, respiratory + mortality + egestion) ($r^2 = 0.960$; $n = 11$; $p<0.05$), both following a similar distribution ($TF = 0.051 \cdot MB - 3.054$) (Fig. 4.5c). Furthermore, total carbon flux was also correlated with surface chlorophyll concentration (see Section 4.3.5), showing a coupled distribution, with a remarkable intensification around the CBU (Fig. 4.5c).

Large copepods (>1000 μm) were by far the major contributors (47% on average) to total active flux along the transect (Fig. 4.6). Except in the proximities of the Equator and within the region of the CBU, where euphausiids were dominant, large copepods and chaetognaths accounted on average for 76% of the total flux. Strikingly, smallest organisms (<500 μm) represented about 25% of the migratory flux within the EU region.

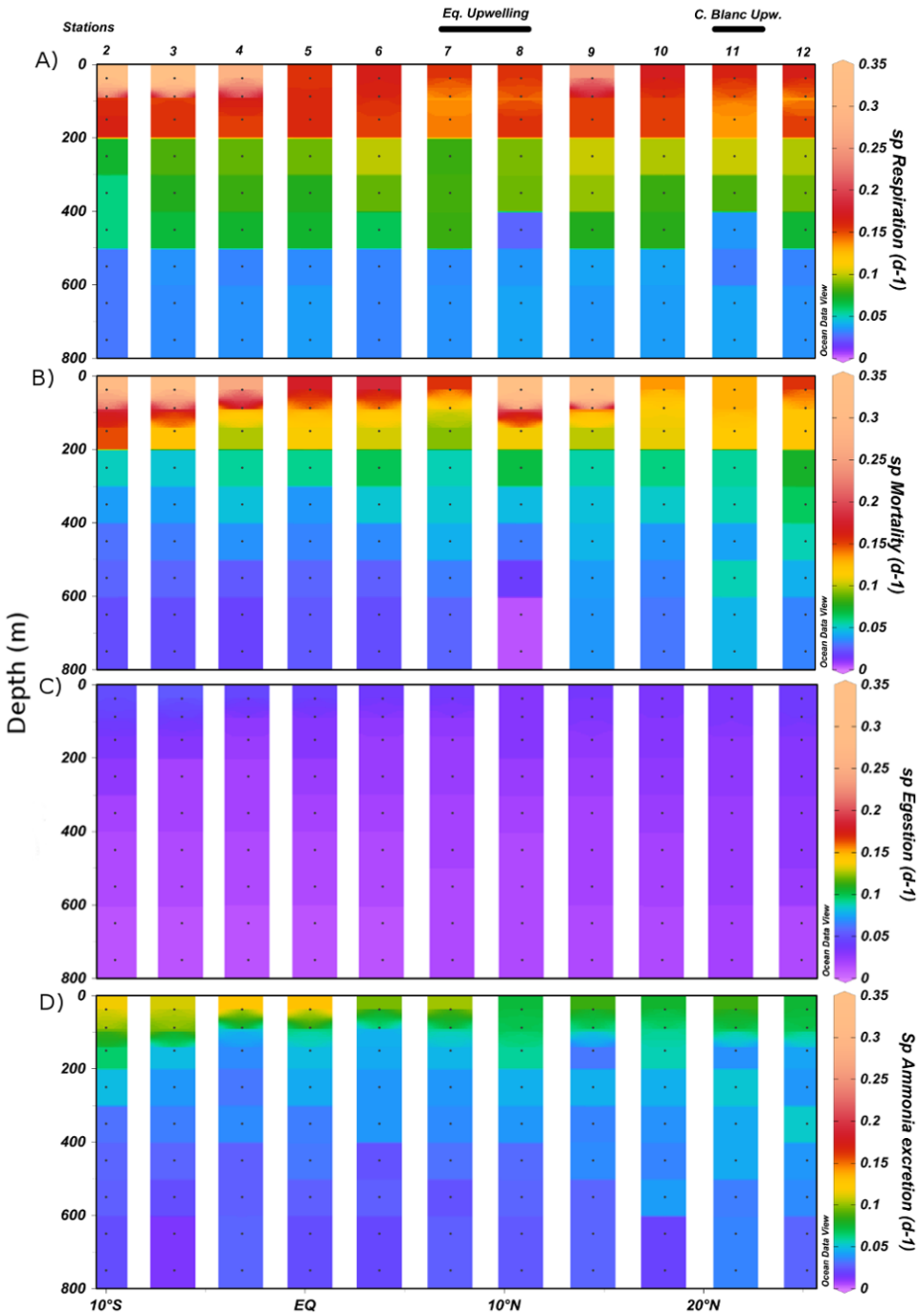

Figure 4.4. Vertical profiles of specific respiration (A), mortality (B), egestion (C) and ammonia excretion (D) rates (d^{-1}) for zooplankton individuals >1000 μm along the study region.

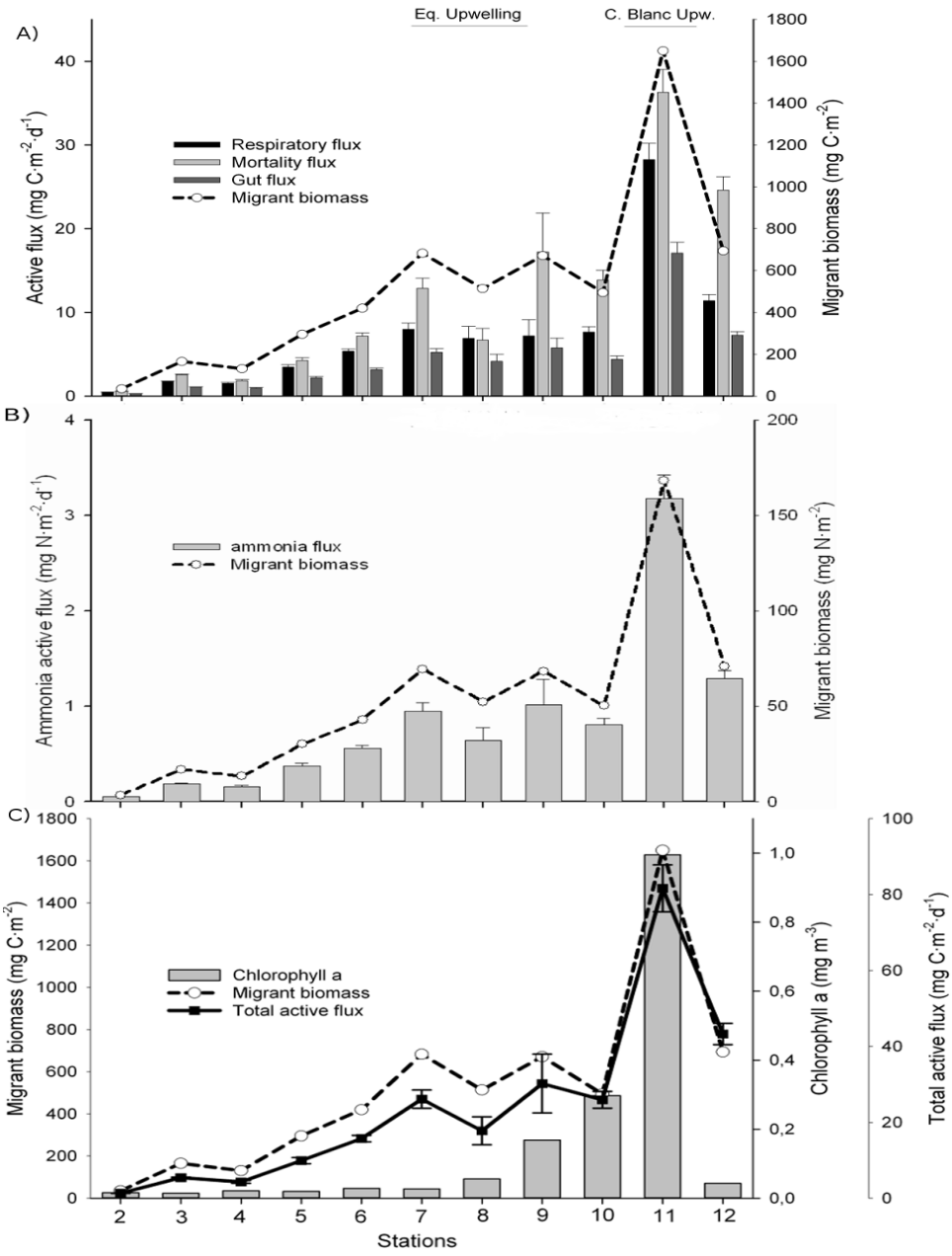

Figure 4.5. Zooplankton migrant biomass (mg C m^{-2}) and active carbon flux (mg C m^{-2} d^{-1}) into the mesopelagic layer (>200 m depth) through respiration, mortality and egestion processes (A), zooplankton migrant biomass in terms of nitrogen (mg N m^{-2}) and ammonia excretion active flux (mg N m^{-2} d^{-1}) (B), and zooplankton migrant biomass (mg C m^{-2}) and total carbon export (R+M+G) (mg C m^{-2} d^{-1}), jointly with the distribution of *in situ* surface chlorophyll concentration (mg m^{-3}) (C) along the cruise. Vertical bars denote standard errors. See Methods section for calculations of migrant biomass and active fluxes.

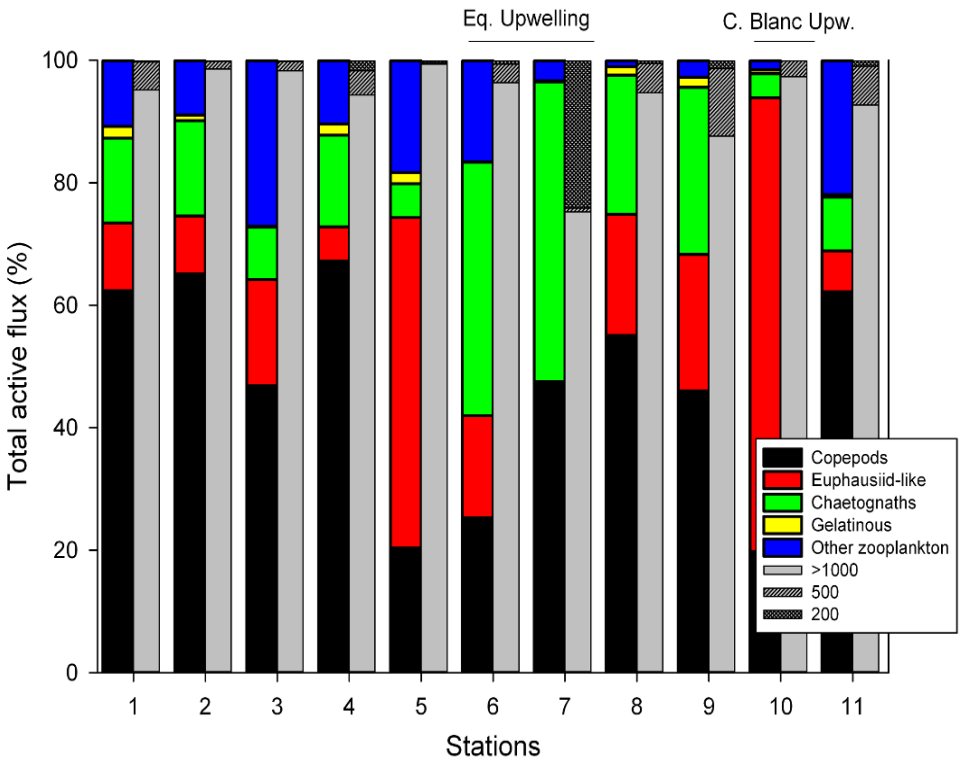

Figure 4.6. Contribution of major taxonomic groups (%), as well as the three size classes (200-500, 500-1000 and >1000 μm), to total active flux (below 200 m depth) through zooplankton along the cruise.

4.3.5 Active flux and remote sensing

Migrant biomass of zooplankton was correlated with *in situ* surface chlorophyll concentration along the cruise ($r^2 = 0.500$; n = 11; $p<0.05$) (Fig. 4.7a). Furthermore, as migratory biomass mainly determined the distribution and magnitude of active fluxes (see above), the different mechanisms (and consequently total carbon flux) were also significantly correlated with surface Chl*a* (Fig. 4.7b, c, d, e). A similar observation was made for ammonia excretion fluxes (Fig. 4.7f). Likewise, we found that migrant biomass was highly (negatively) correlated with *in situ* measurements of SST ($r^2 = 0.661$; n = 11; $p<0.05$). As a consequence, multiple linear regressions relating

zooplankton export and surface Chl*a* and temperature showed rather high correlation coefficients (Table 4.4).

4.4 Discussion

4.4.1 Methodological constraints

Migrant biomass was assumed to be the increment of biomass at the euphotic zone during nighttime. This probably led to a biomass overestimation (and consequently of active flux) due to daytime avoidance of nets by large organisms in the upper sunlit layers (Ianson et al., 2004). Another source of overestimation relies on the assumption that migrant biomass, estimated at night in the euphotic zone, is not predated and reaches the mesopelagic layer to reside during the day. However, it has been suggested that about 1-4% of zooplankton biomass is removed by mesopelagic fish in the upper 200 m depth (Hopkins and Gartner, 1992; Watanabe et al., 2002; Hudson et al., 2014), although it remains unclear which fraction of the migrant zooplankton is impacted by this consumption.

By opposite, migrant biomass and export could be underestimated if assessments are based on daytime increments under 200 m depth, since it is known that organisms may detect the nets even at deeper layers during the day (Ianson et al., 2004). In addition, individuals migrating to the surface layers during nighttime and residing deeper than 800 m during the day (not sampled) would be also unaccounted for. Avoidance of and attraction to nets due to bioluminescence or pressure changes are additional source of bias (Ianson et al., 2004).

Concerning carbon and nitrogen export, we assumed the metabolic rates of individuals in the 300-500 m layer as the average metabolism of the migrant community, since most individuals distributed at this depth during daytime (Fig. 4.3), and therefore releasing most of the carbon and nitrogen within this layer. In this regard, despite individuals were also found at other layers, metabolic rates were not significantly different (ANOVA, $p>0.05$) within the whole mesopelagic region, and therefore this was not considered as a

significant source of uncertainty (Fig. 4.4). Moreover, the influence of taxonomy on zooplankton metabolism is known to be lower than other factors such as body weight and temperature, or even habitat depth (Bode et al., 2013; Ikeda, 2014). In any case, this effect was presumably minimized for estimates of growth rates and ammonia excretion, as different equations and corrections were applied for each group. Predictive equations, relating metabolism and body weight and temperature, are suggested as a reliable alternative to assess zooplankton community fluxes when they fit to the specific conditions of the study region (Garijo and Hernández-León, 2015; Garijo et al., Chapter 3). In these studies, authors observed that estimates using temperature-specific equations were at least comparable to assessments from enzymatic methods, and probably more accurate than using existing relationships for the global ocean, such as those from Ikeda (1985), Hirst et al. (2003), Hirst and Bunker (2003), Zhou et al. (2010) and Ikeda (2014).

4.4.2 Active flux: the importance of migrant biomass

The pattern of vertical distribution of zooplankton community biomass showed two main layers during the day: one in the upper 200 m depth, and another in the 300-500 m layer. The latter coincided with the position of the so-called deep scattering layer (DSL) (see Dietz, 1948 or Boden and Kampa, 1967), regularly observed through acoustic sampling. At night, part of these organisms migrated to the surface layers, since nightly biomass in the upper 200 m was always higher than during daytime. However, mesopelagic biomass also increased during the night. This could be partly attributed to a second migration, coming from deeper layers, in agreement to the hypothesis by Vinogradov (1970), or merely due to the possible increase of effectiveness of hauls by night (Hernández-León et al., 2001).

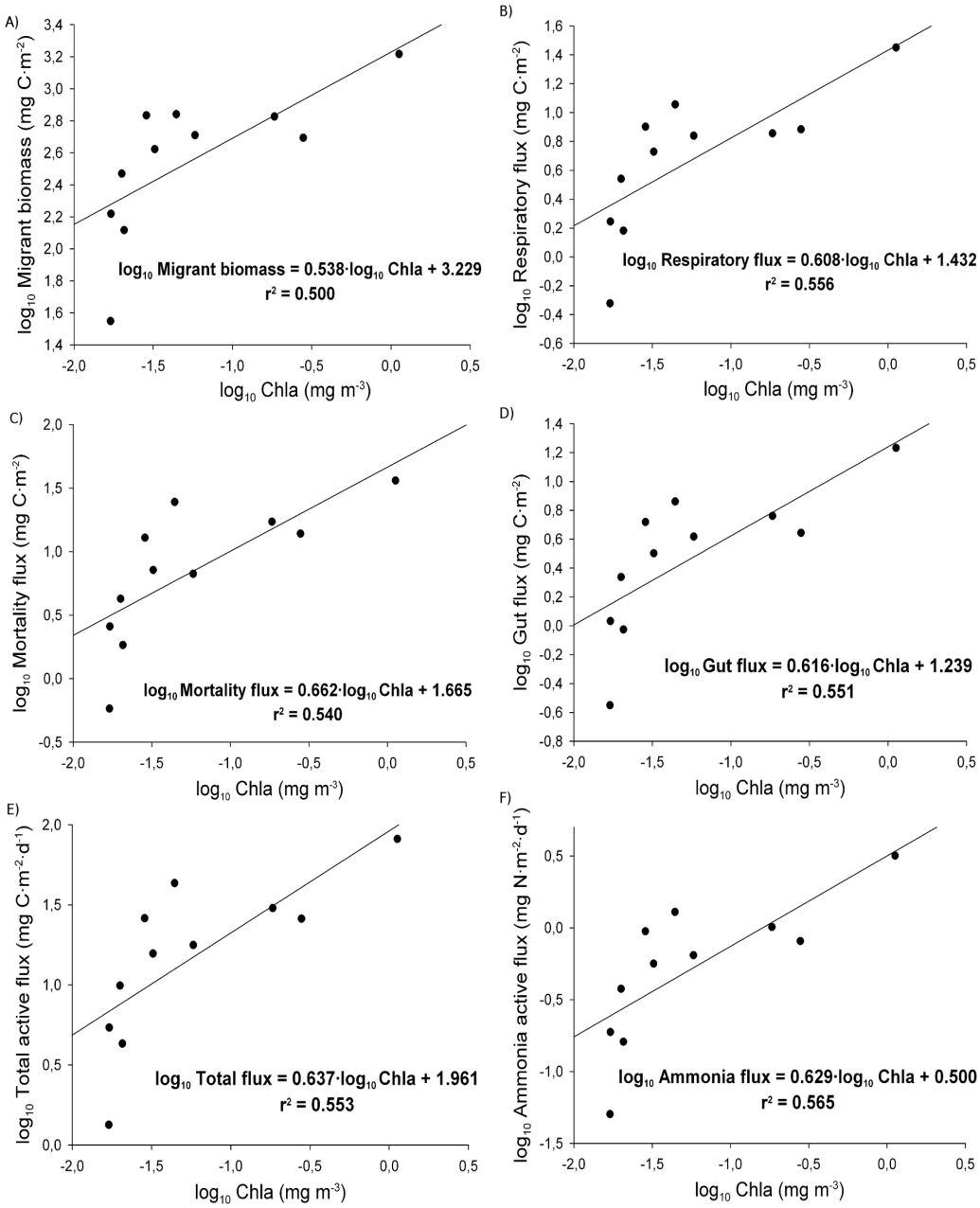

Figure 4.7. Correlations (log_{10}-log_{10}) between surface Chl*a* (*in situ*) and estimates of zooplankton migrant biomass (A), and assessments of the respiratory (B), mortality (C), egestion (D), total (E) and ammonia excretion (F) active fluxes through zooplankton to the mesopelagic layer (below 200 m depth).

Active fluxes through zooplankton (mortality, respiratory, egestion and ammonia excretion) matched the distribution of migrant biomass, rather than specific metabolic rates, in agreement with Huntley and Lopez (1992). These authors observed that, estimating community production of zooplankton, the variation of community biomass was one to three orders of magnitude larger than specific metabolic rates. In our case, this is attributed to the scarce variability observed on estimates of specific metabolism within the mesopelagic layer, homogeneously distributed along the cruise. In this respect, metabolic rates derived from predictive equations depended on body weight and temperature, both parameters showing little variability at depth during the cruise. Therefore, we highlight the importance of precisely estimate the community biomass of zooplankton, since it eventually determines the accuracy of active fluxes through these communities.

Larger organisms (>1000 μm) represented on average more than 93% of total migrant biomass along the cruise (Fig. 4.6), highlighting the relevance of this size class on DVM (*e.g.,* Hernández-León, 2001; Ariza et al., 2015; Isla et al., 2015). On the other hand, some authors argue that active fluxes are mainly driven by euphausiids (Stuckel et al., 2013; Isla et al., 2015). However, these organisms only dominated DVM around the equatorial and the CBU regions (Fig. 4.6). In fact, euphausiids were not certainly abundant along the cruise while copepods, which represented more than 80% of abundance, were the main drivers for export fluxes. It has been suggested elsewhere that predatory pressure (Stuart and Verheye, 1991; Frost and Bollens, 1992), especially from chaetognaths (Ohman, 1990; Irigoien et al., 2004; Isla et al., 2015), could induce this migrant behavior on copepods.

4.4.3 Carbon and nitrogen export: comparisons among studies

As active fluxes were directly dependent on the magnitude of migrant biomass, and the latter increased around the productive waters of both upwelling zones (mostly in the very productive waters of the CBU), downward export of zooplankton also increased in these zones in

comparison with the oligotrophic regions (Fig. 4.5a, b, c). Therefore, it seems that productivity could be a major factor determining active fluxes through zooplankton.

Contribution of mortality flux was larger than respiratory and gut flux, and mainly in the most productive regions, such as both upwelling zones (Fig. 4.5a). On this subject, despite the scarce number of studies addressing specific rates and active fluxes through mortality, some authors agreed that contribution of this mechanism to the biological pump might range in the order of the respiratory flux (Zhang and Dam, 1997) or being even greater (Hidaka et al., 2001) (see Table 4.2).

The geographical location, which determines the hydrographic and productive features of each region, seemed to mainly influence the magnitude of migratory fluxes, as also observed on the wide range of values found in the literature (Tables 4.2 and 4.3). As an example, our estimates of total carbon (R + M + G) exported below 200 m depth within the oligotrophic regions were 2-53% (2-42% in the case of migrant biomass) of those assessed in the eutrophic waters of the CBU (Table 4.2).

Comparing with other studies, our estimates of migrant biomass within the CBU waters (1649.9 mg C m^{-2}) were on the top of the range of values found in the literature, although most of the studies addressing active fluxes were carried out in oligotrophic waters. Alike, total carbon flux in this oceanic upwelling (81.7 mg C m^{-2} d^{-1}) exceeded estimates from the eutrophic waters of the California Current (66.9 mg C m^{-2} d^{-1}) (Stuckel et al., 2013), although our estimates were about 10% lower than those assessed by Steinberg et al. (2008) along the Subarctic North Pacific Ocean (91.6 mg C m^{-2} d^{-1}). However, comparisons are always difficult to evaluate since, unlike estimates from the California Current (which referred to merely the respiratory flux), we also included mortality and egestion pathways in our assessments of total flux. Conversely, our respiration estimates referred to dissolved inorganic carbon (DIC), while Steinberg et al. (2008) also included dissolved organic carbon (DOC) excretion in their assessments. In fact, assuming that DOC

excretion represents 24% of the carbon metabolized (excreted + respired) (Steinberg et al., 2000), our estimates of total flux increased to 90.6 mg C m^{-2} d^{-1}.

Regarding oligotrophic regions, our estimates of migrant biomass and the different mechanisms of the active carbon flux through zooplankton ranged the values found in most of the studies along similar regions, such as Hawaii, Bermuda or the Canary Islands waters (Table 4.2). Remarkably, estimates of respiratory flux from Dam et al. (1995) were, however, about 3.5-fold larger than the highest values observed here.

Concerning mesotrophic regions, our estimates of migrant biomass along the weak EU ranged measurements found in similar regions, such as the oligotrophic waters of the Canary Islands within eddy-induced enhanced production (Yebra et al., 2005b) and the Subarctic North Pacific (Takahashi et al., 2009; Kobari et al., 2013). However, the variability of migrant biomass in the latter region exceeded one order of magnitude among studies, and thus our estimates were more than two-fold higher than assessments of Kobari et al. (2008) and, at the same time, about 50% lower than values estimated by Steinberg et al. (2008). Regarding active fluxes, total export (R + M + G) below 200 m within the EU region was considerably higher in comparison with these studies. This might be explained through (1) they did not consider the three export mechanisms (R, M, G) in their calculations of total flux, and (2) our estimates of mortality flux were out of the range observed in some of these comparative studies (Table 4.2). The only exception was the three-fold higher total export estimated by Steinberg et al. (2008), probably as a direct consequence of the high values of migrant biomass they observed.

Regarding gut flux to the mesopelagic layer by zooplankton, the few studies covering this component were performed along oligotrophic regions. Therein, authors suggested a more than probable underestimation of their measurements, derived from the use of the GF method (Hernández-León et al., 2001, Yebra et al., 2005b; Putzeys et al., 2011). They assumed an

exclusive herbivorous feeding, despite it is well known that organisms in oligotrophic regions are mainly omnivores, with a reduced importance of pigmented food in their diet (*e.g.,* Saiz et al., 1999; Hernández-León et al., 2002). In this sense, larger animals (migrators) prey upon micro- and mesozooplankton to fulfill their physiological demands. Moreover, these authors assumed that organisms exclusively feed at surface layers, and retained their guts full until the mesopelagic. However, it is known that they may feed on aggregates (marine snow) or ungrazed phytoplankton sinking from surface layers while migrating down (Hernández-León et al., 2001). The organic content of this material can provide a rich food source for zooplankton (Alldredge et al., 1998), and there is also evidence of feeding during daytime at mesopelagic layers (Roger, 1973).

By opposite, we estimated total gut content (pigmented + non-pigmented food) to determine egestion rates and fluxes (see Methods section). Moreover, we determined theoretical gut content of organisms according to body size, independently if they feed or not at mesopelagic layers, reducing the bias in this regard. In any case, our measurements ranged the conservative values from previous studies (Table 4.2). As it is known that gut passage times of larger diel migrants considerably decrease when moving down to water of lower temperature and food concentration (Dagg and Walser, 1987; Portillo-Hahnefeld, 1995; Irigoien, 1998), they probably exceed the migration interval (*e.g.*, Wiebe et al., 1992). Therefore, we can presumably accept that these organisms can transport in their guts part of the ingested food at the surface to the mesopelagic layer, where they release fecal pellets during daytime.

Table 4.2. Comparisons among studies measuring zooplankton migrant biomass and active carbon fluxes (updated from Takahashi et al., 2009; Ariza et al., 2015). R, M and G refer to respiratory, mortality and egestion fluxes, respectively. Active fluxes referred to export into the mesopelagic layer (below 150-200 m, depending on the study). EU and CBU corresponded to stations located along the Equatorial upwelling in the Atlantic Ocean (EU) and the Cape Blanc oceanic upwelling, respectively. Ms: mesozooplankton, Mc: macrozooplankton, NC: *Neocalanus cristatus*, NF: *N. flemingeri*, NP: *N. plumchrus*, NT: *N. tonsus*. See Methods section for details on procedures used to estimate migrant biomass and active fluxes through zooplankton.

Location	Terms	Migrant biomass		Respiratory flux	Mortality flux	Gut flux	Total flux	Source
		(mg C m^{-2})	Components	(mg C m^{-2} d^{-1})	(mg C m^{-2} d^{-1})	(mg C m^{-2} d^{-1})	(mg C m^{-2} d^{-1})	
Pacific Ocean								
W. subtropical	R, G	98.5	Ms+Mc	1.7	-	0.5	2.2	Kobari et al. (2013)
W. subarctic gyre (K_2)	R, G	601.2	Ms+Mc	6.8	-	2.3	8.9	Kobari et al. (2013)
W. subarctic gyre (K_2)	R, M	558.2	Ms+Mc	5.7	0.2-2.1	-	5.9-7.8	Takahashi et al.(2009)
W. subarctic gyre (K_2)	R, M, G	78-263	Ms+Mc	1.5-6.4	0.5-1.6	2.0-8.6	4.0-16.6	Kobari et al. (2008)
W. Equator	R	47.2	Ms+Mc	3.8	-	-	3.8	Rodier & Le Borgne (1997)
Central Equator	R	52.8	Ms+Mc	7.9	-	-	7.9	Rodier & Le Borgne (1997)
E. Equator	R, M	96-155	Ms	4.2-7.3	2.9-5.4	-	7.1-12.7	Zhang & Dam (1997)
Hawaii	R	142.0	Ms+Mc	3.6	-	-	3.6	Al-Mutairy & Landry (2001)
W. subarctic gyre (K_2)	R, M, G	1280	Ms+Mc	23.5	-	-	31.1-91.6	Steinberg et al. (2008)
W. Equator	R, M	145-448	Ms+Mc	7.3-19.1	0.8-22.1	-	8.1-41.2	Hidaka et al. (2001)
Hawaii	R, M, G	158.0	Ms+Mc	-	-	-	3.7-13.6	Steinberg et al. (2008)
California Current	R	-	Ms+Mc	2.4-66.9	-	-	2.4-66.9	Stukel et al. (2013)
Ocean Weather (St. P)	R, M	-	NC+NF+NP	-	-	-	13.7	Bradford-Grieve et al. (2001)

Mediterranean Sea								
NW Mediterranean	R	-	Ms+Mc	7.2-28.8	-	-	7.2-28.8	Isla et al. (2015)
Atlantic Ocean								
Sargasso Sea	R	29	Ms+Mc	2.8-8.8	-	-	2.8-8.8	Longhurst et al. (1990)
Bermuda	R	82-536	Ms	6.2-40.6	-	-	6.2-40.6	Dam et al. (1995)
Bermuda	R	49-123	Ms+Mc	2.0-9.9	-	-	2.0-9.9	Steinberg et al. (2000)
Bermuda	G	0.7-468	Ms+Mc	-	-	0.007-4.5	0.007-4.5	Schnetzer & Steinberg (2002)
Canary Islands (island-induced eddies)	R, G	580-1280	Ms	1.9-8.3	-	0.1-0.4	2.0-8.7	Yebra et al. (2005b)
Canary Islands	R, G	125-248	Ms	1.9-4.3	-	0.3-2.4	2.2-6.7	Hernández-León et al. (2001)
Canary Islands (Sept-Oct)	R, G	108-342	Ms	0.5-1.4	-	1.2-3.4	1.7-4.8	Putzeys et al. (2011)
Canary Islands	R	107-412	Ms	1.1-4.5	-	-	1.1-4.5	Ariza et al. (2015)
Off NW Spain	R	-	Ms+Mc	1.2-30	-	-	1.2-30	Isla & Anadón (2004)
Bay of Biscay	R	-	Ms+Mc	6-28.8	-	-	6-28.8	Isla et al. (2004)
Subtropical	R, M	-	NT	-	-	-	4.7-25.5	Bradford-Grieve et al. (2001)
Oligotrophic	M	-	-	-	1.0-18.9	-	1.0-18.9	Peterson & Wroblewsk (1984)
Subtropical (oligot.)	R, M, G	35.4-693.8	Ms+Mc	0.5-11.4	0.6-24.6	0.3-7.3	1.4-43.3	**This study**
EU (mesotrophic)	R, M, G	513.3-682.6	Ms+Mc	6.9-7.2	6.7-17.2	4.2-5.8	17.8-30.2	**This study**
CBU (eutrophic)	R, M, G	1649.9	Ms+Mc	28.3	36.3	17.1	81.7	**This study**

Therefore, valid comparisons of zooplankton export below 200 m depth are difficult to analyze, as a consequence of the heterogeneity of studies, in terms of hydrographic conditions, the components analyzed (meso and/or macrozooplankton) or the mechanisms involved (respiration, defecation, mortality, ammonia excretion). Moreover, we point out the lack of studies addressing zooplankton active fluxes through quantitatively important pathways, such as mortality and ammonia excretion processes. More studies are therefore needed to better understand the contribution of zooplankton active fluxes to the biological pump in the ocean.

Table 4.3. Comparisons among studies addressing zooplankton migrant biomass (mg N m^{-2}) and ammonia export to the mesopelagic layer (below 150-200 m, depending on the study). Updated from Steinberg et al. (2002). EU and CBU corresponded to stations located along the Equatorial upwelling in the Atlantic Ocean (EU) and the Cape Blanc upwelling, respectively. See Methods section for details on procedures used to estimate migrant biomass and active fluxes.

Location	Migrant biomass (mg N m^{-2})	Migratory flux (mg N m^{-2} d^{-1})	Source
Pacific Ocean			
Equatorial (oligot.)	8	3.6	Le Borgne and Rodier (1997)
W. Equat. (oligot.)	9	3.6	Rodier and Le Borgne (1997)
Hawaii	32	0.2-1.6	Al-Mutairi and Landry (2001)
California Current	-	0.3-8.85	Stuckel et al. (2013)
Atlantic Ocean			
North Sargasso Sea	9	0.7	Longhurst et al. (1989)
Sargasso Sea (BATS)	20-133	0.8-5.3	Dam et al. (1995)
Sargasso Sea (BATS)	0-92	0-7.7	Steinberg et al. (2002)
Subtropical (oligot.)	3.6-70.8	0.1-1.3	**This study**
EU (mesotrophic)	52.4-69.6	0.7-1.0	**This study**
CBU (eutrophic)	168.3	3.2	**This study**

4.4.4 Zooplankton export through remote sensing: perspectives

According to the correlations observed between *in situ* surface Chl*a* data and migratory biomass and zooplankton active flux (Fig. 4.7), it seems that chlorophyll concentration influences their distribution and magnitude. In

addition, significant correlations were also observed in relation to *in situ* SST, thus developing predictive equations to approach zooplankton export along the warm Atlantic (Table 4.4), according to these parameters normally measured by remote sensing. Despite it is also possible to obtain primary production data from satellite, we consider the use of surface Chl*a* data more appropriated than measurements of the former, as primary production is based on empirical relationships that use (among other variables) Chl*a* and temperature data from satellites (*e.g.,* Dunne et al., 2007, and references therein), and thus do not represent a truly independent variable.

The highly significant correlations observed between active fluxes and surface Chl*a* and temperature are partly not surprising. Taking into account that active fluxes depended on (migrant) biomass, Irigoien et al. (2014) recently remarked that global biomass of mesopelagic fish is mainly determined by primary production rates. Therefore, the possibility of modeling zooplankton export at the large-scale through remote sensing data could be afforded in a near future. Despite our predictive equations were simply set using results from the tropical and subtropical Atlantic Ocean (10ºS-25ºN), the design of the sampling plan allowed estimating active fluxes through a large gradient of productivity and temperature (Fig. 4.1). As a consequence, our results covered the whole range of active fluxes and zooplankton migrant biomass found in the literature, giving certain robustness to our results.

However, we remark the need to develop more complex and robust models to provide accurate estimates at large spatial scales. Thus, special attention should be taken at particular regions, such as coastal and upwelling waters or oxygen minimum zones (OMZs), where the use of these simple equations could be inappropriate. Due to hypoxic conditions in the water column, organisms in OMZs showed reduced metabolism as an adaptation mechanism (Kiko et al., 2015a, 2015b), and therefore lowering the magnitude of migratory fluxes in relation to similar latitudes. In this respect, specific work should be carried out, specially taking into account that OMZs occupy approximately 9% of the ocean's surface (Fuenzalida et al., 2009),

highlighting the importance of these regions on approaches of zooplankton export at a global scale. Nevertheless, our main purpose is to illustrate the potential use of remote sensing to regularly explore the role of zooplankton in the biological pump.

Table 4.4. Multiple linear (mechanistic) regressions between surface Chl*a* and temperature data (in situ), and zooplankton migrant biomass, and active fluxes to the mesopelagic layer (below 200 m depth) through respiration (R), mortality (M), egestion (G) and ammonia excretion processes along the warm Atlantic. Regression for total carbon flux (R + M + G) was also estimated.

	Stepwise multiple linear regression $a + b[\log_{10} \text{Chl}a] + c \cdot SST$				
	n	**a**	**b**	**c**	**r^2**
$\log_{10}$ Migrant biomass (mg C m^{-2})	11	4.269	0.161	-0.061	0.600
$\log_{10}$ Respiratory flux (mg C m^{-2} d^{-1})	11	2.744	0.127	-0.078	0.688
$\log_{10}$ Mortality flux (mg C m^{-2} d^{-1})	11	3.410	0.022	-0.104	0.731
$\log_{10}$ Gut flux (mg C m^{-2} d^{-1})	11	2.565	0.132	-0.079	0.683
$\log_{10}$ Total flux (mg C m^{-2} d^{-1})	11	3.494	0.075	-0.091	0.716
$\log_{10}$ Ammonia flux (mg N m^{-2} d^{-1})	11	1.836	0.140	-0.079	0.695

Conclusions

Our results provide the first assessment of the active downward export of dissolved nitrogen and carbon, mediated by zooplankton DVM, at the basin scale along the tropical and subtropical Atlantic Ocean (10ºS-25ºN). We highlight the prominent role of zooplankton in the biological pump since our assessments were, in some cases, higher than in previous studies where

active fluxes exceeded the passive flux. We explored a wide latitudinal gradient, and as a consequence, our estimates covered the whole range of values of zooplankton active flux and migrant biomass found in the literature. The magnitude of mortality, egestion and ammonia excretion fluxes indicated that these processes should not be disregarded in studies addressing zooplankton downward export. As previously reported by Dam et al. (1995), we observed that active fluxes were entirely dependent on the magnitude of migrant biomass. On the other hand, zooplankton active fluxes were highly correlated with *in situ* surface chlorophyll and temperature data, and simple models based on predictive equations were then developed. Data from these variables may be regularly obtained from satellite measurements. In this sense, here we show the possibility of using remote sensing to regularly explore zooplankton active fluxes at large spatial scales in a near future. In any case, we consider that more complex models are needed in order to obtain accurate estimates. More studies (including gravitational flux) are equally necessary to increase the geographical coverage of models, while specific work should be carried out on certain regions (*e.g.,* coastal and upwelling waters, regions of high mesoscale activity, OMZs, etc.).

Acknowledgements

This research was conducted under the Mafia project, funded by the Spanish Ministry of Economy and Competitiveness (CTM2012-39587-C04). Thanks are due to all colleagues who contributed to acquire the data during the cruise, especially Jose Varela, Iñaki Franco and Maite Álvarez who performed a hard work on board to obtain the zooplankton samples. Also the UTM team of the "Hespérides" who developed an excellent technical assistance with the MOCNESS net. Mikhail Emelianov provided the physical data and Alejandro Ariza helped with the processing of measurements from the ship's thermosalinometer and fluorometer. We are also grateful to Fabien Lombard, who contributed to improve the ecophysiological correlations presented in this work, and Lars Stemmann

who welcomed me on his lab, enabling the development of part of this study. Juan Carlos Garijo was supported by a postgraduate grant (FPU-AP2010-5080) from the Spanish Ministry of Education.

Synthesis and future research

I. General discussion

I.1 IBS as alternative methodology

Results achieved during the present thesis suggest that IBS may be as reliable as traditional and enzymatic methods to ascertain zooplankton community biomass and metabolic fluxes. Estimates were not significantly different (see *Chapters 1 and 3*), although the former resulted in a faster and inexpensive process. Organisms are individually measured and classify into taxonomic groups in a semi-automated process (*e.g.,* MacLeod et al., 2010; Gorsky et al., 2010; Bachiller et al., 2012). This allows working on studies addressing size spectra distribution and the related ecological implications. Furthermore, using empirical relationships and predictive equations, body weight and metabolism may be also achieved for each individual separately. In addition, samples are not destroyed, resting available for further analysis. As a consequence, it is expected a growing application of these systems on studies covering large regions and temporal scales in a near future (*e.g.,* Benfield et al., 2007; Gislason and Silva, 2009; Gorsky et al., 2010; Di Mauro et al., 2011; Vandromme et al., 2012, 2013; Basedow et al., 2014).

However, equations or empirical relationships used to estimate metabolism and body weight seem to be of paramount importance to accurately use the IBS (Garijo and Hernández-León, 2015). Thus, we observed in *Chapter 3* that growth and respiration of zooplankton are probably more accurately estimated when metabolic equations are fitted to the environmental conditions of each region, instead of using generalist equations for the global ocean (see Section I.3 for further discussion). In the case of biomass, Lehette and Hernández-León (2009) developed empirical relationships between body area and body weight showing rather high coefficients of determination (r^2>0.80, Table 1.1). Besides, as they established their relationships according to individuals that were frozen before being digitized and weighed, they avoided the problem of weight-loss normally observed in planktonic organisms when preserved with formaldehyde (*e.g.,* Omori, 1970; Durbin and Durbin, 1978; Landry, 1978). On the other hand, no shrinkage effect of organisms has been previously detected using this chemical as

preservative (Landry, 1978; Durbin and Durbin, 1978; Viitasalo, 1995; Pollupüü, 2007). Therefore, IBS using this kind of relationships based on the area of individuals could be eventually considered as a reliable alternative to assess community biomass of zooplankton (*Chapter 1*).

Additionally to use these systems to study growth and respiration of zooplankton, we also proposed IBS to assess egestion rates of these communities in a faster procedure (*Chapter 1*). Our developed relationship between body weight and gut content of organisms, combined with that given by Irigoien (1998) relating temperature and gut evacuation rates, led to realistic results in comparison to previous estimates using the *gut fluorescence* method (*e.g.,* Hernández-León et al., 2004, 2007) (see Fig. 1.7). As expected, estimates derived from the *gut fluorescence* method –based exclusively on measurements of pigmented food– were lower, as organisms in oligotrophic regions are normally omnivores (*e.g.,* Saiz et al., 1999; Hernández-León et al., 2001, 2001). Based on reliable assessments of growth, respiration and egestion of zooplankton through IBS, we suggested this methodology as an alternative to estimate community ingestion fluxes mediated by these communities along large spatial scales (see Fig. 1.8).

Nevertheless, based on results from *Chapter 1*, we suggest that enzymatic methods might be more appropriated to assess specific metabolic rates of zooplankton, since these methods seemed to better represent the variability of specific rates along a sampling period. Although it is known that ETS and AARS methods suffer from the uncertainty to approach respiration and growth rates respectively (Hernández-León et al., 1995; Hernández-León and Gómez, 1996), estimates using these methods were assumed as valid references. This allowed to test the suitability of different predictive equations and methodologies according to comparisons between two random variables (linear regression type II).

I.2 Physical-biological coupling on mesoscale structures

Results from *Chapter 2* showed that IBS might be also useful to determine community biomass and metabolism of zooplankton within regions with a high mesoscale activity. Strikingly, we observed a physical-biological coupling along the *Coastal Transition Zone* (CTZ) off Northwest Africa, since mesoscale structures clearly influenced the three-dimensional distribution of organisms in terms of size and biomass, and influenced their metabolism, normally following the physical signatures.

We observed that coastal filaments and the upwelling jet along the CTZ exported organic matter from the upwelling waters to the oligotrophic region near the Canary Islands, as previously observed by *e.g.,* Hernández-Guerra et al. (1993) and Basterretxea and Arístegui (2000) for Chl*a*, Baltar et al. (2009) for nano- and picoplankton, Hernández-León et al. (2002) for zooplankton, and Bécognée et al. (2009) and Moyano et al. (2014) for ichtyoplankton. Moreover, interactions between these filaments and island-induced eddies increased the biomass of zooplankton within the latter structures, as previously reported by Hernández-León et al. (2002). The deepening or elevation of isotherms, depending on cyclonic or anticyclonic eddies, influenced the vertical distribution of organisms (see Fig. 2.3 and 2.4). Individuals were normally concentrated at deeper layers on anticyclonic eddies, in agreement with Arístegui et al. (1997) for Chl*a* and bacteria. Conversely, the opposite pattern was observed on cyclonic structures, where these authors suggested that isotherm elevation and nutrient pumping into the sunlit layers could enhance biomass at the surface. Additionally, as metabolism primarily depends on temperature (*e.g.,* Hirst and Lampitt, 1998; Hirst et al., 2003), deepening or elevation of isotherms affected growth, respiration and egestion of individuals within these structures (see Fig. 2.7).

Frontal systems may also influence the distribution of organisms. As observed in *Chapter 2*, the front generated between the cold and productive waters of the coastal upwelling, and the warmer and oligotrophic waters to the ocean, acted as a natural barrier retaining Chl*a* and zooplankton (see Fig.

2.5), as previously observed by Moyano et al. (2014) for ichtyoplankton in the same frontal system.

Export of zooplankton from the upwelling region to the vicinities of the Canary Archipelago, and their retention in fronts and eddies, might eventually influence local fisheries, since zooplankton constitute the natural food for fish larvae. However, further studies are needed to fully understand possible effects. Nevertheless, productivity of the upwelling is not constant throughout the year. Comparing our results (weak upwelling season) and those from Hernández-León et al. (2002) during the most productive period, it seems that seasonality is the most important factor determining the capacity of mesoscale structures to export zooplankton towards the ocean.

I.3 Specific metabolic equations for specific regions

Estimates of zooplankton metabolism from enzymatic methods were better correlated with measurements obtained using the temperature-specific equations developed in this thesis (*Chapters 1 and 3*) respect to those from generalist equations for the global ocean, previously developed by Hirst et al. (2003), Hirst and Bunker (2003) and Zhou et al. (2010) for growth, and Ikeda (1985, 2014) for respiration. Comparisons were ascertained along the subtropical waters of the Canary Islands and the upper 2000 m depth of the 40ºN-40ºS region, according to a wide range of temperatures (2.5–30ºC) and productivity (oligo-, meso- and eutrophic). Hence, these specific equations could be helpful to assess metabolism of zooplankton along the epi-, meso- and bathypelagic layers of a large latitudinal band of the ocean.

As metabolism of zooplankton seems mainly determined by habitat temperature and body weight (*e.g.,* Ikeda et al., 2014), we reasonably developed different equations according to ranges of values of these parameters in order to increase the accuracy of metabolic estimates (see Tables 3.2 and 3.3). Hence, specific equations developed in *Chapter 3* were fitted to the specific ranges of temperature of the main ocean regions and depth layers found along the Malaspina cruise, in agreement with the average

values given by Longhurst (2007) for the main geographical provinces. Additionally, estimates of body weight assessed during the Malaspina circumnavigation were used as reference to establish mesozooplankton ranges of this parameter.

The accuracy (r^2) of our temperature-specific equations increased in many cases respect to those of previous relationships for the global ocean. However, it is still imperative the need to improve some relationships, as it is the case of equations predicting growth for copepods, due to their ubiquity and abundance in the global ocean. Besides, the influence of food quantity and quality, as well as other parameters such as depth or oxygen concentration, on metabolic rates still needs to be more profoundly investigated in order to develop more appropriated and accurate equations.

According to the results, both temperature-specific and generalist equations might be considered as a useful alternative to measure zooplankton metabolism on large spatial (geographical and vertical) and temporal scales. In fact, in the case of respiration, estimates from both kinds of equations matched the global distribution and ranged the values given by Hernández-León and Ikeda (2005). However, it is true that growth and respiration assessments using generalist equations were significantly lower than measurements from enzymatic methods and specific equations. Hence, it seems that the latter equations could be more accurate depending on the conditions of each environment.

This could be due to different reasons. For example, the use of extreme data (-2.0ºC > T > 30ºC) to configure the generalist equations given by Hirst et al. (Hirst et al., 2003), Hirst and Bunker (Hirst and Bunker, 2003) and Ikeda (Ikeda, 1985, 2014) could introduce considerable bias in certain regions, as it is known that temperature highly influences metabolism: it increases with temperature and vice versa (*e.g.,* Hirst and Lampitt, 1998; Hirst et al., 2003). In turn, the Chl*a* values managed by Hirst and Bunker (2003) and Zhou et al. (2010) as references to configure their equations were up to two orders of magnitude greater than usually found in oligotrophic waters (Garijo and

Hernández-León, 2015), therefore suggesting the suitability of these equations for more eutrophic systems. Moreover, as zooplankton in these regions are mainly omnivores (*e.g.,* Saiz et al., 1999), it seems that using Chl*a* as a food *proxy* of the availability and quality of food resources could result inappropriate. Considering that oligotrophic regions constitute the major fraction of the global ocean, we should be especially cautious when using this kind of equations on global assessments (*e.g.,* Stock et al., 2014), since they could underestimate production on these vast regions of the ocean. Finally, inclusion of data from individuals larger than mesozooplankton could also introduce some bias, since metabolism decreases with body weight (*e.g.,* Hirst and Lampitt, 1998). In this sense, estimates of growth using the Hirst et al. (Hirst et al., 2003) equations could be underestimated, as these relationships were configured using data from organisms up to two orders of magnitude greater than usually given for mesozooplankton (Garijo and Hernández-León, 2015).

Hence, the temperature-specific equations developed in this thesis could represent an alternative to enzymatic methods to assess zooplankton community fluxes at the large-scale. Besides, the accuracy of estimates using this kind of equations is expected to be higher respect to using generalist equations for the global ocean. However, there are still many constraints to solve. We claim for the need to set up more taxonomically-specified equations, while specific work should be carried out on particular regions, such as upwelling and coastal waters or OMZs. In the latter, it seems that organisms adapted their metabolism to the existing critical levels of oxygen (Kiko et al, 2015a; Kiko et al, 2015b), and consequently equations should be adapted in this regard.

I.4 Contribution of zooplankton to the biological pump

Our estimates of zooplankton active fluxes from *Chapter 4* have contributed to increase the number of existing assessments of this important component of the biological pump. Results from this thesis roughly covered the whole range of values for migrant biomass and active fluxes existing in the

literature (see Table 4.2). This was probably due to the high heterogeneity of regions explored in terms of productivity and temperature (see Fig. 4.1). Thus, assessments on the Cape Blanc oceanic upwelling region were on the top of the range of values found in the literature, while the opposite was observed along the oligotrophic region south the Equator. In this respect, it seems that the geographical location, which determines the hydrographic features, is the factor mainly influencing migratory fluxes.

This thesis contributed to reduce the evident lack of studies addressing active fluxes through mortality, egestion and ammonia excretion of zooplankton (see Tables 4.2 and 4.3). We highlight the importance of these mechanisms in relation to their contribution to the biological pump, and the need to be included on assessments of total zooplankton export. Thus, mortality fluxes, often not taken into account when estimating zooplankton migratory fluxes, could be even greater than respiratory fluxes (see Fig. 4.5), as already observed by Zhang and Dam (1997) and Hidaka et al. (2001). Many authors have pointed out the importance of active fluxes through zooplankton, as they even exceeded passive flux in some cases (*e.g.,* Lampitt et al., 1993; Dam et al., 1995; Hidaka et al., 2001; Schnetzer and Steinberg, 2002; Steinberg et al., 2002; Kobari et al., 2013; Stuckel et al., 2013). On this subject, despite we were not able to measure gravitational flux, our assessments of zooplankton total export were, in some regions, higher than in other studies from similar environments where active fluxes were comparable or even higher than passive flux.

Comparisons of active fluxes among regions are always difficult, since their magnitude depends on the components included in the calculations (meso-, macrozooplankton) and the mechanisms involved (respiration, mortality, egestion, ammonia excretion). Besides, results are also influenced by factors such as the depth layer considered for export calculations (150, 200, 300 m, etc.), how migrant biomass is assessed or the methods employed to estimate zooplankton metabolism. In this regard, we consider that methodologies and procedures to assess zooplankton active fluxes should be standardized.

Nevertheless, we highlight the importance of migrant biomass in the magnitude of zooplankton downward export, in agreement with Dam et al. (1995). In this sense, we observed that active fluxes clearly matched the distribution of migrant biomass, instead of that of specific metabolic rates (see Fig. 4.4 and 4.5). This agreed with observations of Huntley and Lopez (1992), who suggested community biomass as the main factor determining production rates of zooplankton. As a consequence, the influence of surface chlorophyll concentration on zooplankton migratory biomass determined that zooplankton export was equally influenced by Chl*a*. Hence, we point out the importance of accurately determining the migrant biomass of these communities when assessing migratory fluxes, despite important bias may be introduced depending on the way we estimate this parameter (see Section 4.4.1).

Hence, our assessments also support the idea that zooplankton active flux is one of the main mechanisms involved in the export of organic matter to the mesopelagic layer. The contribution of these communities to the biological pump may be comparable or even greater than passive flux (Dam et al., 1995; Hidaka et al., 2001; Steinberg et al., 2002, 2008; Stuckel et al., 2013). Therefore, we consider that more studies addressing this important component, including mortality, egestion and ammonia excretion mechanisms, should be carried out in order to reduce our present uncertainty of the biological pump in the ocean.

I.5 Exploring zooplankton by remote sensing

We observed strong correlations between zooplankton and environmental data easily available from remote sensing (*Chapter 4*). Thus, migrant biomass and active fluxes through zooplankton were correlated with *in situ* surface temperature and chlorophyll concentration along the tropical and subtropical Atlantic Ocean. According to these correlations, simple models based on these two variables were developed (see Table 4.4). In this respect, Irigoien et al. (2014) also observed that global biomass of mesopelagic fish was mainly determined by the distribution of primary production. Despite

our results only referred to a transect in the Atlantic Ocean, the sampling program allowed to explore a wide latitudinal gradient, highly heterogeneous in terms of productivity and temperature (see Fig. 4.1), increasing the robustness of our results. In any case, it is obvious that more complex models combining much more variables should still be developed. In this regard, many researchers are already working on this aspect, as they try to understand all possible relationships between export fluxes and data of the ocean's surface obtained through current technology (*e.g.,* EXPORTS, http://exports.oceancolor.ucsb.edu).

Nevertheless, our main intention is to illustrate the future potential of using remote sensing to regularly assess the role of zooplankton in the biological pump in the ocean. The required data may be relatively easy acquired, allowing exploring remote regions, otherwise too expensive and challenging to sample directly. In any case, there are still many constraints to solve. In addition to developing more sophisticated models, the accuracy of metabolic equations should be improved, while more relationships for more taxonomic levels are still needed (see Section I.3). Moreover, as occurs with equations modeling in particular regions, such as OMZs occupying 9% of the ocean's surface (Fuenzalida et al., 2009), coastal and upwelling systems or regions with high mesoscale activity, will require specific work. In this respect, global data compilations for prominent environmental factors within the water column (*e.g.,* temperature, dissolved oxygen, phosphate and nitrate concentration, salinity, etc.), such as that of the *World Ocean Atlas* (WOA, https://www.nodc.noaa.gov/OC5/SELECT/woaselect/woaselect.html) of the *National Oceanic and Atmospheric Administration* (NOAA), might be helpful to reduce the uncertainty of the estimates obtained from models only using remote sensing data.

II. Conclusions

In general, the main conclusions arising from the research conducted during the present thesis are:

1. Image-based systems (IBS) are as reliable as traditional and enzymatic methods to ascertain community biomass and metabolic fluxes of zooplankton along large spatial and temporal scales, although the former resulted in a faster and inexpensive process. In turn, enzymatic methods could be a better alternative to assess specific metabolic rates along temporal-series.

2. The proposed relationship between body weight and gut content of organisms, in combination with an IBS, is a helpful tool to assess egestion rates of zooplankton in a relatively rapid procedure.

3. Mesoscale structures in the *Coastal Transition Zone* off Northwest Africa determined the three-dimensional distribution of zooplankton biomass and metabolism. This led to a physical-biological coupling, where zooplankton followed the signatures of physical structures. As these communities represent the natural feeding for fish larvae, export of zooplankton through filaments from the upwelling waters to the vicinities of the Canary Archipelago might eventually influence local fisheries. Nevertheless, seasonality seems to be the most important factor determining the export extent.

4. The temperature-specific metabolic equations for zooplankton respiration and growth developed in this thesis, fitted to the conditions of each region, increased the accuracy of metabolic flux estimates using an IBS respect to existing global equations. However, more relationships addressing current constraints are still required.

5. Our estimates of zooplankton migrant biomass and active fluxes along a wide latitudinal gradient through the tropical and subtropical Atlantic Ocean covered the entire range of assessments found in the literature.

Moreover, zooplankton export fluxes were highly correlated with surface temperature and chlorophyll concentration, and their prediction through remote sensing could be considered in a near future.

III. Future research

Due to certain limitations of traditional methodologies to estimate zooplankton biomass and metabolic fluxes along large spatial scales, the research conducted during this thesis aimed to develop and test alternative tools to approach these parameters in a faster and inexpensive way. However, certain methodological limitations should still be solved in a near future. Although some of the constraints have been already addressed along the previous chapters, it might be convenient to compile the main limitations detected during the present research, and providing some guidance in this regard:

1. IBS seem to constitute an alternative to measure community biomass and metabolic fluxes of zooplankton along large spatial scales. However, one of the main limitations facing the IBS at the present is their inability to classify organisms into more detailed taxonomic levels, and the associated error to the automated classification process. As a solution, the recently-developed software *Ecotaxa* (http://ecotaxa.sb-roscoff.fr) allows the visual exploration of images after automated classification, enabling correcting the errors made by the software or classifying individuals into more detailed taxonomic levels. The compromise between time, effort and accuracy will always depend on the particular objectives of each study. Hence, using this kind of software in future studies will help to reduce the present error associated to taxonomy, while more detailed categorization will be achieved. Based on more precise classifications, estimates of biomass will be also more accurate, since different relationships are used to convert body area into body weight for each taxonomic group.

2. As previously referred in *Chapters 1* and *3*, although temperature-specific metabolic equations for zooplankton developed during this thesis, fitted to the specific conditions of each study region, are expected to increase the accuracy of growth and respiration estimates respect to generalist

equations, they still need to be refined. The accuracy (r^2) of growth equations for copepods is rather low, while the abundance of these organisms normally represents more than 80% in all oceanic environments. Additionally, the inclusion of other variables (*e.g.,* oxygen concentration, habitat depth, etc.) should be further studied. In this sense, specific equations predicting metabolism of zooplankton on particular regions should be investigated. As discussed in *Chapter 3*, organisms inhabiting the water column of *oxygen minimum zones* seem to reduce their metabolism as a response to the lack of oxygen, in comparison with communities with similar conditions of temperature and productivity. On the other hand, zooplankton in eutrophic waters such as upwelling and coastal regions, could present different metabolic rates than individuals in less productive waters. In this respect, the influence of food (quantity and quality) on zooplankton metabolism should be more deeply investigated. Finally, as metabolism differs between taxonomic groups, we also claim for the need to develop more taxonomically-specified equations, in order to accurately estimate zooplankton-mediated fluxes.

3. According to results showed in *Chapter 4* it is highlighted the important contribution of zooplankton to the biological pump in the ocean. In this sense, assessments of migrant biomass and active flux from this thesis could result helpful, since they roughly covered the whole range of values found in the literature. Moreover, valuable estimates of zooplankton export through mortality, egestion and ammonia excretion processes were also ascertained, highlighting the importance of addressing studies including these mechanisms, normally disregarded. However, comparatives of active fluxes among different studies are rather difficult as a consequence of the variety of methods and processes existing to estimate migrant biomass and metabolism (see Section I.4). In this regard, we consider of paramount importance the standardization of methodologies and processes to estimate active fluxes.

4. The observed correlations between zooplankton and environmental data easily obtained from remote sensing could illustrate future possibilities of exploring zooplankton downward export at large spatial scales (*Chapter 4*). Nevertheless, it is evident that more complex and robust models will be required. In this sense, the influence of all variables available from remote sensing should be further investigated. Finally, as occurs with metabolic equations, the development of this kind of models will require specific work on certain regions (*e.g.,* OMZs, upwellings, coastal environments, high physical activity regions, etc.), where specific conditions are given.

5. Increasing the spatial coverage of zooplankton sampling to explore correlations with environmental data at larger scales will require the use of equipment saving time and effort respect to current methodologies. In this sense, the use of modern devices, such as the *Underwater Vision Profiler* (UVP, http://www.hydroptic.com/uvp.html, Picheral et al., 2010), allows image acquisition of zooplankton organisms greater than 100 μm directly in the water column, and up to 6000 m depth. These systems, attached to oceanographic instruments currently used, such as the rossete sampler, enable acquiring zooplankton images while measuring physical-chemical components, saving the time normally dedicated to sampling nets. On this subject, *UVP* systems also avoid time-consuming steps between net sampling and digitalization of organisms, since images are directly available to be processed by the IBS immediately after sampling. Furthermore, these systems also allow exploring the distribution of organisms in the water column according to a resolution of centimeters, providing valuable information.

Resumen en español
(Spanish summary)

I. Introducción general

I.1 Antecedentes

I.1.1 El papel del zooplancton en la bomba biológica y el ciclo del carbono

El zooplancton juega un papel fundamental en los ciclos biogeoquímicos en el océano, pues ocupa una posición central en la cadena trófica. Consume materia orgánica para satisfacer sus demandas energéticas, reciclando y redistribuyendo nutrientes y materia orgánica, no sólo a diferentes niveles de la cadena alimenticia, sino también horizontal y verticalmente en la columna de agua (Banse, 1995). Por tanto, resulta un componente clave de la bomba biológica en el océano. Ésta constituye una compleja red de procesos fisiológicos y ecológicos que provocan un ciclo de materia en la columna de agua y un transporte de dióxido de carbono atmosférico hacia el océano profundo (Véase Fig. I.1). Brevemente, el fitoplancton fija el carbono inorgánico de la atmósfera por medio de la fotosíntesis, que a su vez es ingerido por el zooplancton. Estos últimos, además, consumen y producen materia orgánica particulada (POM) y liberan nutrientes orgánicos e inorgánicos por medio de procesos de excreción, los cuales pueden ser reutilizados por el fitoplancton en las capas superficiales. Paralelamente, el bucle microbiano constituye una vía de regeneración de nutrientes, pues emplea además POM y materia orgánica disuelta (DOM) para satisfacer sus demandas.

El desequilibrio causado por los productores en el sistema de carbono atmosférico puede remineralizarse de nuevo vía respiración a través de los consumidores epipelágicos (Del Giorgio y Duarte, 2002), o bien ser transportado a la capa mesopelágica por medio de diversos mecanismos. A este respecto, las partículas de materia orgánica pueden conformar agregados, que pueden hundirse gravitacionalmente a capas más profundas, conformando el denominado "flujo pasivo". Adicionalmente, los nutrientes disueltos y la materia orgánica pueden ser transportados activamente a la capa mesopelágica por medio de la migración zooplanctónica (*e.g.,*

Longhurst et al., 1990, Zhang y Dam, 1997, Steinberg et al., 2000). Finalmente, procesos físicos, como la difusión y la mezcla vertical (*e.g.*, Arístegui et al., 2003), pueden igualmente contribuir al carbono orgánico e inorgánico (DOC y DIC) exportado. En conjunto, estos procesos constituyen la bomba oceánica de carbono.

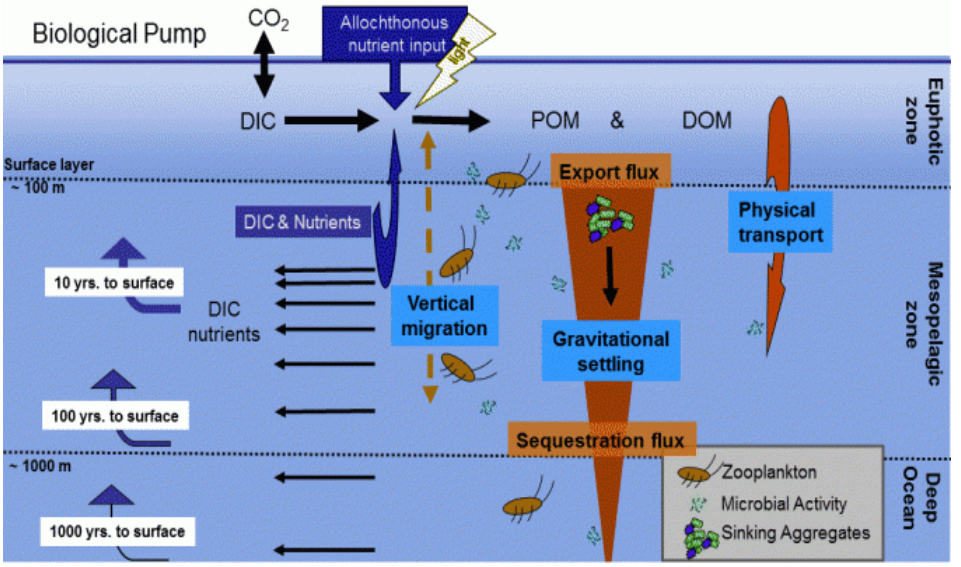

Figura I.1. Esquema de la bomba oceánica de carbono. Extraído de Passow y Carlson (2012).

Llama la atención que sólo un tercio del carbono que se exporta hacia capas profundas se atribuye a procesos físicos (Passow y Carlson, 2012), subrayando por tanto la importancia de los flujos activo y pasivo. A pesar de que éste último ha recibido mucha más atención (*e.g.,* Buesseler et al., 2007), estudios llevados a cabo durante las últimas décadas muestran que el flujo activo por medio del zooplancton migrante puede explicar una importante porción (en el rango del flujo pasivo en algunos casos) del carbono total exportado (*e.g.,* Longhurst et al., 1990; Steinberg et al., 2000, 2008; Kobari et al., 2013).

En este sentido, se sabe que los flujos de producción, respiración o egestión mediados por el zooplancton dependen principalmente de la biomasa comunitaria. Así, Huntley y Lopez (1992) observaron que la variabilidad de ésta última era entre uno y tres órdenes de magnitud superior a la de las tasas metabólicas específicas. Por su parte, la migración vertical del zooplancton ocurre en todos los ambientes marinos y es probablemente uno de los mayores movimientos de biomasa en el océano. Los organismos migran cada día para alimentarse en la capa epipelágica por la noche, y vuelven al amanecer a capas más profundas (Lampert, 1989), donde pueden excretar parte del nitrógeno y liberar carbono por medio de procesos tales como la mortalidad, la respiración o la defecación. Por tanto, parece claro el importante papel que juegan estas comunidades en el ciclo del carbono en el océano.

Durante las últimas décadas se han llevado a cabo estudios centrados en el flujo activo por medio de la respiración zooplanctónica (y de la mortalidad en menor medida) (*e.g.,* Zhang y Dam, 1997; Al-Mutairy y Landry, 2001; Hidaka et al., 2001; Takahashi et al., 2009; Stuckel et al., 2013), remarcando su importancia con respecto al flujo pasivo. Sin embargo, es cierto que ha recibido menor atención el proceso conocido como "flujo de tracto digestivo". Parte del alimento no asimilado, ingerido en la capa epipelágica, puede ser transportado en el tracto digestivo de los organismos migrantes mientras se mueven a capas más profundas, donde finalmente procesan la totalidad o parte del material ingerido, liberando POM durante el día (Angel, 1989). A pesar de que se sabe que el método de *fluorescencia del tracto digestivo* (Nemoto, 1968; Mackas and Bohrer, 1976) subestima este flujo en organismos omnívoros, y que las estimaciones presentan cierto error en relación a varias asunciones, algunos autores han remarcado la importante contribución del flujo de tracto digestivo al carbono exportado total (Lampitt et al., 1993; Hernández-León et al., 2001; Schnetzer y Steinberg, 2002; Yebra et al., 2005b; Kobari et al., 2008; Putzeys et al. 2011; Kobari et al., 2013). Lo mismo ocurre con el flujo activo de excreción de amonio. A pesar de que algunos estudios han señalado su importancia (Dam et al., 1995; Steinberg et al., 2002; Stuckel et al., 2013), superando en algunos casos

la magnitud del flujo pasivo, el nitrógeno exportado a través de la migración del zooplancton ha sido, de hecho, raramente estudiado.

En la actualidad, los océanos se han convertido en uno de los mayores protagonistas en el escenario de cambio climático. Teniendo en cuenta que el CO_2 es el máximo responsable del calentamiento global, es de destacar que los océanos contienen cerca de 50 veces más carbono que la atmósfera. Consecuentemente, los modeladores que se dediquen a estimar balances de carbono a nivel global requerirán un mayor conocimiento de la bomba biológica y, por tanto, de la participación del zooplancton en la misma, así como en los ciclos de carbono en el océano (Usbeck et al., 2003). En definitiva, la realización de estimaciones precisas de la biomasa y de los flujos metabólicos del zooplancton a escala global resultan de suma importancia a este respecto.

I.1.2 Limitaciones de la metodología actual de biomasa y metabolismo

Las estimaciones de la biomasa del zooplancton son relativamente fáciles de abordar en comparación con las determinaciones metabólicas. Sin embargo, la metodología estándar, basada en medidas del peso seco de los individuos (Lovegrove, 1966), ha quedado desfasada. Con dicho proceso no es posible obtener estimaciones de biomasa ni taxonómica ni individualmente, mientras que las muestras son destruidas, imposibilitando su reutilización para estudios futuros.

Por su parte, los métodos clásicos para estudiar la fisiología del zooplancton, como es el caso de las incubaciones, consumen mucho tiempo y son procesos muy tediosos, imposibilitando su aplicación a escalas espaciales grandes. Además, presentan otras limitaciones, como es la relación del número óptimo de individuos incubados, el estrés de los animales capturados, crecimiento de bacterias, inanición (Ikeda et al., 2000) o la baja concentración de organismos por debajo de la zona eufótica (Yebra et al., 2005b), remarcando así la dificultad de estimar el metabolismo en la zona profunda del océano. Durante las últimas décadas se han desarrollado otros

métodos para estimar las tasas de crecimiento, por medio de la producción de huevos o del factor RNA/DNA (Véase Basedow et al., 2014). Sin embargo, estos métodos sufren igualmente fuertes limitaciones, impidiendo su acoplamiento con medidas físicas y químicas en oceanografía. Como alternativa, los métodos enzimáticos se basan en medidas de la actividad celular para aproximar la respiración o el crecimiento del zooplancton. Los individuos se congelan a bordo de los barcos, permitiendo la determinación de la actividad enzimática posteriormente en el laboratorio. Esto permite el estudio del metabolismo a lo largo de grandes regiones, algo imposible de acometer por medio de incubaciones. Además, la actividad enzimática puede medirse en individuos de capas más profundas, permitiendo realizar un mapeado horizontal y vertical del metabolismo del zooplancton (Véase Packard et al., 1985). Es el caso del método del sistema de transporte de electrones (ETS, Packard, 1971), usado para aproximar la respiración, o de los métodos de medida de la aspartato transcarbamilasa (ATC, Biegala y Bergeron, 1998) y aminoacil-tRNA-sintetasas (AARS, Yebra y Hernández-León, 2004) con los que se determina el crecimiento.

Sin embargo, a pesar de que estas aproximaciones supusieron un gran avance con respecto a los métodos estándar, todavía presentan algunas incertidumbres en cuanto a la relación entre la actividad de las enzimas y las tasas metabólicas (Hernández-León et al., 1995; Hernández-León y Gómez, 1996). Por ejemplo, la actividad ETS se mide en condiciones de saturación de sustrato, algo que no siempre ocurre en la naturaleza, provocando una sobreestimación de la respiración. Por el contrario, un factor R/ETS igual a 0.5 podría subestimar las tasas cuando los sustratos no están limitados *in vivo* (Hernández-León y Gómez, 1996; Hernández-León y Torres, 1997; Putzeys et al., 2005). Por tanto, encontrar relaciones adecuadas, ajustadas a las condiciones específicas de cada región, es todavía el cuello de botella de los métodos enzimáticos.

I.1.3 Sistemas de análisis de imágenes (IBS)

La concepción de los sistemas semi-automatizados de análisis de imágenes nace de la necesidad de solventar 1) las limitaciones de los métodos existentes y 2) las demandas de la oceanografía moderna en cuanto a conocer la influencia del zooplancton en los ciclos de carbono. Estos sistemas permiten realizar estimaciones de abundancias taxonómicas y por grupos de tallas en un proceso semi-automatizado, resultando mucho más rápido que usando la metodología tradicional. Como consecuencia, las escalas espaciales de los estudios pueden incrementarse notablemente (Grosjean et al., 2004; Benfield et al., 2007; Gislason y Silva, 2009; MacLeod et al., 2010; Gorsky et al., 2010; Bachiller et al., 2012). Adicionalmente, usando relaciones empíricas basadas en parámetros medidos con el IBS, como el diámetro esférico equivalente (ESD) o el área corporal y la talla de los individuos (*e.g.,* Grosjean y Denis, 2007; Lehette y Hernández-León, 2009), el peso corporal puede igualmente estimarse en un proceso rápido y en el que las muestras no son destruidas (Véase Fig. I.2).

Las estimaciones de biomasa individual mediante los IBS permiten la aplicación de ecuaciones predictivas a cada organismo por separado, relacionando la temperatura y el peso corporal con las tasas metabólicas de respiración (Ikeda, 1985), crecimiento (Ikeda y Motoda, 1978; Hirst y Lampitt, 1998; Hirst et al., 2003) y mortalidad (Peterson y Wroblewski, 1984; Hidaka et al., 2001). Además, Hirst y Bunker (2003) y Zhou et al. (2010) decidieron incluir la Chl*a* como *proxy* de la cantidad de alimento en sus ecuaciones, mientras que Ikeda (2014) además introdujo factores de corrección para la profundidad y el grupo taxonómico en sus ecuaciones de respiración y excreción de amonio. Empleando este tipo de ecuaciones metabólicas se han obtenido estimaciones razonables (Peterson et al., 2002; Basedow et al., 2014; Stock et al., 2014), aunque todavía no está totalmente claro el efecto de la disponibilidad de alimento en el metabolismo de estos organismos (*e.g.,* Webber y Roff, 1995; Richardson y Verheye, 1999). Con respecto a la contribución relativa de cada una de las variables a la variabilidad total de las tasas metabólicas, Ikeda (2014) ha observado recientemente que la respiración y la excreción de amonio están

determinadas principalmente por el peso individual y la temperatura, mientras que la profundidad y la taxonomía son factores de menor importancia. Se remarca así el potencial de estos dos parámetros, junto con la biomasa de la comunidad, a la hora de determinar los flujos metabólicos a partir de ecuaciones predictivas.

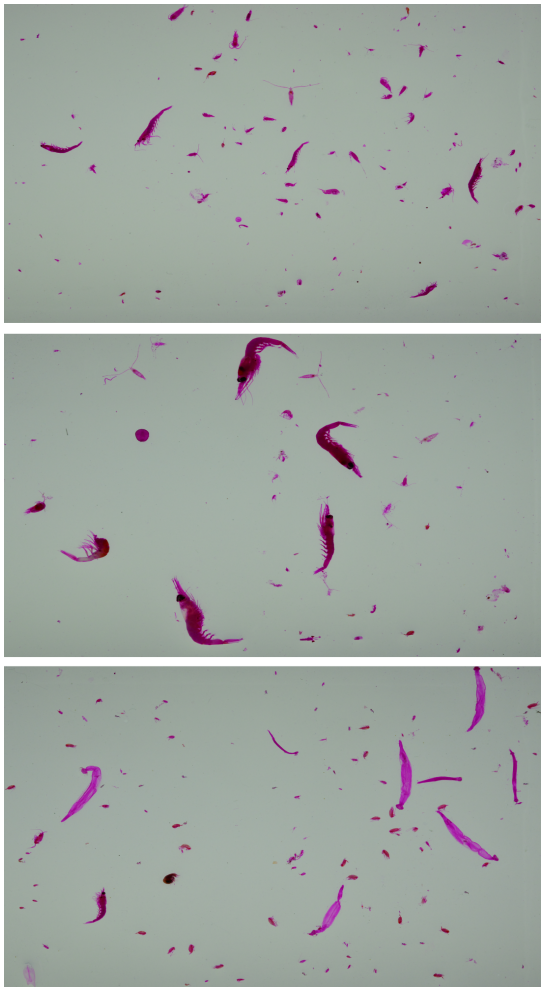

Figura I.2. Imágenes de mesozooplancton para análisis mediante un IBS. En este caso, los individuos se tiñeron usando *Rosa de Bengala* y se digitalizaron a una resolución de 1850 ppp. Para ello se usó una cámara *Nikon D800* y una lente Macro (*Micro Nikkor* 60mm f/2.8G ED). Se emplearon luces LED con el fin de garantizar una iluminación uniforme de fondo.

I.1.4 Teledetección y zooplancton

Comprender la contribución del zooplancton en la bomba biológica y el balance de carbono oceánico requiere la estimación de flujos mediados por estas comunidades a gran escala. Sin embargo, esto es algo inabordable en términos de tiempo, recursos y esfuerzo mediante la metodología actualmente empleada. Debido a diversos factores (*e.g.,* condiciones climáticas, distancia a tierra firme, etc.), extensas regiones del océano permanecen casi inexploradas, mientras que las campañas oceanográficas normalmente se concentran en las mismas zonas (Reid et al., 2003; Isla et al., 2004; San Martin et al., 2006). Alternativamente, los modelos ecosistémicos a escala global podrían resultar útiles para estudiar vastas y remotas regiones del océano, aproximando flujos de carbono de diferentes comunidades de acuerdo a funciones macroecológicas, el tamaño corporal o la ecología de la red trófica (López-Urrutia et al. 2006, Jennings et al. 2009).

La existencia de avances recientes en teledetección por satélite podrían igualmente valorarse como una herramienta alternativa, capaz de proporcionar información del estado de la superficie oceánica a escala planetaria. Así, los satélites proporcionan regularmente datos de parámetros clave de la superficie del océano, como la temperatura, Chl*a*, anomalías del nivel del océano o la producción primaria. A este respecto, determinados investigadores sostienen la hipótesis de que ciertos parámetros de la superficie del océano, medidos por satélite e instrumentos automatizados, podrían utilizarse para alimentar modelos que predigan la magnitud de los flujos de carbono en el océano (*e.g.,* EXPORTS, http://exports.oceancolor.ucsb.edu) (Véase Fig. I.3), De este modo, correlaciones entre el zooplancton y datos de ciertas variables medidas por teledetección podrían resultar muy útiles para desarrollar este tipo de modelos, con los cuales continuamente explorar los flujos de carbono mediados por estas comunidades, incluso en regiones que supondrían todo un reto a la hora de muestrearse directamente (Stock et al., 2014).

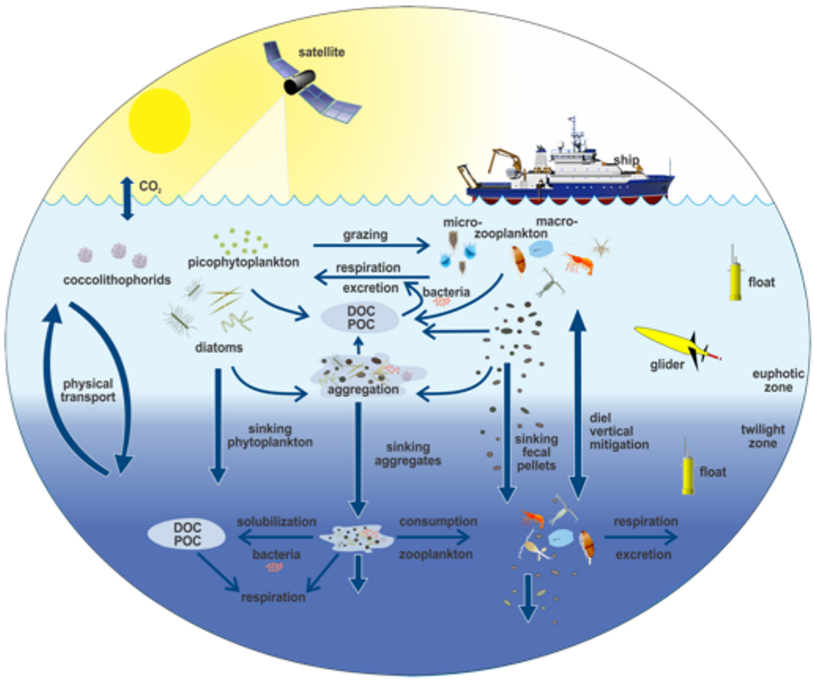

Figura I.3 Esquema del proyecto científico EXPORTS de la NASA. Éste sostiene la hipótesis de que la magnitud de la materia orgánica transferida a capas profundas podría predecirse por medio del estado del ecosistema superficial del océano. Los modelos desarrollados utilizarán datos procedentes de teledetección y dispositivos autónomos (robots submarinos, boyas, etc.), calibrados por medio de datos *in situ* procedentes de campañas oceanográficas.

En cualquier caso, los modeladores todavía necesitan medidas *in situ* de biomasa y flujos mediados por el zooplancton sobre grandes áreas, a fin de poder desarrollar modelos globales. Ello requerirá el uso de la tecnología disponible (*e.g.,* sistemas de imágenes) y ecuaciones adecuadas para predecir el metabolismo de estos organismos. Para ello, debería llevarse a cabo un trabajo específico en ciertas regiones que requieren especial atención, como es el caso de aguas costeras y afloramientos o zonas de mínimo de oxígeno (OMZs), donde los modelos basados exclusivamente en datos de la superficie del océano podrían introducir un error considerable. En estas últimas regiones, los organismos que habitan la columna de agua presentan un metabolismo limitado como adaptación a los bajos niveles de oxígeno

(Kiko et al., 2015a, 2015b). En buena lógica, esto debería repercutir en cuanto a la contribución de estas comunidades a la bomba biológica y los ciclos de carbono.

I.2 Objetivos y planteamiento de la investigación

Los métodos tradicionales empleados para estimar el metabolismo del zooplancton son procesos que consumen mucho tiempo, impidiendo su uso en estudios sobre grandes regiones. Del mismo modo, los métodos enzimáticos, aunque muy útiles, presentan algunas incertidumbres a la hora de aproximar las tasas metabólicas a partir de la actividad enzimática. A este respecto, los sistemas semi-automatizados, basados en el análisis de imágenes (IBS), combinados con ecuaciones metabólicas, podrían representar una alternativa válida para predecir flujos mediados por el zooplancton a gran escala. Por otro lado, estudiar las posibles relaciones entre el zooplancton y datos de ciertas variables ambientales, obtenidos por medio de teledetección, podrían finalmente resultar útiles para desarrollar modelos con los que investigar los flujos de carbono mediados por estas comunidades.

Comprender el papel del zooplancton en la bomba biológica requiere estimaciones adecuadas de la biomasa y el metabolismo de estas comunidades. El objetivo de esta tesis fue desarrollar y probar la validez de herramientas alternativas con las que poder estimar la biomasa y los flujos metabólicos mediados por el zooplancton a gran escala. Los objetivos específicos de este trabajo se detallan a continuación, a fin de responder a las siguientes cuestiones:

¿Son los IBS una alternativa válida para estudiar la biomasa y el metabolismo del zooplancton? **Capítulo 1**

Tratamos de abordar esta cuestión mediante una comparativa entre estimaciones de biomasa y tasas de crecimiento, respiración, egestión e ingestión a partir de métodos tradicionales y enzimáticos, y las obtenidas usando un IBS. Este estudio se llevó cabo a lo largo de una serie temporal en aguas subtropicales.

¿Existe un acoplamiento físico-biológico en regiones con una elevada actividad mesoescalar? ¿Son los IBS igualmente útiles para estudiar el zooplancton en estas regiones? **Capítulo 2**

Esta doble cuestión fue respondida aplicando el IBS y las ecuaciones metabólicas previamente desarrolladas y probadas en la región subtropical, a la zona de transición entre el afloramiento costero del noroeste de África y las aguas de las Islas Canarias. Las numerosas estructuras físicas detectadas en esta región, como es el caso de filamentos, remolinos inducidos por las islas y frentes, fueron estudiados tridimensionalmente en términos de biomasa y flujos metabólicos del zooplancton.

El ajuste de las ecuaciones metabólicas a las condiciones específicas de cada región, ¿incrementa la precisión de las estimaciones? **Capítulo 3**

Para responder esta cuestión desarrollamos ecuaciones metabólicas específicas, ajustadas a los rangos de temperatura de las principales regiones y capas de profundidad del océano. Se realizó una comparativa entre estimaciones usando estas ecuaciones específicas y las generalistas existentes para el océano global, con medidas a partir de métodos enzimáticos. Para ello se usaron muestras procedentes de la circunnavegación Malaspina-2010, a lo largo de las capas epi-, meso- y batipelágica de la banda ~40ºN-40ºS.

¿Cuál es la magnitud del flujo activo por medio del zooplancton en el océano cálido? ¿Está correlacionado con variables ambientales medidas por teledetección? **Capítulo 4**

Estas cuestiones fueron abordadas aplicando las ecuaciones metabólicas específicas para la temperatura previamente desarrolladas y probadas. Usando un IBS, estudiamos la contribución del flujo activo mediado por el zooplancton a través de la respiración, la mortalidad, la egestión y la excreción de amonio a lo largo de una sección latitudinal en el Atlántico tropical y subtropical (10ºS-25ºN), y su posible correlación con datos regularmente medidos por teledetección (Chl*a* y SST).

II. Material y métodos

Con el objeto de llevar a cabo los objetivos planteados en la presente tesis doctoral se emplearon diferentes técnicas de muestreo y análisis. A continuación se presenta, en términos generales, la metodología empleada.

II.1. Medidas oceanográficas

Los muestreos oceanográficos se llevaron a cabo a bordo de dos buques de investigación españoles: "Hespérides" y "Atlantic Explorer". En las diferentes campañas se realizaron perfiles verticales de medidas de la temperatura y la conductividad, por medio de un sistema CTD montado sobre una roseta oceanográfica, equipada con 6-24 botellas Niskin de 4-10 litros de capacidad, dependiendo de cada campaña en concreto. La roseta contó además con un fluorímetro, cuyas medidas *in situ* se utilizaron para finalmente derivar la clorofila, calibrada mediante una serie de muestras recogidas en la columna de agua. En algunos de los estudios se emplearon igualmente imágenes de satélite de SST y Chl*a* derivadas del sensor MODIS, a bordo del satélite Aqua (EOS PM) de la NASA (http://oceancolor.gsfc.nasa.gov/cms/data/aqua). Del mismo modo, la producción primaria se obtuvo de la web *Ocean Productivity* (http://www.science.oregonstate.edu/ocean.productivity/index.php; Behrenfeld y Falkowski 1997), cuyas estimaciones se basan en medidas de Chl*a* y temperatura del sensor MODIS igualmente.

II.2 Muestreos de zooplancton

Para muestrear las comunidades de zooplancton se emplearon diferentes tipos de redes, luz de malla y tipo de arrastre, dependiendo del tipo de estudio y sus objetivos. Así, en el *Capítulo 1* se empleó una red WP-2 doble (UNESCO, 1968), equipada con una malla de 100 μm para realizar muestreos verticales de la columna de agua hasta 200 m de profundidad. En cambio en el *Capítulo 2*, se utilizó una red tipo *Longhurst-Hardy Plankton*

Recorder (LHPR, Longhurst y Williams, 1976), equipada con una malla de 200 μm, con la que se realizaron arrastres oblicuos a una velocidad del buque de aproximadamente 3 nudos. Las muestras se estratificaron cada 20 metros en los primeros 200 m de profundidad. Por su parte, para desarrollar el *Capítulo 3* las muestras se tomaron en cuatro estratos de profundidad (0-200, 200-500, 500-1000 y 1000-2000 m) mediante arrastres oblicuos de una red Multinet (0.7 x 0.7 m, malla de 300 μm), a una velocidad del buque de unos 3 nudos. Finalmente, las muestras usadas en el *Capítulo 4* fueron tomadas mediante una red tipo *Multiple Opening and Closing Net and Environmental Sensing System* (MOCNESS) de 1 m^2 de área e incorporando una malla de 200 μm, en 8 estratos de profundidad (0-50, 50-100, 100-200, 200-300, 300-400, 400-500, 500-600 y 600-800 m) mediante arrastres oblicuos, a una velocidad constante del buque de aproximadamente 3 nudos.

II.3 Estimaciones de biomasa del zooplancton

En los *Capítulos 1 y 3,* en los que se realizaron comparaciones entre las estimaciones metabólicas de zooplancton a partir de métodos enzimáticos (ETS y AARS), y a partir de ecuaciones predictivas en combinación con el IBS, las muestras se fraccionaron a bordo del buque, a fin de poder realizar análisis según ambos tipos de metodologías en el laboratorio. Por su parte, en los *Capítulos 2 y 4* el conjunto de la muestra se empleó para realizar medidas por medio del IBS. En cualquiera de los casos, las muestras reservadas para realizar estimaciones con el IBS se conservaron a bordo usando formol al 4%, mientras que las destinadas a analizarse mediante métodos enzimáticos se congelaron inmediatamente tras el muestreo con el uso de nitrógeno líquido (-196ºC).

Una vez en el laboratorio, se realizaron estimaciones de biomasa de la comunidad de zooplancton de acuerdo con diferentes técnicas. Por un lado, las muestras se secaron en la estufa a 60ºC durante 24 horas siguiendo el método de Lovegrove (Lovegrove, 1966), estimándose el peso seco de la comunidad por medio de una microbalanza. Por otra parte, se usó el sistema

IBS para conocer el peso de cada uno de los individuos presentes en las muestras. Para ello, se introdujeron los organismos en un matraz y se homogeneizaron. Dependiendo de su abundancia, se tomaron diferentes alícuotas o submuestras mediante una pipeta Hensen, las cuales se colocaron en bandejas de poliestireno (90 x 130 mm). Éstas se digitalizaron usando un escáner *Epson Perfection 4990 PHOTO* (con el software *VueScan Professional Edition 8.4.77*), a una resolución de 1200 ppp. Una variante de este modo de obtención de imágenes de zooplancton es el que se utilizó en el *Capítulo 4,* en el que las submuestras se tiñeron con 1 ml de *Rosa de Bengala* (5g l^{-1}). Tras 24 horas, estas submuestras se colocaron en bandejas de metacrilato de 70 x 105 mm, con el fin de obtener entre 5 y 10 réplicas de cada submuestra. En este caso, las imágenes se obtuvieron con una cámara digital *Nikon D800* (36 Mega Pixel) con una lente Macro (*Model Micro Nikkor* 60 mm f/2.8G ED). Para conseguir una luz de fondo uniforme se empleó luz LED blanca. Las submuestras se fotografiaron entonces a 1850 puntos por pulgada (ppp).

En ambos casos, las imágenes se procesaron con el software *ZooImage 1 versión 1.2-1* (http://www.sciviews.org/zooimage), de acuerdo con Grosjean y Denis (2007). Los organismos se enumeraron, midieron, pesaron y clasificaron de forma automatizada en cinco grupos taxonómicos: copépodos, quetognatos, tipo eufausiáceos, gelatinosos y otro zooplancton. Para ello se realizó un *training set* manual a partir de imágenes de individuos de las propias muestras, con el fin de ayudar al programa a establecer patrones de clasificación para cada uno de los grupos taxonómicos. Se eligió el algoritmo *Random Forest* para realizar la clasificación, de acuerdo a Grosjean et al. (2004). La nieve marina, fibras, burbujas, sombras y otros compuestos inorgánicos se reunieron en un grupo extra, de forma que el programa fuese capaz de descartarlos a la hora de realizar estimaciones de abundancias o biomasas. El error en la clasificación automatizada se estimó en el rango 4-8.5%.

Una vez clasificados los individuos, se realizaron estimaciones de biomasa según el sistema IBS (B_{IBS}) de acuerdo a relaciones entre el área de los individuos y su peso seco. Para ello, empleamos las relaciones empíricas

desarrolladas por Lehette y Hernández-León (2009), empleando una ecuación diferente para cada grupo taxonómico (Tabla 1.1). Las estimaciones de biomasa en peso seco (todos los métodos) se convirtieron a unidades de carbono de acuerdo con Kiørboe (2013), usando una relación diferente para cada grupo taxonómico (Véase Tabla 1.1).

II.4 Estimaciones de la actividad enzimática: AARS y ETS

La actividad del sistema enzimático aminoacil-tRNA-sintetasas (método AARS) fue empleada como *proxy* de las tasas de crecimiento del zooplancton. De forma breve, las muestras congeladas en nitrógeno líquido se homogeneizaron en Tris-HCl (20 mM, pH 7.8) y se centrifugaron (10 min, 0ºC), con el fin de medir la actividad del sistema de enzimas siguiendo el método de Yebra y Hernández-León (Yebra y Hernández-León, 2004), ligeramente modificado por Herrera et al. (2012). La actividad AARS se corrigió a la temperatura *in situ,* aplicando una energía de activación de 8.57 kcal mol^{-1} (Yebra et al., 2005a), con el fin de obtener la actividad *in situ* normalizada según el contenido proteico de las muestras. Para ello, una fracción del homogeneizado inicial se analizó siguiendo el método de Lowry et al. (Lowry et al., 1951), adaptado por Rutter (1967), usando *Albúmina Sérica Bovina* como estándar. La actividad específica (spAARS) se convirtió a crecimiento específico (G_{AARS}) usando la ecuación desarrollada por Hernández-León et al. (en preparación):

$$G_{AARS}\ (d^{-1}) = -0.0117 + 0.0038 \cdot \text{spAARS} \quad (r^2 = 0.738;\ p<0.001) \qquad (II.1)$$

donde spAARS se expresó en nmol PPi mg $prot^{-1}$ h^{-1}, y PPi es pirofosfato.

De la misma forma, la actividad enzimática del sistema de transporte de electrones (método ETS) fue empleado como *proxy* de las tasas de respiración del zooplancton. Para ello se tomó una alícuota del mismo homogeneizado usado para analizar la actividad AARS, y se siguió la metodología desarrollada por Packard (1971) para zooplancton. Detalles del proceso se pueden encontrar en Hernández-León y Gómez (1996). La

actividad ETS se corrigió a la temperatura *in situ* usando la ecuación de Arrhenius y una energía de activación de 15 Kcal mol^{-1} (Packard et al., 1975), y finalmente se normalizó al contenido proteico de las muestras (µl O_2 mg $prot^{-1}$ h^{-1}). La actividad ETS se aproximó finalmente a la respiración potencial (d^{-1}) de la comunidad asumiendo una relación C:*dw* = 0.48 (Kiørboe, 2013) y usando la relación entre el *dw* y las proteínas desarrollada por Hernández-León et al. (2001):

$$dw = 1.445 + 4.283 \cdot \text{mg prot} \qquad (r^2 = 0.900;\ n = 306;\ p<0.001) \qquad \text{(II.2)}$$

Finalmente, la respiración potencial se aproximó a la respiración específica de la comunidad (d^{-1}) asumiendo relaciones R/ETS iguales a 0.5 y 1.0 (Hernández-León y Gómez, 1996). El consumo de oxígeno se convirtió en unidades de carbono utilizando un coeficiente respiratorio de 0.97 (Omori e Ikeda, 1984).

II.5 Método de la fluorescencia del tracto digestivo (*gut fluorescence)*

Las tasas de egestión (alimentación pigmentada) se estimaron mediante el método de *gut fluorescence*. Para ello, una alícuota del mismo homogeneizado usado para analizar la actividad enzimática de los sistemas AARS y ETS se colocó en un tubo de 10 ml con 90% de acetona, y se conservó a -20ºC durante 24 horas en la oscuridad. La fluorescencia de las muestras se midió antes y después de acidificación con tres gotas de HCl al 10% en un fluorímetro *Turner Design* (*model 10-AU-005-CE*). Los pigmentos se calcularon según las indicaciones de Strickland y Parsons (Strickland y Parsons, 1972), modificado por Hernández-León et al. (2001):

$$\text{clorofila } a = k \cdot (Fo - Fa) \cdot \text{mg prot}^{-1} \qquad \text{(II.3)}$$

$$\text{feopigmentos} = k \cdot (R \cdot Fo - Fa) \cdot \text{mg prot}^{-1} \qquad \text{(II.4)}$$

donde k es la calibración del instrumento, *Fo* y *Fa* son las medidas de fluorescencia antes y después de la acidificación y *R* es el coeficiente de acidificación. El contenido en pigmentos se normalizó según el contenido

proteico (μg pigmento mg prot^{-1}), y finalmente las tasas específicas de egestión (material pigmentado) (d^{-1}) se obtuvieron asumiendo relaciones C:Chl*a* de 50 (*e.g.*, Reigstad et al., 2008) y C:*dw* de 0.48 (Kiørboe, 2013). Además, se empleó la relación entre el *dw* y las proteínas especificado arriba (ecuación II.2) y una tasa de evacuación de 0.056 min^{-1} (Hernández-León et al., 2002).

II.6 Metabolismo mediante IBS y ecuaciones predictivas

Se empleó un sistema IBS en combinación con ecuaciones predictivas que relacionaron la actividad metabólica de los organismos con parámetros tales como la temperatura, el peso de los individuos (*bw*) y la clorofila. De este modo, se estimaron las tasas específicas de crecimiento, respiración, egestión, ingestión y excreción de amonio de cada individuo por separado.

Por un lado, las tasas específicas de crecimiento (G) del zooplancton (d^{-1}) fueron estimadas de acuerdo a diferentes ecuaciones predictivas. Por ejemplo, Hirst y Bunker (Hirst y Bunker, 2003) y Zhou et al. (Zhou et al, 2010) relacionaron las tasas fisiológicas con el peso individual, la temperatura y la clorofila (*proxy* de la disponibilidad de alimento) en una ecuación única para todo el océano (Tabla 1.2). Por otro lado, Hirst et al. (Hirst et al., 2003) desarrollaron diferentes ecuaciones para los principales grupos taxonómicos, relacionando las tasas de crecimiento con el peso individual y la temperatura (Tabla 1.3). Para ello usaron datos de experimentos provenientes desde aguas polares a ecuatoriales, y de sistemas tanto oligotróficos como de afloramientos. Al igual que Hirst et al. (Hirst et al., 2003), en esta tesis se desarrollaron diferentes ecuaciones para los principales grupos taxonómicos, empleando la temperatura y el peso de los individuos como variables en las ecuaciones (*Capítulos 1 y 3*). Sin embargo, en nuestro caso se desarrollaron diferentes relaciones de acuerdo a las condiciones específicas de temperatura de las principales regiones del océano. Igualmente, sólo se utilizaron datos de individuos cuyo peso se encontró dentro del rango observado para el mesozooplancton (Tablas 1.3 y 3.3).

En el caso de la respiración específica (R) se utilizaron las ecuaciones de Ikeda (Ikeda, 1985, 2014) y las ecuaciones desarrolladas durante la presente tesis doctoral (*Capítulos 1 y 3*). En todas ellas se relacionaron las tasas metabólicas con el peso de los individuos y la temperatura. Las ecuaciones de Ikeda (ambas) son de carácter generalista, de modo que en cada estudio se desarrolló una única ecuación para estimar la respiración del zooplancton a lo largo de todo el planeta (Tabla 1.4; Tabla 4 en Ikeda, 2014). Sin embargo, en nuestro caso, al igual que para el crecimiento, se desarrollaron diferentes ecuaciones, ajustadas exclusivamente al mesozooplancton y de acuerdo a los rangos de temperatura dados en las principales regiones del océano (Tablas 1.4 y 3.2).

Las tasas específicas de egestión (E) del zooplancton (ng C ind^{-1} min^{-1}) se estimaron a partir de dos relaciones empíricas. Así, se empleó la relación entre el peso de los individuos y su contenido estomacal (GC), desarrollado por Garijo y Hernández-León (2015):

$$\log_{10} GC = 0.852 \log_{10} bw - 1.160 \quad (r^2 = 0.769;\ n = 208;\ p<0.05) \quad \text{(II.5)}$$

en combinación con la relación entre la temperatura y las tasas de evacuación (e) del zooplancton, de acuerdo a Irigoien (1998):

$$e = 0.0026T + 0.012 \quad (r^2 = 0.940;\ n = 19) \quad \text{(II.6)}$$

Se asumió una relación C:Chl*a* de 50 (*e.g.,* Reigstad et al., 2008).

Como resultado, las tasas específicas de ingestión (I) se derivaron a partir de las estimaciones previas de crecimiento, respiración y egestión:

$$I = G + R + E \quad \text{(II.7)}$$

Finalmente, se estimaron las tasas específicas de excreción de amonio (d^{-1}) empleando la ecuación generalista de Ikeda (Ikeda, 2014) para el océano global, de acuerdo al peso de los individuos, la temperatura y la profundidad. Además, las tasas fueron corregidas para los principales grupos taxonómicos (Tabla 2.1).

Los flujos metabólicos comunitarios mediados por el zooplancton (mg C m^{-2} d^{-1}), tanto derivados de medidas de la actividad enzimática, como usando el sistema IBS (producción, respiración, egestión, ingestión y excreción de amonio), se estimaron empleando las respectivas tasas metabólicas específicas y las medidas de biomasa comunitaria.

II.7 Biomasa migrante y flujo activo del zooplancton

La biomasa migrante del zooplancton se estimó en el *Capítulo 4* usando una red tipo MOCNESS para realizar los muestreos. Los organismos más grandes, tales como decápodos y peces, fueron eliminados pues normalmente estos individuos escapan a este tipo de redes. Así, se estimó la biomasa migrante como el aumento de la biomasa nocturna respecto a la diurna en la capa epipelágica (por encima de 200 m de profundidad).

Por otro lado, el flujo activo hacia la capa mesopelágica (>200 m profundidad) por medio de la respiración (R), la mortalidad (M) y la egestión (G) (mg C m^{-2} d^{-1}), así como la excreción de amonio (N) del zooplancton, se calculó de acuerdo a Zhang y Dam (1997). De este modo, aplicamos la ecuación:

$$F = B * M * T \quad \text{(II.8)}$$

donde B es la biomasa migrante diaria de zooplancton (mg C m^{-2} o mg N m^{-2}); M es la tasa metabólica específica (d^{-1}) promedio de los organismos durante el día en la capa 300-500 m de profundidad; y T es el número promedio de horas de luz diaria (12h). Para el cálculo de las tasas metabólicas se empleó un IBS en combinación con las ecuaciones desarrolladas en la presente tesis, tal y como se ha descrito anteriormente en el apartado II.6. En el caso de la excreción de amonio se utilizó la ecuación de Ikeda (Ikeda, 2014), mientras que la mortalidad se aproximó a partir de las tasas específicas de crecimiento, pues en una comunidad en balance (*steady-state)* ambos conceptos se asumen equivalentes. Por su parte, para estimar el flujo de egestión se asumió (1) que no se produjo defecación

durante la migración vertical hacia capas profundas y (2) que el tiempo de residencia del alimento ingerido en el tracto digestivo fue lo suficientemente alto como para permitir que se lleve a cabo defecación en la capa mesopelágica.

II.8 Desarrollo de ecuaciones metabólicas

Para el desarrollo de las ecuaciones de crecimiento del zooplancton de los *Capítulos 1 y 3* se emplearon datos recogidos en los Apéndices 1 y 2 de Hirst et al. (2003). Por su parte, para el desarrollo de las ecuaciones de respiración de los mismos capítulos se usaron los datos compilados por Hernández-León e Ikeda (2005). Todas las ecuaciones se obtuvieron usando un programa de regresiones lineales en *SYSTAT versión 13*, así como el programa *SPSS-IBM, versión 22*.

Por su parte, las correlaciones (r^2) entre estimaciones procedentes de diferentes métodos se obtuvieron mediante comparativas de acuerdo al modelo de regresión lineal *reduced major axis* (RMA) (regresión lineal tipo II) (Véase Tablas 1.5, 1.6, 3.4 y 3.5), pues se asumió que ambas variables fueron medidas con un cierto error (ver Smith, 2009; y las referencias dentro de éste).

III. Principales resultados y discusión

III.1 El uso de un sistema basado en el análisis de imágenes para estudiar las tasas fisiológicas del zooplancton: comparativa con métodos enzimáticos

Juan Carlos Garijo y Santiago Hernández León (2015)
Journal of Plankton Research, 37: 923-938

Los sistemas tradicionales presentan grandes limitaciones a la hora de determinar la biomasa (Lovegrove, 1966) y el metabolismo del zooplancton (Véase Basedow et al., 2014) a escalas espaciales grandes. Por ello, en este estudio pretendíamos verificar las posibilidades de uso de un sistema basado en el análisis de imágenes (IBS) para determinar estos importantes parámetros del zooplancton a dicha escala. Para ello, en primer lugar realizamos una comparativa entre estimaciones de biomasa, a partir del método de peso seco (Lovegrove, 1966) tradicionalmente usado, y las obtenidas mediante un IBS en combinación con las relaciones empíricas existentes entre el área y el peso seco de los individuos, desarrolladas por Lehette y Hernández-León (2009).

Por otro lado, comparamos el crecimiento y la respiración obtenido a partir de la actividad enzimática de los métodos de AARS y ETS respectivamente, y las estimaciones usando el IBS en combinación con ecuaciones generalistas existentes para el océano global, así como con ecuaciones desarrolladas en este estudio, ajustadas a las condiciones de la región subtropical. Del mismo modo, se estimó la egestión de los organismos mediante el IBS, combinando una relación entre el peso de los individuos y su contenido estomacal, desarrollada en esta tesis, y la relación entre la temperatura y la tasa de evacuación desarrollada por Irigoien (1998). Las tasas de egestión obtenidas por esta vía se compararon con medidas a partir del método de *fluorescencia del tracto digestivo*. Finalmente, se estudió la viabilidad del IBS para estudiar la ingestión de los organismos a partir de las estimaciones de crecimiento, respiración y egestión previas.

Nuestros resultados sugieren la utilidad del IBS para determinar la biomasa comunitaria de zooplancton, pues las estimaciones usando este método no mostraron diferencias significativas (ANOVA, $p>0.05$) con respecto a la metodología tradicional. Tras una revisión bibliográfica, no se han detectado problemas de reducción del área de los individuos a causa de su conservación en formol (Landry, 1978; Durbin y Durbin, 1978; Viitasalo et al., 1995; Pollupüü, 2007). Teniendo en cuenta que se usaron relaciones entre el área y el peso de los individuos (Lehette y Hernández-León, 2009), sugerimos la fiabilidad de estos sistemas a la hora de estimar la biomasa comunitaria de zooplancton.

Las estimaciones de crecimiento y respiración usando ecuaciones específicas, ajustadas a las condiciones de la región subtropical, resultaron mejor correlacionadas con las medidas a partir de métodos enzimáticos, que las estimaciones obtenidas a partir de las ecuaciones globales desarrolladas por Hirst et al. (Hirst et al., 2003), Hirst y Bunker (Hirst y Bunker, 2003) y Zhou et al. (Zhou et al., 2010) para el crecimiento, así como la desarrollada por Ikeda (Ikeda, 1985) para la respiración. De hecho, las estimaciones comunitarias de producción y respiración usando nuestras ecuaciones específicas fueron las únicas que no presentaron diferencias significativas (ANOVA, $p>0.05$) con respecto a las obtenidas por métodos enzimáticos. Se ha comprobado la importancia de la temperatura y el peso de los individuos en el metabolismo del zooplancton (Ikeda, 2014). Por ello, el uso de datos extremos de temperatura (*e.g.,* $-2.0ºC>T>30ºC$) o provenientes de individuos demasiado grandes, fuera del rango del mesozooplancton, usados por Hirst et al. (Hirst et al., 2003) e Ikeda (Ikeda, 1985) para configurar sus ecuaciones, podría ser la causa de la menor correlación observada usando estas ecuaciones. En el caso de Hirst y Bunker (Hirst y Bunker, 2003) y Zhou et al. (Zhou et al., 2010) posiblemente fue debido a los elevados valores de Chl*a* (muy superiores a los que se observan normalmente en zonas oligotróficas) empleados como valores de referencia a la hora de desarrollar sus ecuaciones.

La relación entre el peso de los individuos y su contenido estomacal desarrollada en este trabajo resultó ser útil para determinar la egestión de los organismos a través del IBS de una forma sencilla. Así, las estimaciones de egestión se encontraron dentro del rango de valores observados por Hernández-León et al. (2004, 2007) en la misma región. Sin embargo, las medidas a partir del método de *fluorescencia del tracto digestivo* fueron significativamente inferiores (ANOVA, $p<0.05$), algo normal considerando que este método sólo mide el material pigmentado. A este respecto, se sabe que el zooplancton en aguas oligotróficas es fundamentalmente omnívoro, con una importancia reducida del fitoplancton en su dieta (*e.g.,* Saiz et al. 1999; Hernández-León et al., 2001).

Finalmente, se observó una elevada correlación entre la ingestión a partir de las estimaciones previas de crecimiento, respiración y egestión, usando por un lado el IBS en combinación con nuestras ecuaciones y, por el otro, los métodos enzimáticos. Asimismo, observamos una elevada correlación entre la ingestión derivada de nuestras ecuaciones y las estimaciones obtenidas a partir de las ecuaciones planteadas por Saiz y Calbet (2007). Esto sugiere la utilidad del IBS, así como de las ecuaciones desarrolladas en este estudio, para determinar el metabolismo de estos organismos.

En definitiva, los resultados indican que los IBS pueden ser tan fiables como los métodos enzimáticos para determinar el metabolismo y los flujos mediados por el zooplancton. Sin embargo, los primeros resultan más apropiados en estudios a gran escala pues, entre otras ventajas, se trata de procesos más rápidos y económicos. Por otro lado, el metabolismo obtenido por medio de un IBS podría resultar más preciso cuando las ecuaciones se ajustan a las condiciones de la región de estudio, en comparación con las estimaciones de ecuaciones generalistas para el océano global. En cualquier caso, parece que los métodos enzimáticos resultan más apropiados para estudiar tasas metabólicas específicas del zooplancton en determinados casos, como son las series temporales.

III.2 La influencia de las estructuras mesoescalares en la biomasa y el metabolismo del zooplancton en la Zona de Transición Costera del Noroeste de África durante la estación de afloramiento debilitado

Juan Carlos Garijo y Santiago Hernández León

En este estudio se exploró la zona de transición entre las aguas productivas y frías del afloramiento del Noroeste de África, y las aguas cálidas y oligotróficas próximas a las Islas Canarias, durante la estación de débil afloramiento (final de invierno-primavera). Con ello se buscaba comprender la influencia de estructuras físicas persistentes, como es el caso de frentes, filamentos, remolinos inducidos por las islas, etc., en la distribución tridimensional de la biomasa y los flujos metabólicos del zooplancton en esta región. Así, este estudio pretendía dar continuidad al trabajo previo desarrollado en la misma región por Hernández-León et al. (2002) durante la estación de alta productividad del afloramiento. Por tanto, nuestros resultados suponen un elemento de contraste ante un escenario claramente diferenciado. Para llevar a cabo el trabajo nos basamos en los estudios previos llevados a cabo por Benítez-Barrios et al. (2011) y Moyano et al. (2014) en los que detallaron respectivamente la distribución de estructuras mesoescalares y de larvas de peces en la misma campaña.

Para ello, combinamos un IBS con la ecuación de crecimiento desarrollada por Hirst y Bunker (Hirst y Bunker, 2003) y la ecuación de respiración desarrollada en el *Capítulo 1* para la región subtropical. Además, aplicamos la relación entre el peso y el contenido estomacal de los individuos desarrollada en el *Capítulo 1* para estimar la egestión. Finalmente, la ingestión fue determinada a partir de las estimaciones previas de los tres componentes anteriores. Por otro lado, se usaron las ecuaciones planteadas por Ikeda (Ikeda, 2014) para determinar la excreción de amonio.

Nuestros resultados mostraron que el IBS empleado podría resultar útil para estudiar la distribución de la biomasa, el metabolismo y los flujos mediados

por el zooplancton en estas estructuras mesoescalares. Lo más sorprendente del estudio es, sin duda, el claro acoplamiento físico-biológico observado. Así, el zooplancton normalmente estuvo relacionado con las estructuras físicas, de modo que éstas influyeron en la distribución tridimensional de su biomasa y su metabolismo.

Se observó que tanto los filamentos provenientes del afloramiento como el *jet* costero en dirección noreste-suroeste eran capaces de transportar Chl*a* y zooplancton hacia la zona oligotrófica cercana al Archipiélago Canario. Esto es algo que previamente se ha observado en la misma zona. Por ejemplo, Hernández-Guerra et al. (1993) y Basterretxea y Arístegui (2000) observaron un transporte de Chl*a*, mientras que Baltar et al. (2009) observó el mismo patrón en el caso del nano- y picoplancton. Por su parte, Hernández-León et al. (2002) observó transporte de zooplancton y, por ejemplo, Bécognée et al. (2009) y Moyano et al. (2014) constataron el mismo fenómeno en el caso del ictioplancton.

Además, las interacciones entre estos filamentos y los remolinos inducidos por las islas incrementaban la concentración de Chl*a* en estos últimos, de acuerdo con lo observado previamente por Van Camp (1991) y Arístegui et al. (1997, 2004). De la misma forma, estas interacciones incrementaron la biomasa de zooplancton dentro de estas estructuras, en concordancia con las observaciones de Hernández-León et al. (2002) en la misma región. Es remarcable que la concentración de organismos fue superior en las regiones de interacción entre los remolinos y sus correspondientes filamentos, coincidiendo con la zona donde se acumulaba una cantidad más elevada de Chl*a*. En este sentido, se observó una correspondencia generalizada entre la distribución de la Chl*a* y el zooplancton a lo largo de la estructura tridimensional de filamentos y remolinos. Posibles desacoples en este sentido, observados en algún remolino, se explican por medio de la ageostrofía que pueden presentar estas estructuras. Así, muchos de los remolinos generados al sur de las Islas Canarias cambian continuamente su estructura vertical y horizontal, mostrando formas elípticas y asimétricas (Sangrá, 1995; Arístegui et al., 1997).

En cualquier caso, la mayor concentración de organismos observada en las capas más profundas de los remolinos anticiclónicos muestreados coincidió con las observaciones de Arístegui et al. (1997), atribuido al hundimiento de isotermas e isopicnas (Véase Fig. 2.3 y 2.4). Lo mismo se observó en el caso de remolinos ciclónicos, donde estos mismos autores sugirieron que la elevación de las isotermas y el bombeo de nutrientes hacia la capa más somera e iluminada podría provocar un aumento de la biomasa en la superficie. Por otro lado, debido a que el metabolismo depende fundamentalmente de la temperatura (*e.g.,* Hirst y Lampitt, 1998; Hirst et al., 2003; Ikeda, 2014), el hundimiento o la elevación de las isotermas ejerció una clara influencia en la distribución y magnitud de las tasas de crecimiento, respiración, egestión, ingestión y excreción de amonio del zooplancton en dichos remolinos (Véase Fig. 2.7).

Los frentes que se generan en estas regiones de transición pueden influir igualmente en la distribución de los individuos. En este caso, el frente existente entre el afloramiento y la región oligotrófica, retuvo una cierta cantidad de Chl*a* y zooplancton (Véase Fig. 2.5), actuando así como una barrera natural paralela a la costa. Este fenómeno fue observado igualmente por Moyano et al. (2014) en este mismo frente en el caso del ictioplancton.

El zooplancton exportado desde la región productiva del afloramiento hasta las aguas próximas al Archipiélago por medio de filamentos, así como su retención en frentes y remolinos, podría finalmente repercutir en las pesquerías locales, ya que estas comunidades representan el alimento natural de las larvas de peces. En cualquier caso, es necesario que se lleven a cabo estudios específicos a fin de comprender si efectivamente se dan estos efectos. De todos modos, parece ser que la productividad del afloramiento, fuertemente variable a lo largo del año, es el factor determinante en cuanto a la capacidad de exportación de estas estructuras mesoescalares hacia las aguas del Archipiélago. A este respecto, nuestros resultados de biomasa y flujos metabólicos mediados por el zooplancton son ciertamente inferiores a los observados por Hernández-León et al. (2002) durante la estación de máxima productividad del afloramiento.

III.3 Estimación del metabolismo del zooplancton a gran escala mediante un sistema de análisis de imágenes: comparativa entre métodos

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La estimación de flujos de carbono mediados por el zooplancton es esencial para comprender el papel de estas comunidades en la bomba biológica en el océano. Los métodos tradicionalmente usados para estimar la respiración o el crecimiento de estos organismos requieren mucho tiempo, impidiendo su aplicación a escalas espaciales grandes. Con el objetivo de presentar una solución válida a este problema, en este estudio nos basamos en los resultados previos obtenidos por Garijo y Hernández-León (2015). Estos autores mostraron que los IBS, en combinación con ecuaciones ajustadas a las condiciones de la región subtropical, pueden resultar tan fiables como los métodos enzimáticos para estudiar los flujos mediados por el zooplancton, si bien resultando ser un proceso más rápido y económico.

De este modo, utilizando los datos publicados por Hirst et al. (2003), así como los compilados por Hernández-León e Ikeda (2005), desarrollamos una serie de ecuaciones para determinar el crecimiento y la respiración del mesozooplancton. Éstas fueron ajustadas a los rangos de temperatura dados en las principales regiones oceánicas y capas de profundidad, relacionando las tasas metabólicas con la temperatura del hábitat y el peso de los individuos. Con el objeto de verificar su posible utilidad, realizamos una comparativa entre las tasas metabólicas obtenidas mediante un IBS, en combinación con estas ecuaciones específicas para la temperatura y con las existentes para el océano global, con medidas a partir de métodos enzimáticos. Para llevar a cabo dicha comparativa se empleó la colección de muestras compiladas durante la circunnavegación Malaspina-2010 (~40ºN-40ºS) en las capas epi- (0-200 m), meso- (200-1000 m) y batipelágica (1000-2000 m). Como resultado, el programa de muestreo cubrió de forma aproximada el rango de temperaturas que puede encontrase en casi cualquier

parte del océano entre dichas latitudes (2.5-30ºC), explorando además zonas oligo-, meso- y eutróficas, dando lugar a una elevada heterogeneidad en este sentido (Véase Tabla 3.1).

El metabolismo del zooplancton está principalmente influenciado por la temperatura y el peso de los organismos (*e.g.,* Ikeda, 2014). Por ello, con el objetivo de mejorar la precisión de las estimaciones, consideramos conveniente ajustar nuestras ecuaciones a los rangos específicos de temperatura de cada región de estudio (Véase Tablas 3.2 y 3.3). Así, los rangos establecidos coincidieron de forma aproximada con los valores dados por Longhurst (2007) para las principales provincias oceanográficas. Igualmente, datos provenientes de organismos de un tamaño superior o inferior al del mesozooplancton fueron descartados a la hora de ajustar las ecuaciones. Para ello, el peso corporal estimado a lo largo de la campaña Malaspina-2010 sirvió para establecer los límites de referencia para el mesozooplancton a este respecto. Así, es de esperar que las ecuaciones desarrolladas en este estudio pudieran aplicarse a la capa superficial de (1) Regiones polares a templadas: ecuaciones 1-8ºC, (2) Regiones templadas a subtropicales: ecuaciones 8-16ºC, (3) Regiones subtropicales a ecuatoriales: ecuaciones 16~28ºC, y a las diferentes capas de profundidad (4) Meso-batipelágica (hasta 2000 m): ecuaciones 1-8ºC y 8-16ºC.

Con estas premisas, las estimaciones de metabolismo a partir de métodos enzimáticos mostraron una correlación más elevada con las obtenidas mediante nuestras ecuaciones específicas, que usando las ecuaciones generalistas de Hirst et al. (Hirst et al., 2003) y Hirst y Bunker (Hirst y Bunker, 2003) para el crecimiento, e Ikeda (Ikeda, 2014) para la respiración (Véase Tablas 3.4 y 3.5). Todo ello desde la superficie a la capa batipelágica, a lo largo de la región ~40ºN-40ºS. En cualquier caso, a la luz de los resultados y dada la magnitud espacial del estudio, tanto las ecuaciones específicas como las globales podrían considerarse como una alternativa potencialmente válida con respecto a los métodos enzimáticos, a la hora de obtener estimaciones a gran escala (Véase Fig. 3.2, 3.3 y 3.4). No en vano, en el caso de la respiración, las estimaciones obtenidas a partir del IBS usando

ecuaciones específicas y las desarrolladas por Ikeda (Ikeda, 2014) coincidieron, de forma aproximada, con la distribución del metabolismo a nivel global de la revisión llevada a cabo por Hernández-León e Ikeda (2005).

Sin embargo, es importante remarcar que no hubo diferencias significativas entre las estimaciones medidas a partir de métodos enzimáticos y usando ecuaciones específicas, mientras que el metabolismo obtenido usando ecuaciones generalistas sí era significativamente inferior al derivado de la actividad de las enzimas. Por ello, en este estudio se remarca que el tipo de ecuaciones usadas en combinación con un IBS puede finalmente influir en la calidad de los resultados obtenidos. Las ecuaciones específicas parecen ser más precisas, al menos tanto como los métodos enzimáticos, dependiendo de las condiciones específicas que se dan en cada ambiente.

El hecho de que las estimaciones procedentes de ecuaciones generalistas pudieran resultar menos precisas podría atribuirse a diferentes factores. Por un lado, conociendo que la temperatura es el principal elemento que determina el metabolismo (*e.g.,* Hirst y Lampitt, 1998; Hirst et al., 2003; Ikeda, 2014), el uso de datos extremos (*e.g.*, -2.0ºC>T>30ºC) para configurar ciertas ecuaciones generalistas, como las de Hirst et al. (Hirst et al., 2003) y Hirst y Bunker (Hirst y Bunker, 2003) para el crecimiento, y las de Ikeda (Ikeda, 1985, 2014) para determinar la respiración, podrían introducir imprecisiones a la hora de estimar el metabolismo en ciertas regiones. Por otro lado, el peso de los individuos es el segundo factor más importante en cuanto a su influencia en el metabolismo del zooplancton (Ikeda, 2014), y se sabe que éste decrece con el peso corporal (*e.g.,* Hirst y Lampitt, 1998). Así, desarrollar las ecuaciones incluyendo datos de individuos de tamaño superior al observado para el mesozooplancton, podría introducir igualmente cierto error a la hora de estimar el metabolismo de estas comunidades. Como ejemplo, Hirst et al. (Hirst et al., 2003) configuraron sus ecuaciones incluyendo datos de organismos hasta dos órdenes de magnitud superiores a los rangos propios del mesozooplancton (Garijo y Hernández-León, 2015). Esto podría provocar una subestimación de las tasas de crecimiento.

Asimismo, los valores de Chl*a* empleados como referencia a la hora de configurar las ecuaciones que incluyen un *proxy* de la disponibilidad de alimento, deberían tratarse con cautela dependiendo de la zona de estudio para la que se diseñen. Por ejemplo, en el caso de la ecuación de crecimiento de Hirst y Bunker (Hirst y Bunker, 2003) se tomaron como referencia valores de Chl*a* hasta dos órdenes de magnitud superiores a los normalmente dados en regiones oligotróficas (Garijo y Hernández-León, 2015). Por lo tanto, parece ser que dicha ecuación resultaría más adecuada para zonas más productivas. En aguas oligotróficas, que representan la mayor fracción del área de los océanos, la clorofila tal vez no sería el parámetro más adecuado para indicar la disponibilidad de alimento, pues el zooplancton en estas regiones es generalmente omnívoro (*e.g.,* Saiz et al., 1999). Es por ello que el uso de este tipo de ecuaciones en zonas menos productivas podría subestimar el crecimiento de los individuos, por lo que debería ponerse mucha atención cuando se realizan estimaciones a gran escala (*e.g.,* Stock et al., 2014).

Aunque es cierto que los coeficientes de determinación (r^2) de las ecuaciones específicas desarrolladas en este estudio se incrementaron en muchos casos con respecto a las ecuaciones globales existentes, resulta necesario mejorarlas todavía. Por ejemplo, en el caso de las ecuaciones de crecimiento para copépodos, resulta alarmante el bajo coeficiente (r^2) conseguido, teniendo en cuenta su ubicuidad en todas las regiones oceánicas y que su abundancia normalmente supera el 80% del total. Del mismo modo, las estimaciones usando nuestras ecuaciones específicas para regiones cálidas (16-28ºC) son las que presentaron unas correlaciones más bajas con respecto a los métodos enzimáticos (Véase Fig. 3.2 y 3.4), sugiriendo que tal vez deberían mejorarse a fin de incrementar la precisión de los resultados. En cualquier caso, parece ser que la biomasa es el factor determinante a la hora de estimar los flujos metabólicos mediados por el zooplancton. Así, Huntley y Lopez (1992) observaron que la variabilidad de la biomasa comunitaria era entre uno y tres órdenes de magnitud superior a la de las tasas específicas del metabolismo. Por tanto, resulta evidente la necesidad de estimar de forma precisa la

biomasa de estas comunidades, a fin de reducir nuestra incertidumbre sobre sus flujos metabólicos.

En definitiva, se espera que las ecuaciones específicas para la temperatura desarrolladas en este estudio puedan resultar útiles a la hora de estimar los flujos metabólicos mediados por el zooplancton a gran escala. Sin embargo, todavía restan muchas limitaciones por resolver. Así, el papel del alimento (cantidad y calidad) en el metabolismo, así como de otras variables (oxígeno, profundidad, etc.) todavía debería estudiarse con mayor detenimiento. Del mismo modo, deberían desarrollarse ecuaciones adecuadas a niveles taxonómicos más detallados, con el objeto de reducir la imprecisión debida a la taxonomía. Igualmente, es muy probable que se requieran ecuaciones adaptadas a zonas con condiciones diferenciadas, como es el caso de las *zonas de mínimo de oxígeno* (OMZs, Kiko et al, 2015a, 2015b), afloramientos, zonas de elevada actividad mesoescalar, zonas eutróficas, costeras, etc.

III.4 Flujo activo del zooplancton en el océano cálido: relaciones con la clorofila y la temperatura

Juan Carlos Garijo, Maria Luz Fernández de Puelles y Santiago Hernández León

En este trabajo se examina la distribución vertical de la biomasa del zooplancton, así como sus tasas respiratorias, de mortalidad, egestión y excreción de amonio, con el fin de estimar el flujo activo de carbono y nitrógeno debido a la migración vertical de estos organismos en el Atlántico tropical y subtropical (10ºS-25ºN). Para ello, empleamos un IBS en combinación con ecuaciones metabólicas recientemente desarrolladas (Garijo et al., Capítulo 3), relacionando las tasas fisiológicas con la temperatura y el peso corporal. Además de estudiar la contribución de la exportación de carbono de estas comunidades a la bomba biológica e identificar los principales grupos taxonómicos responsables, se estudiaron

posibles correlaciones entre datos de variables ambientales de la superficie del océano, normalmente medidas mediante teledetección, y la exportación de carbono realizada por el zooplancton migrante.

Nuestros resultados señalan la importancia del flujo activo por parte del zooplancton en cuanto a su contribución a la bomba biológica. Muchos autores ya lo remarcaron previamente indicando que, en algunos casos, el flujo activo podría ser incluso superior al flujo pasivo (*e.g.,* Lampitt et al., 1993; Dam et al., 1995; Hidaka et al., 2001; Schnetzer y Steinberg, 2002; Steinberg et al., 2002; Kobari et al., 2013; Stuckel et al., 2013). En nuestro caso, a pesar de que no pudimos medir el flujo gravitacional, algunas de nuestras estimaciones de flujo activo mediado por el zooplancton fueron superiores a las de otros estudios con condiciones similares, en los que dicho flujo fue similar, e incluso superior, al flujo pasivo.

La biomasa migrante parece ser el factor que determina la magnitud del flujo activo tal y como observó previamente Dam et al (1995) pues, en nuestro caso, éste siguió la distribución de la primera en lugar de la de las tasas metabólicas (Véase Fig. 4.4 y 4.5). Ello coincide con las observaciones previas de Huntley y Lopez (1992), quienes observaron que, a la hora de estimar la producción, la variabilidad de la biomasa de la comunidad de zooplancton fue entre uno y tres órdenes de magnitud superior a la de las tasas metabólicas específicas. Por otro lado, la localización geográfica y las condiciones hidrográficas de cada región influyen claramente en la magnitud del flujo activo por parte del zooplancton. A este respecto, a pesar de que nuestro estudio se desarrolló a lo largo de una única sección latitudinal, es cierto que se exploraron regiones con una elevada heterogeneidad en términos de temperatura y productividad (Véase Fig. 4.1). Como consecuencia, nuestras estimaciones de biomasa migrante y flujo activo cubrieron el rango completo de valores encontrados en la literatura (Véase Tabla 4.2). Así, nuestras estimaciones en la región del afloramiento oceánico de Cabo Blanco se encontraban entre los valores más altos encontrados en todos los estudios (1649.4 mg C m^{-2}), mientras que se observó el fenómeno contrario en la región oligotrófica al sur del Ecuador (35.4 mg C m^{-2}).

En este estudio se remarca además la importancia de estimar el flujo activo debido a la egestión y la excreción de amonio de estos organismos, pero sobre todo debido a la mortalidad (Véase Fig. 4.5). Así, observamos que éste último, escasamente tenido en cuenta en trabajos en los que se estudia el flujo migrante por parte del zooplancton, podría ser incluso superior al flujo respiratorio, algo que ya vieron anteriormente otros autores, como Zhang y Dam (1997) e Hidaka et al. (2001). En cuanto a la egestión, coincidimos con estudios previos como el de Lampitt et al. (1993), Hernández-León et al. (2001), Schnetzer y Steinberg (2002), Yebra et al. (2005b), Kobari et al. (2008), Putzeys et al. (2011) y Kobari et al. (2013) en los que se remarca la importante contribución de este flujo con respecto al carbono total exportado por parte del zooplancton, y la necesidad de llevar a cabo estudios en los que dicho mecanismo de la bomba biológica sea tenido en cuenta. Lo mismo ocurre con el nitrógeno exportado por medio de la excreción de amonio. Nuestros resultados fueron muy similares a los valores encontrados en estudios en los que el flujo activo fue similar o superior al flujo pasivo, apoyando la argumentación de otros autores en cuanto a desarrollar estudios abarcando dicho componente de la bomba biológica (Dam et al., 1995; Steinberg et al., 2002; Stuckel et al., 2013).

En cualquier caso, la comparativa de estimaciones de flujo activo entre estudios y regiones es algo siempre complicado, pues no se dispone de una metodología estandarizada para llevar a cabo los cálculos. Así, debido al peso de la biomasa migrante en la magnitud del flujo activo, los resultados se ven principalmente influenciados por la forma en la que se estima este parámetro. Por ejemplo, cuando la estimación de la biomasa migrante se basa en incrementos en la capa epipelágica durante la noche es muy probable que se produzca una sobreestimación de la misma, pues los organismos más grandes son capaces de evitar la red durante el día en las capas iluminadas (Ianson et al., 2004). En cambio, es muy posible que la biomasa migrante sea subestimada cuando los cálculos se basan en incrementos diurnos por debajo de la capa eufótica, en la que los organismos pueden detectar la red durante el día incluso en capas profundas (Ianson et al., 2004). Por tanto, señalamos la importancia de estimar de forma precisa la

biomasa migrante, ya que influye de forma notable en las estimaciones de flujo activo. Además, existen otros factores que también influyen en los resultados, como por ejemplo, los componentes tenidos en cuenta en las estimaciones (meso- y/o macrozooplancton), los mecanismos considerados (respiración, mortalidad, egestión, excreción de amonio), la profundidad considerada para calcular el carbono exportado (150, 200, 300 m, etc.) o el método utilizado para estimar el metabolismo del zooplancton (Véase Sección 4.4.1).

Finalmente, teniendo en cuenta las correlaciones observadas entre datos *in situ* de la concentración de clorofila y temperatura en la superficie del océano, y la biomasa y el carbono exportado por medio del zooplancton migrador (Véase Tabla 4.4), parece ser que estos factores influyen en la magnitud del flujo activo por parte de estas comunidades a lo largo del Atlántico tropical y subtropical. Puesto que el flujo activo pareció depender fundamentalmente de la magnitud de biomasa (migrante), estas elevadas correlaciones no sorprenden pues Irigoien et al. (2014) sugirieron recientemente que la biomasa de peces mesopelágicos está principalmente determinada por la producción primaria a nivel global.

A pesar de que es igualmente posible obtener medidas de producción primaria a partir de teledetección para correlacionar con el zooplancton, consideramos más apropiado el uso de clorofila superficial, puesto que las estimaciones de producción primaria se basan en relaciones empíricas que usan, entre otros datos, la clorofila y la temperatura medidas por satélite (*e.g.,* Dunne et al., 2007). Por lo tanto, la producción primaria no representa una variable independiente, medida con un mayor porcentaje de error.

A pesar de que nuestros datos proceden de una única sección latitudinal a lo largo del Atlántico tropical y subtropical, es importante remarcar que nuestros resultados cubrieron el rango de biomasa migrante y flujo activo encontrados en la literatura. En cualquier caso, nuestra intención fue la de ilustrar el posible potencial de la teledetección para explorar de forma regular los flujos activos del zooplancton a gran escala en un futuro próximo.

Todavía existen muchas limitaciones, por lo que consideramos necesario el desarrollo de modelos mucho más complejos con el fin de obtener estimaciones precisas. Resulta igualmente necesaria la realización de más estudios (incluyendo el flujo gravitacional) para poder incrementar la cobertura espacial de los modelos, mientras que determinadas regiones (afloramientos, aguas costeras, regiones con elevada actividad mesoescalar, OMZs, etc.) en las que se dan unas condiciones especiales, requerirán el desarrollo de trabajo específico.

IV. Síntesis y trabajo futuro

IV.1 Discusión general

IV.1.1 El uso de IBS como metodología alternativa

Los resultados obtenidos durante la presente tesis sugieren que los IBS pueden ser tan fiables como la metodología enzimática y tradicional para determinar la biomasa comunitaria y flujos metabólicos del zooplancton. Las estimaciones a este respecto no fueron significativamente diferentes (Véase los *Capítulos 1* y *3),* aunque los primeros resultaron ser procesos más rápidos y económicos. Por tanto, es de esperar que estos sistemas pudiesen ser aplicados en estudios cubriendo amplias regiones o escalas temporales grandes (*e.g.,* Benfield et al., 2007; Gislason y Silva, 2009; Gorsky et al., 2010). Además, tienen otras ventajas. Por ejemplo, los organismos se miden de forma individualizada y son clasificados en grupos taxonómicos de forma semi-automatizada (*e.g.,* MacLeod et al., 2010; Gorsky et al., 2010; Bachiller et al., 2012). Esto permite trabajar en estudios en los que se determina la distribución del espectro de tallas y sus implicaciones ecológicas, y además estimar el peso corporal y el metabolismo de cada individuo de forma separada. Adicionalmente, las muestras no se destruyen, quedando disponibles para estudios posteriores.

Sin embargo, el tipo de ecuación metabólica o relación empírica que se emplea para determinar el metabolismo y la biomasa influye notablemente en los resultados (Garijo y Hernández-León, 2015). Así, observamos en los *Capítulos 1 y 3* que el crecimiento y la respiración son probablemente estimados de forma más precisa cuando las ecuaciones metabólicas se ajustan a las condiciones específicas de cada región, en lugar de aplicar ecuaciones generalistas, únicas para todo el océano global (Véase Sección IV.1.3). En el caso de la biomasa, Lehette y Hernández-León (2009) ajustaron relaciones empíricas entre el área y el peso corporal con coeficientes de determinación bastante altos (r^2>0.80, Tabla 1.1). Además, puesto que los individuos fueron congelados antes de ser fotografiados y pesados a fin de establecer las relaciones empíricas, se evitó el problema de

pérdida de peso que normalmente ocurre en plancton cuando se utiliza formol como conservante (*e.g.,* Omori, 1970; Durbin y Durbin, 1978; Landry, 1978). Por otro lado, no se ha detectado previamente ningún efecto de reducción del área de los organismos usando este conservante químico (Landry, 1978; Durbin y Durbin, 1978; Viitasalo, 1995; Pollupüü, 2007). Por tanto, dado que las estimaciones de biomasa mediante un IBS normalmente se basan en determinaciones del área de los individuos, estos sistemas podrían ser finalmente considerados como alternativa válida a los métodos tradicionales a la hora de determinar la biomasa comunitaria de zooplancton (*Capítulo 1*).

Aparte de usar los IBS para estudiar el crecimiento y la respiración del zooplancton, proponemos estos sistemas para determinar las tasas de egestión de estas comunidades, mediante un proceso más rápido que la metodología normalmente utilizada (*Capítulo 1*). La relación desarrollada en esta tesis entre el peso de los individuos y su contenido estomacal, combinada con la de Irigoien (1998) relacionando la temperatura y las tasas de evacuación, condujo a resultados realistas de las tasas de egestión en comparación con estimaciones previas en la misma zona (*e.g.,* Hernández-León et al., 2004, 2007), así como con medidas de *fluorescencia del tracto digestivo* en nuestras propias muestras (Véase Fig. 1.7). Como era de esperar, las estimaciones usando este método fueron inferiores a las obtenidas con el IBS, pues los organismos en aguas oligotróficas son normalmente omnívoros (*e.g.*, Saiz et al., 1999; Hernández-León et al., 2001, 2001). Basados en las estimaciones de crecimiento, respiración y egestión usando el IBS, finalmente parece que estos sistemas suponen una alternativa válida para estudiar los flujos de ingestión mediados por el zooplancton a escalas espaciales grandes (Véase Fig. 1.8).

En cualquier caso, los resultados del *Capítulo 1* sugieren que los métodos enzimáticos podrían ser más apropiados para estudiar las tasas metabólicas específicas. Aunque se sabe que estos métodos presentan algunas incertidumbres (*e.g.,* Hernández-León et al, 1995; Hernández-León y Gómez, 1996), las estimaciones a partir de la actividad enzimática fueron

empleadas como referencia. Ello permitió comprobar la posible utilidad del IBS y las diferentes ecuaciones predictivas, a través de comparativas entre dos variables aleatorias (regresión lineal tipo II).

IV.1.2 Acoplamiento físico-biológico en estructuras de mesoescala

Los resultados incluidos en el *Capítulo 2* mostraron que el IBS podría ser además útil para determinar la biomasa y el metabolismo del zooplancton en regiones con una elevada actividad mesoescalar. Sorprendentemente, observamos un claro acoplamiento físico-biológico, ya que las estructuras mesoescalares claramente influyeron en la distribución tridimensional de los organismos, en términos de tallas y biomasa, e influenciaron su metabolismo, puesto que el zooplancton normalmente seguía las señales de las estructuras físicas.

En la *Zona de Transición Costera* (CTZ) del Noroeste de África, observamos que los filamentos y los *jet* costeros transportaron zooplancton desde las aguas del afloramiento hasta la región oligotrófica cercana a las Islas Canarias, como previamente se ha visto en el caso de la Chl*a*, nano- y picoplancton, zooplancton e ictioplancton (*e.g.,* Hernández-Guerra et al., 1993; Basterretxea y Arístegui, 2000; Hernández-León et al., 2002; Baltar et al., 2009; Bécognée et al., 2009; Moyano et al., 2014). Además, las interacciones entre estos filamentos y remolinos inducidos por las islas, incrementaron la biomasa de zooplancton en estos últimos, como previamente observaron Hernández-León et al. (2002). El hundimiento o elevación de las isotermas, dependiendo de remolinos ciclónicos o anticiclónicos, influenció la distribución vertical de los organismos (Véase Fig. 2.3 y 2.4). Así, los individuos normalmente fueron arrastrados a capas más profundas en remolinos anticiclónicos, tal y como previamente observaron Arístegui et al. (1997). Y lo contrario en el caso de estructuras ciclónicas, donde estos mismos autores sugirieron que la elevación de las isotermas y el bombeo de nutrientes hacia la capa más somera e iluminada podría provocar un aumento de la biomasa en la superficie. Adicionalmente,

como parece ser que el metabolismo del zooplancton depende principalmente de la temperatura (*e.g.,* Hirst y Lampitt, 1998; Hirst et al., 2003), el hundimiento o la elevación de las isotermas tuvo consecuencias en el crecimiento, respiración y egestión de los individuos dentro de dichas estructuras (Véase Fig. 2.7).

Los sistemas frontales también pueden influir en la distribución de los organismos. Como hemos observado, el frente generado entre las aguas frías y productivas del afloramiento costero, y las más cálidas y oligotróficas hacia el océano, actuó como barrera natural reteniendo Chl*a* y zooplancton (Véase Fig. 2.5). A este respecto, Moyano et al. (2014) observaron el mismo efecto para el ictioplancton en este mismo frente.

El zooplancton exportado desde las aguas del afloramiento hasta las proximidades del Archipiélago Canario, y su retención en frentes y remolinos, podría finalmente repercutir en las pesquerías locales, pues estas comunidades constituyen el alimento natural de las larvas de peces. Sin embargo, todavía son necesarios más estudios para comprender los posibles efectos en su totalidad. En cualquier caso, la productividad del afloramiento no es constante a lo largo de todo el año. Así, comparando nuestros resultados (época de afloramiento debilitado) y los obtenidos por Hernández-León et al. (2002), durante la estación de máxima productividad, sugiere que la estacionalidad es el factor determinante en cuanto a la capacidad de las estructuras físicas para exportar zooplancton hacia el océano.

IV.1.3 Ecuaciones metabólicas específicas para regiones específicas

Las estimaciones de metabolismo a partir de nuestras ecuaciones específicas para la temperatura (*Capítulos 1* y *3)* presentaron una mejor correlación con medidas de métodos enzimáticos que las estimadas a partir de ecuaciones generalistas, previamente desarrolladas por Hirst et al. (Hirst et al., 2003), Hirst y Bunker (Hirst y Bunker, 2003) y Zhou et al. (Zhou et al., 2010) para el crecimiento, e Ikeda (Ikeda, 1985, 2014) para la respiración.

Las comparaciones entre métodos se realizaron a lo largo de la región subtropical de las Islas Canarias y de los 2000 primeros metros de la banda latitudinal 40ºN-40ºS, donde la temperatura variaba entre 2.5 y 30ºC. Además, se exploraron regiones oligo-, meso- y eutróficas, mostrando por tanto la posible utilidad de estas ecuaciones para determinar el metabolismo del zooplancton en un escenario heterogéneo y cuasi-global, desde la superficie hasta la capa batipelágica.

Como el metabolismo parece estar determinado en su mayoría por la temperatura y el peso de los individuos (*e.g.,* Ikeda et al., 2014), desarrollamos nuestras ecuaciones de acuerdo a rangos ajustados de estos dos parámetros, a fin de conseguir aumentar la precisión de las estimaciones (Véase Tablas 3.2 y 3.3). De este modo, las ecuaciones específicas se ajustaron a los rangos de temperatura encontrados en las principales regiones oceánicas y capas de profundidad, en concordancia con los valores dados por Longhurst (2007) en las principales provincias oceanográficas. De forma similar, el peso corporal estimado durante la circunnavegación Malaspina-2010 sirvió como referencia para establecer los rangos de dicho parámetro para las comunidades de mesozooplancton.

La precisión (r^2) de nuestras ecuaciones específicas se incrementó en muchos casos respecto a la de ecuaciones previas, aunque es imperativa la necesidad de mejorar algunas relaciones todavía. Así, las ecuaciones de crecimiento para copépodos, dada su ubicuidad y abundancia en el océano global, presentaban una r^2 demasiado baja. De acuerdo a los resultados obtenidos, tanto las ecuaciones generalistas como las específicas podrían considerarse como alternativas válidas a los métodos enzimáticos para estimar el metabolismo del zooplancton a escalas espaciales (geográficas y verticales) y temporales grandes. De hecho, en el caso de la respiración, las estimaciones usando ambos tipos de ecuaciones coincidían con la distribución y los rangos de valores de la revisión realizada por Hernández-León e Ikeda (2005). Sin embargo, es cierto que el crecimiento y la respiración usando ecuaciones generalistas fue significativamente inferior al estimado mediante métodos enzimáticos y ecuaciones específicas. Por tanto, parece que éstas

últimas podrían resultar más precisas para estimar el metabolismo, dependiendo de las condiciones específicas de cada ambiente.

La posible menor precisión usando ecuaciones generalistas podría deberse a diferentes razones. Por ejemplo, el uso de datos extremos (-2.0ºC>T>30ºC) usados para configurar las ecuaciones de Hirst et al. (Hirst et al., 2003), Hirst y Bunker (Hirst y Bunker, 2003) e Ikeda (Ikeda, 1985, 2014) podría introducir considerables incertidumbres en los resultados en ciertas regiones. A este respecto, se sabe que la temperatura influye notablemente en el metabolismo: éste incrementa con la temperatura y viceversa (*e.g.,* Hirst y Lampitt, 1998; Hirst et al., 2003). Por otro lado, los valores de Chl*a* manejados por Hirst y Bunker (2003) y Zhou et al. (2010) como referencia en sus ecuaciones, fueron hasta dos órdenes de magnitud superiores a los que normalmente se dan en aguas oligotróficas (Garijo y Hernández-León, 2015), sugiriendo el uso de estas ecuaciones para sistemas productivos. Por otro lado, dado que el zooplancton en estas regiones es en su mayoría omnívoro (*e.g.,* Saiz et al., 1999), parece que el uso de la Chl*a* como único *proxy* de la disponibilidad de alimento pudiera resultar inapropiado. Teniendo en cuenta que las regiones oligotróficas constituyen la mayor fracción de los océanos, debería ponerse especial atención cuando se usan estas ecuaciones para realizar estimaciones a gran escala, pues podrían subestimar el metabolismo en estas vastas regiones del océano (*e.g.,* Stock et al., 2014).

Finalmente, la inclusión de datos de individuos de talla superior a la que normalmente se observa en el mesozooplancton, podría igualmente introducir un cierto error, ya que el metabolismo decrece con el peso corporal (*e.g.,* Hirst y Lampitt, 1998). Así, la ecuación de Hirst et al. (Hirst et al., 2003) tal vez podría subestimar el crecimiento pues, para configurar sus ecuaciones, estos autores usaron datos de organismos de un tamaño hasta dos órdenes de magnitud superior a los que normalmente se dan en el mesozooplancton (Garijo y Hernández-León, 2015).

Por tanto, es posible que las ecuaciones específicas para la temperatura desarrolladas en esta tesis puedan resultar útiles a la hora de estimar los

flujos metabólicos del zooplancton a escala espacial grande, tal vez incrementando la precisión de las estimaciones con respecto a las ecuaciones globales existentes. Sin embargo, existen todavía muchas limitaciones a resolver. Consideramos clave el desarrollo de más ecuaciones, adaptadas a niveles taxonómicos más específicos, así como la realización de trabajos más exhaustivos en determinadas regiones con unas condiciones diferenciadas, como es el caso de los afloramientos, aguas costeras o las OMZs. En estas últimas regiones, los individuos han adaptado su metabolismo a los bajos niveles de oxígeno (Kiko et al, 2015a, 2015b), por lo que deberían desarrollarse ecuaciones especiales teniendo en cuenta las condiciones dadas.

IV.1.4 Contribución del zooplancton a la bomba biológica

Nuestras estimaciones de flujo activo del zooplancton mostradas en el *Capítulo 4* han contribuido a ampliar las medidas existentes de este importante componente de la bomba biológica. Los resultados que se desprenden de estas tesis podrían resultar útiles, pues cubren el rango completo de valores de biomasa y flujo migrante existentes en la literatura (véanse Tablas 4.2 y 4.3). Esto se dio probablemente como consecuencia de la elevada variabilidad de las regiones exploradas a lo largo del Atlántico tropical y subtropical, en términos de productividad y temperatura (Véase Fig. 4.1). Así, las estimaciones en la región del afloramiento oceánico de Cabo Blanco se encontraron entre los valores máximos de biomasa migrante y flujo activo encontrados de la literatura, mientras que lo opuesto se observó a lo largo de la región oligotrófica al sur del Ecuador. A este respecto, parece que la localización geográfica, que determina las condiciones hidrográficas de cada región, es uno de los factores que influye en mayor grado los flujos migrantes.

Con este trabajo se contribuye a reducir la evidente falta de estudios abordando estimaciones de flujo activo por medio de la mortalidad, egestión y excreción de amonio por parte del zooplancton migrador (Véase Tablas 4.2 y 4.3). En este sentido, señalamos la importancia de estos mecanismos en

cuanto a su contribución a la bomba biológica y la necesidad de ser incluidos en estimaciones de carbono total exportado por parte del zooplancton. Así, observamos que el flujo debido a la mortalidad, normalmente no tenido en cuenta en trabajos estudiando el flujo activo por parte del zooplancton, podría ser incluso superior al flujo respiratorio (Véase Fig. 4.5). A este respecto, Zhang y Dam (1997) e Hidaka et al. (2001) ya mostraron la importancia de estimar este importante componente del exporte mediado por el zooplancton.

Muchos autores han remarcado la importancia del flujo activo en el funcionamiento de la bomba biológica, puesto que en algunos casos éste puede ser incluso superior al flujo pasivo (*e.g.,* Lampitt et al., 1993; Dam et al., 1995; Hidaka et al., 2001; Schnetzer y Steinberg, 2002; Steinberg et al., 2002; Kobari et al., 2013; Stuckel et al., 2013). En nuestro caso, a pesar de que no pudimos medir el flujo gravitacional, nuestras estimaciones de carbono exportado por el zooplancton fueron, en algunas regiones, superiores a las de otros estudios con condiciones similares en los que el flujo activo fue similar o incluso superior al flujo pasivo.

Por otro lado, las comparativas de flujo activo entre regiones son siempre complicadas, pues la magnitud de las estimaciones depende de los componentes incluidos en los cálculos (meso-, macrozooplancton) y de los mecanismos considerados (respiración, mortalidad, egestión, excreción de amonio). Además, los resultados están influenciados por otros factores, como son la profundidad considerada para calcular el carbono exportado (150, 200, 300 m, etc.), cómo se calcula la biomasa migrante o el método utilizado para estimar el metabolismo del zooplancton (Véase Sección 4.4.1).

En cualquier caso, señalamos la importancia de la biomasa migrante en la magnitud del flujo activo mediado por el zooplancton, tal y como mostraron previamente Dam et al. (1995). En este sentido, observamos que el carbono exportado por parte de estas comunidades claramente seguía el patrón de distribución de la biomasa migrante, en lugar del de las tasas metabólicas específicas (Véase Fig. 4.4 y 4.5). Esto coincide con las observaciones de

Huntley y Lopez (1992), quienes sugirieron la biomasa comunitaria como el factor determinante en la estimación de la producción en zooplancton. Por tanto, señalamos la importancia de estimar de forma precisa la biomasa migrante de estas comunidades, más si cabe teniendo en cuenta que su magnitud varía dependiendo del proceso elegido para su estimación (Véase Sección 4.4.1).

En definitiva, nuestras estimaciones sostienen igualmente la idea de que el flujo activo mediado por el zooplancton es uno de los mecanismos más importantes en el proceso de exporte de materia orgánica hacia la capa mesopelágica. La contribución de estas comunidades a la bomba biológica puede llegar a ser comparativa o incluso superior a la del flujo pasivo (Dam et al., 1995; Hidaka et al., 2001; Steinberg et al., 2002, 2008; Stuckel et al., 2013). Por tanto, consideramos que deberían realizarse más estudios de este tipo, incluyendo por supuesto el flujo activo a través de la mortalidad, la egestión y la excreción de amonio, a fin de reducir nuestra incertidumbre actual acerca de la naturaleza de la bomba biológica en el océano.

IV.1.5 Exploración del zooplancton por medio de la teledetección

Se han observado importantes correlaciones entre la biomasa migrante y el flujo activo por medio del zooplancton y datos *in situ* de la concentración superficial de clorofila y temperatura a lo largo de la región tropical y subtropical del Océano Atlántico (*Capítulo 4)*. Los datos de estas variables pueden obtenerse de forma regular por teledetección. De acuerdo a estas correlaciones, se desarrollaron modelos simples (Véase Tabla 4.4). A este respecto, Irigoien et al. (2014) también observaron recientemente que la biomasa de peces mesopelágicos estaba principalmente determinada por la distribución de la producción primaria a nivel global. A pesar de que nuestros resultados provenían simplemente de una sección latitudinal en el Atlántico, el programa de muestreo planteado permitió explorar un amplio gradiente latitudinal, altamente heterogéneo en términos de productividad y temperatura (Véase Fig. 4.1), incrementándose en cierto modo la robustez de nuestros resultados. En cualquier caso, es obvio que todavía deberían

desarrollarse modelos más complejos, combinando muchas más variables. En este sentido, algunos investigadores ya están trabajando en este aspecto, intentando comprender todas las posibles relaciones existentes entre los flujos de carbono exportado por parte del zooplancton y datos de la superficie del océano obtenidos por medio de la tecnología actual (*e.g.,* EXPORTS, http://exports.oceancolor.ucsb.edu).

En cualquier caso, nuestra intención fue ilustrar el posible potencial futuro de usar datos medidos por teledetección para determinar de forma regular el papel del zooplancton en la bomba biológica en el océano. Los datos requeridos para ello pueden ser adquiridos con relativa facilidad, permitiendo la exploración de regiones remotas, demasiado caras de muestrear directamente. De todos modos, todavía restan muchas limitaciones por resolver. Además de desarrollar modelos mucho más sofisticados, la precisión de las ecuaciones metabólicas debería mejorarse, mientras que resultaría igualmente necesario desarrollar más ecuaciones, ajustadas a más grupos taxonómicos. Además, como ocurre con las ecuaciones metabólicas, el modelado en ciertas regiones, como es el caso de las OMZs -que ocupan el 9% de la superficie del océano (Fuenzalida et al., 2009)-, sistemas costeros y de afloramiento o regiones con una elevada actividad mesoescalar, requerirán el desarrollo de trabajo específico.

IV.2 Conclusiones

En general, las principales conclusiones que se desprenden de la investigación llevada a cabo durante la presente tesis son las siguientes:

1. Los sistemas de análisis de imágenes (IBS) son tan precisos como los métodos tradicionales y enzimáticos para estudiar la biomasa y los flujos metabólicos de zooplancton a escalas espaciales y temporales grandes, aunque los primeros resultaron ser procesos más rápidos y económicos. En cambio, los métodos enzimáticos parecen resultar más apropiados para estimar las tasas metabólicas específicas a lo largo de series temporales.

2. La relación propuesta entre el peso y el contenido estomacal de los individuos, en combinación con un IBS, es una herramienta útil para estimar las tasas de egestión del zooplancton por medio de un proceso relativamente rápido.

3. Las estructuras mesoescalares en la *Zona de Transición Costera* del Noroeste de África determinaron la distribución tridimensional de la biomasa y el metabolismo del zooplancton. Como consecuencia, se observó un acoplamiento físico-biológico, donde los patrones del zooplancton siguieron las señales de las estructuras físicas. Dado que estas comunidades representan el alimento natural de las larvas de peces, el zooplancton exportado desde las aguas del afloramiento a las proximidades del Archipiélago Canario podría ejercer una influencia sobre las pesquerías locales. En cualquier caso, la estacionalidad parece ser el factor más determinante en este proceso.

4. Las ecuaciones metabólicas específicas para la temperatura, desarrolladas en esta tesis de acuerdo a las condiciones de cada región, incrementaron la precisión de las estimaciones de flujos metabólicos usando un IBS con respecto a las ecuaciones globales existentes. Sin embargo, todavía se precisan nuevas ecuaciones con las que abordar las limitaciones presentes.

5. Nuestras estimaciones de biomasa migrante y flujo activo mediado por el zooplancton a lo largo del Atlántico tropical y subtropical cubrieron el rango total de valores encontrados en la literatura. Asimismo, observamos una importante correlación entre el carbono exportado por parte del zooplancton y la concentración de clorofila y temperatura superficiales, por lo que su predicción por medio de teledetección podría considerarse en un futuro próximo.

IV.3 Investigación futura

Debido a ciertas limitaciones de los métodos tradicionalmente utilizados para estimar la biomasa y los flujos metabólicos del zooplancton a lo largo de escalas espaciales grandes, la investigación llevada a cabo durante la presente tesis tenía por objetivo desarrollar y probar herramientas alternativas, con el fin de estimar estos parámetros de forma más rápida y económica. Sin embargo, existen todavía una serie de limitaciones metodológicas que deberían resolverse en un futuro próximo. Aunque algunas de éstas ya se han ido relatando en los capítulos anteriores, parece razonable compilar la problemática identificada, así como proporcionar algunas pautas a este respecto:

1. Los IBS parecen constituir una alternativa para medir la biomasa comunitaria y los flujos metabólicos del zooplancton a gran escala. Sin embargo, una de las mayores limitaciones a las que se enfrentan estos sistemas a día de hoy es su reducida capacidad para clasificar los organismos de acuerdo a niveles taxonómicos detallados, así como el error cometido durante la clasificación automatizada. Como solución, ciertos programas recientemente desarrollados, como es el caso de *Ecotaxa* (http://ecotaxa.sb-roscoff.fr), permiten una exploración visual de las imágenes, bien para corregir los errores cometidos durante la clasificación automática, bien para organizar los individuos en niveles taxonómicos más precisos. Todo ello, a través de un compromiso entre el tiempo, el esfuerzo y la precisión conseguida en los resultados. Todo esto dependerá de los objetivos particulares de cada estudio. Por tanto, el uso de este tipo de programas en estudios futuros conducirá a un incremento en la calidad de los resultados. Incluso en términos de biomasa, pues hay que tener en cuenta que se usan diferentes relaciones área-peso individual para cada grupo. Así, obtener una clasificación taxonómica de los individuos sin errores permitirá aplicar cada relación de una forma más acertada.

2. Tal y como se ha detallado en capítulos previos, aunque se espera que las ecuaciones metabólicas específicas para la temperatura, desarrolladas en esta tesis de acuerdo a las condiciones específicas de cada región (*Capítulos 1 y 3),* incrementen la precisión de las estimaciones de crecimiento y respiración con respecto a las ecuaciones globales, todavía son necesarias ciertas mejoras. La precisión (r^2) de las ecuaciones de crecimiento para copépodos son bastante bajas, más si cabe teniendo en cuenta que su abundancia normalmente representa más del 80% del total en todos los hábitats oceánicos. Además, la inclusión de otras variables (*e.g.,* concentración de oxígeno, profundidad, etc.) debería estudiarse con mayor detenimiento. En este sentido, debería investigarse si resultaría necesario desarrollar ecuaciones especificas para estimar de forma precisa el metabolismo del zooplancton en regiones con unas condiciones diferenciadas. Así, como se ha discutido en el *Capítulo 3*, los organismos presentes en la columna de agua de las OMZs presentan un metabolismo reducido como respuesta a los niveles críticos de oxígeno que se dan en estas regiones, en comparación con comunidades que habitan bajo condiciones comparables de temperatura y productividad. Por otro lado, los organismos en aguas eutróficas, como las regiones de afloramiento o los sistemas costeros, podrían presentar un metabolismo bastante diferente al de individuos en aguas menos productivas. En este sentido, debería estudiarse en profundidad la posible influencia del alimento (cantidad y calidad) en el metabolismo del zooplancton. Finalmente, puesto que el metabolismo difiere entre grupos taxonómicos, consideramos igualmente necesario el desarrollo de más ecuaciones, para niveles taxonómicos más complejos, a fin de incrementar la precisión de las estimaciones.

3. De acuerdo a los resultados presentados en el *Capítulo 4*, remarcamos la importante contribución del zooplancton en el funcionamiento de la bomba biológica en el océano. En este sentido, esta tesis podría suponer una importante contribución, pues las estimaciones de flujo activo

cubrieron la totalidad del rango de valores encontrados en la literatura. Además, se obtuvieron estimaciones del carbono exportado por medio de la mortalidad, egestión y excreción de amonio del zooplancton, señalando la importancia de llevar a cabo estudios incluyendo estos mecanismos, normalmente obviados en las estimaciones de flujos mediados por el zooplancton. En cualquier caso, las comparativas de flujo activo entre estudios son más bien complicadas como consecuencia de la variedad de métodos y procesos existentes a la hora de estimar la biomasa migrante y el carbono exportado por parte de estas comunidades (Véase Sección I.4). En este sentido, consideramos totalmente necesario una estandarización de las metodologías y los cálculos involucrados en la estimación del mismo.

4. Las correlaciones observadas entre el zooplancton y datos de variables ambientales, normalmente obtenidos por medio de satélites (*Capítulo 4*), podrían ilustrar las posibilidades futuras de exploración del carbono exportado por parte de estas comunidades a gran escala. En cualquier caso, es evidente que se requieren modelos más complejos y robustos. En este sentido, debería investigarse con detalle la influencia de todas las variables disponibles por medio de teledetección en los resultados. Finalmente, como ocurre con las ecuaciones metabólicas, este tipo de modelos requerirán el desarrollo de trabajo específico en determinadas regiones (*e.g.,* OMZs, afloramientos, sistemas costeros, regiones con elevada actividad mesoescalar, etc.), donde se dan unas condiciones particulares.

5. Incrementar la cobertura espacial de los muestreos requerirá el uso de equipos que permitan ahorrar tiempo y esfuerzo con respecto a las metodologías actuales. En este sentido, el uso de aparatos de reciente creación, como es el *Underwater Vision Profiler* (UVP, http://www.hydroptic.com/uvp.html, Picheral et al., 2010), permite la

adquisición de imágenes de zooplancton mayor de 100 μm directamente en la columna de agua, y hasta 6000 m de profundidad. Estos sistemas, acoplados a la Roseta oceanográfica, permiten el muestreo de zooplancton al mismo tiempo que se miden variables físicas y químicas, ahorrando así el tiempo que se dedica normalmente a las redes de muestreo durante las campañas. En este sentido, los sistemas *UVP* además evitan el consumo de tiempo entre el muestreo de redes y la digitalización de los organismos, puesto que las imágenes están disponibles para ser procesadas por el IBS directamente después del muestreo. Al mismo tiempo, estos equipos permiten explorar la distribución del zooplancton en la columna de agua de acuerdo a una resolución de centímetros, con la gran información que ello supone.

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