UNIVERSIDAD DE LAS PALMAS
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Facultad de Ciencias del Mar
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TESIS DOCTORAL

Identification, Abundance and Rearing of Mysids from Gran Canaria: Application to Laboratory and Oceanographic Respiratory Metabolism Studies

Alicia Herrera Ulibarri

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UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

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UNIVERSIDAD DE LAS PALMAS
DE GRAN CANARIA

Tesis Doctoral
Programa de Doctorado en Oceanografía
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Identification, Abundance and Rearing of Mysids from Gran Canaria: Application to Laboratory and Oceanographic Respiratory Metabolism Studies

(Identificación, abundancia y cultivo de misidáceos de Gran Canaria: aplicación en estudios de laboratorio y oceanográficos de metabolismo respiratorio)

Memoria presentada por D.ª Alicia Herrera Ulibarri para la obtención del Doctorado en Oceanografía en la Universidad de Las Palmas de Gran Canaria y dirigida por los Doctores D.ª María M. Gómez Cabrera y D. Ted Packard.

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Septiembre 2013

Al Universo

Cuando escuché al docto astrónomo,
cuando me presentaron en columnas
las pruebas y guarismos,
cuando me mostraron las tablas y diagramas
para medir, sumar y dividir,
cuando escuché al astrónomo discurrir
con gran aplauso de la sala,
qué pronto me sentí inexplicablemente
hastiado,
hasta que me escabullí de mi asiento y
me fui a caminar solo,
en el húmedo y místico aire nocturno,
mirando de rato en rato,
en silencio perfecto a las estrellas.
Walt Whitman

Agradecimientos

Cuando dedico esta tesis al Universo es porque estoy convencida que no existen las casualidades sino las causalidades, así que de alguna forma he llegado aquí desde el sur del sur, de alguna forma May y Ted se han puesto en mi camino y de alguna forma hoy estoy aquí escribiendo estos agradecimientos. A ellos dos debo agradecerles el haber confiado en mí, y por haber priorizado siempre las relaciones personales y los sentimientos frente a las publicaciones. Por la comprensión y el apoyo frente a las dificultades y por mantener la alegría y la pasión por entender el océano.

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También cuando lo dedico al Universo es para ahorrarme la larga lista de personas a las que debería agradecerles su ayuda en estos cuatro años, pero la verdad es que de todos modos me siento en la obligación de hacerlo.

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A mi familia en Uruguay: Matilde, Abú, Atá, Mica y Emi que siempre

estarán para apoyarme en todos mis proyectos.

A mi padre por enseñarme dos cosas fundamentales: a reírme mucho de todo, especiamente de mí; y a diferenciar cuál es el verdadero éxito.

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Resumen

Los misidáceos son crustáceos peracáridos que tienen una gran relevancia ecológica, sobre todo en zonas costeras donde son muy abundantes. En los últimos tiempos ha aumentado el interés en su estudio debido a su importancia en la cadena trófica ya que son el principal alimento de muchos peces costeros, especialmente juveniles. Son uno de los principales componentes del suprabentos, y recientemente se ha demostrado que intervienen en la remineralización de una gran parte de los detritos y en la regeneración de nitrógeno.

A pesar de su importancia, hasta la actualidad no se habían llevado a cabo estudios sobre la abundancia de estos organismos en Gran Canaria. La presente tesis supone un avance en el conocimiento de las especies que habitan la costa de Gran Canaria, particularmente en los "sebadales" o praderas de *Cymodocea nodosa*. Estos estudios han puesto de manifiesto el importante papel que juegan los misidáceos en estos ecosistemas, así como también la necesidad de preservar su hábitat.

El cultivo de dos especies de misidáceos y el análisis de su calidad nutricional demuestran que son un alimento de excelente calidad para ser utilizado como presa viva de peces y cefalópodos. Estos estudios también son un primer paso en la línea de investigación de nuevas especies en el campo de la acuicultura.

Los estudios del metabolismo respiratorio de *L. lingvura*, una de las especies de misidáceos que ha sido cultivada exitosamente en nuestras instalaciones, han permitido importantes descubrimientos en el campo de la ecofisiología del zooplancton. Se ha demostrado que factores como la inanición afectan a la respiración pero no a la actividad del sistema de transporte de electrones (ETS), por lo tanto la relación R/ETS se ve afectada de la misma

forma que la respiración. Asimismo, se ha comprobado que la disponibilidad de alimento a largo plazo afecta a la relación ETS-biomasa.

Estos resultados han posibilitado la mejora en la interpretación de los datos obtenidos con la técnica ETS y han sido aplicados en investigaciones oceanográficas de suprabentos y zooplancton en las que se han corroborado los resultados obtenidos con misidáceos de cultivo.

Abtract

Mysids are peracarida crustaceans with a highly relevant ecological role, especially in coastal areas where they are abundant. Lately mysids have become topical because of their importance in the food chain; they are the main food for many coastal fish, especially in their juvenile stages. In addition, mysids are one of the principal components of suprabenthos, and have recently been shown to remineralize a large portion of detritus and to regenerate inorganic nitrogen salts.

Despite their importance, until now there have been few studies on the abundance of mysids in the waters off Gran Canaria. This thesis advances our knowledge of Canary Island mysid species especially those that inhabit the coastal *Cymodocea nodosa* seagrass meadows of Gran Canaria. It highlights the important role of mysids in these coastal waters, as well as the need to preserve their habitat.

The cultivation of two species of mysids and a study of their nutritional quality shows that they are an excellent food to be used as live prey for fish and cephalopods. This part of the thesis is the first step in the research of new zooplankton species for aquaculture.

Studies of the respiratory metabolism of *L. lingvura*, one of the species of mysids that has been grown successfully in our laboratory, have led to significant discoveries in zooplankton ecophysiology. They have shown that factors such as starvation affect the respiration, but not the electron transport system (ETS) activity. Consequently, the relationship, R/ETS, is affected in parallel with the effect on respiration. It was also found that the long-term availability of food affects the ETS- biomass relationship.

These results have facilitated the interpretation of ETS measurements and have been applied in zooplankton and suprabenthos oceanographic research corroborating results obtained on cultured mysids.

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Introducción general

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Introducción General

1.1. Antecedentes

Los misidáceos son pequeños organismos pertenecientes al filum Arthropoda, subfilum Crustacea. Actualmente no se clasifican dentro de un mismo orden, sino que pertenecen a tres órdenes diferentes (Lophogastrida, Mysida, Stygiomysida) según Anderson (2010a) o a dos órdenes (Lophogastrida y Mysida) según la clasificación de crustáceos publicada por Zhang (2011) dentro del superorden Peracarida.

Son un grupo altamente adaptativo, la mayoría son marinos, aunque existen unas pocas especies de agua dulce. Habitan distintos ambientes, la columna de agua, justo sobre el sustrato o dentro de él, o en cuevas submarinas (Murano, 1999). Diversos estudios demuestran que los misidáceos son un importante componente de la cadena trófica, especialmente en zonas costeras donde son muy abundantes. Son alimento de peces, cefalópodos y ballenas, sin embargo, durante muchos años se ha subestimado la importancia de este grupo debido principalmente a que las redes tradicionales de plancton o bentos han resultado no ser eficaces para su captura (Mauchline, 1980).

La mayoría de los misidáceos costeros realizan una migración vertical, ascendiendo y dispersándose en la columna de agua durante la noche, y descendiendo durante el día (Mauchline, 1980; Murano, 1999). Son rápidos nadadores y se agrupan en "enjambres", lo que dificulta muchas veces los estudios de abundancia. Se han diseñado diversos métodos específicos para su cap-

tura que incluyen redes de mano, patines bentónicos y bombas de succión, dependiendo del hábitat.

La variedad de ambientes que han colonizado hace que exploten una gran diversidad de recursos, y por lo tanto, que presenten distintos tipos de apéndices para la alimentación. Por lo general son omnívoros, en el contenido estomacal de especies costeras se han encontrado detritos, restos de diatomeas, tintínidos y cuerpos y apéndices de otros crustáceos (Mauchline, 1980; Murano, 1999).

Al igual que otros crustáceos peracáridos, las hembras presentan un marsupio donde se lleva a cabo el desarrollo de los embriones, cuando los misidáceos emergen de este marsupio tienen una forma similar a la de los adultos en miniatura (Figura 1.1). El desarrollo se divide en tres etapas que están marcadas por la puesta y dos mudas. Las etapas del desarrollo marsupial en misidáceos según Mauchline (1980) son:
Estadío I: "huevo", durante este período el embrión podría asemejarse a la estructura de un huevo. Posteriormente aparecen rudimentos de antenas y comienza el desarrollo del abdomen.
Estadío II: comienzan a desarrollarse antenas y apéndices torácicos y los ojos comienzan a pigmentarse. Esta etapa finaliza con una muda.
Estadío III: la larva presenta ojos pedunculados. No presenta estatolito en la vesícula del estatocisto. Esta etapa termina con una muda en el momento de la eclosión o inmediatamente después de la misma.

Wittmann (1981), en un extenso trabajo sobre el desarrollo marsupial en *Leptomysis lingvura* y otras especies mediterráneas, propone una nueva terminología para las mencionadas tres etapas: estadío embrionario (embryonic stage), estadío nauplioide (nauplioid stage) y estadío postnauplioide (postnauplioid stage), cada uno con subdivisiones que se corresponden a importantes cambios morfológicos (Figure 1.2). El tiempo de desarrollo marsupial en *L. lingvura* a 16°C es 0-4.6 días para el estadío embrionario, 4.6-11.2 días para el nauplioide y 11-12 días para el postnauplioide.

La duración del desarrollo en el marsupio varía según cada especie y depende de la temperatura ambiental (Mauchline, 1980). Por ejemplo en *Neomysis intermedia* el tiempo de desarrollo en el marsupio varía entre 7 y

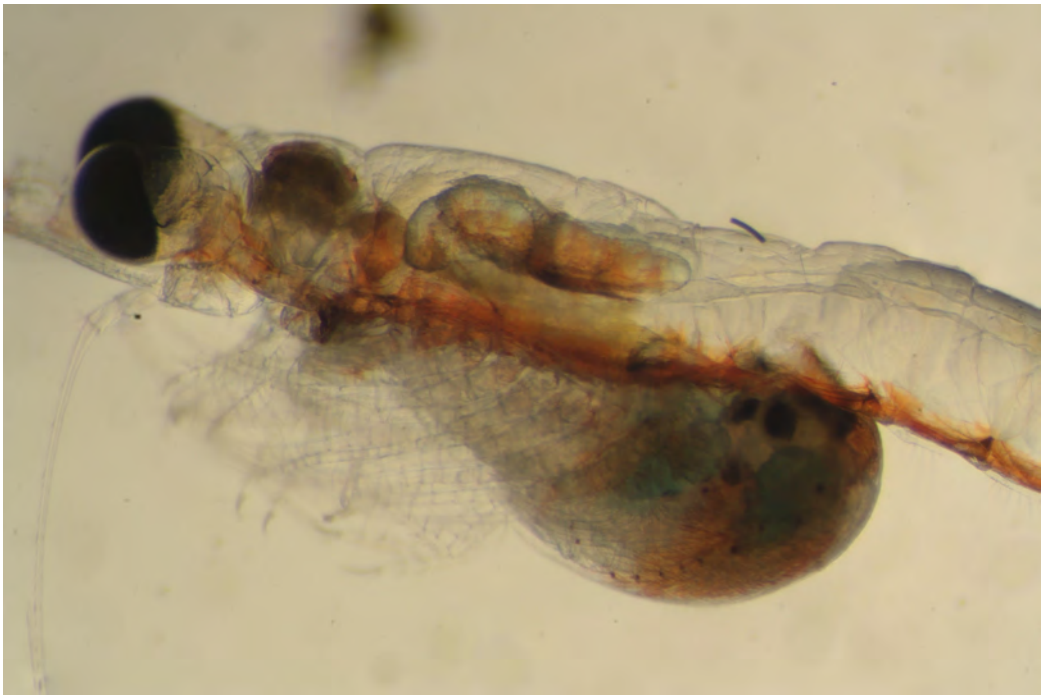


Figura 1.1: Hembra de misidáceo con embriones en el marsupio.

15 días dependiendo de la temperatura del agua (Murano, 1999).

Después de la eclosión y la subsiguiente muda los misidáceos se encuentran en la fase juvenil. Uno de los fenómenos más destacables dentro de esta etapa es la formación de los estatolitos dentro de la cámara del estatocisto, completando así su desarrollo. A partir de este momento los juveniles tienen la forma de un adulto en miniatura pero sin los caracteres sexuales secundarios desarrollados.

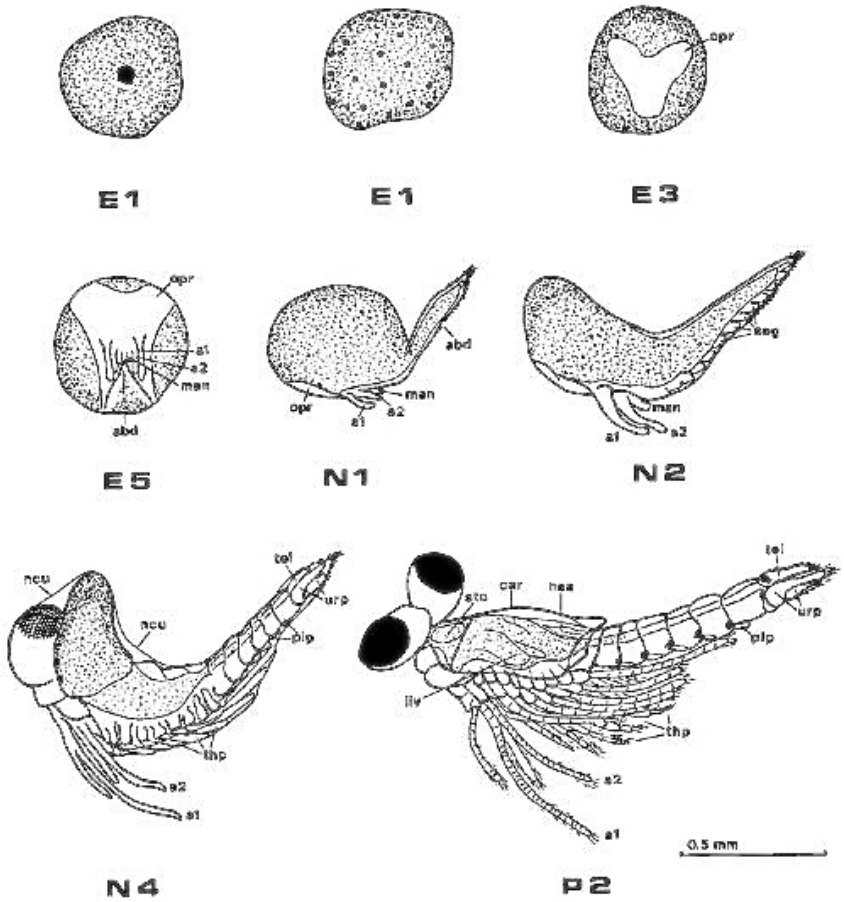


Figura 1.2: Desarrollo embrionario en *Leptomysis lingvura*. E1-E4 estadío embrionario, N1-N4 estadío nauplioide, P2 estadío postnauplioide. Tomado de Wittmann (1981).

En Gran Canaria, los estudios realizados hasta el momento donde se hayan identificado misidáceos son: el estudio de Castro (1995), que identifica varias especies de misidáceos a partir de restos encontrados en el contenido estomacal de *Scomber japonicus*, un primer inventario llevado a cabo por Wittmann and Wirtz (1998) que identifica algunas de las especies costeras, el trabajo de Wittmann et al. (2003) sobre especies epi y batipelágicas, el catálogo de especies planctónicas (Soldevilla et al., 2006) y el primer registro de *Gastrosaccus roscoffensis* publicado recientemente por Wittmann et al. (2010). Según Wittmann and Wirtz (1998) y Wittmann et al. (2010), las especies costeras de misidáceos citadas para el Archipiélago Canario son 18, de las cuales 9 están citadas para Gran Canaria (marcadas con asterisco):

Orden Mysida Haworth, 1825
Familia Mysidae Haworth, 1825

Subfamilia Siriellinae Czerniavsky, 1882
Siriella armata (Milne-Edwards, 1837)∗
Siriella claussi (G.O. Sars, 1877)
Siriella gracilipes (Nouvel, 1942)

Subfamilia Gastrosaccinae Norman, 1892
Anchialina agilis (G.O. Sars, 1877)
Gastrosaccus sanctus (Van Beneden, 1861)∗
Gastrosaccus roscoffensis (Bacescu, 1970)∗
Haplostylus bacescui (Hatzakis, 1977)∗
Haplostylus lobatus (Nouvel, 1951)

Subfamilia Erythropinae Hansen, 1910
Erythrops elegans (G.O. Sars, 1863)

Subfamilia Leptomysinae Hansen, 1910
Leptomysis sp. A (aff heterophila)∗
Leptomysis lingvura ssp. B.
Leptomysis sp. C (aff mediterranea)∗
Paraleptomysis banyulensis (Bacescu, 1966)
Mysidopsis sp. A (aff gibbosa)

Subfamilia Mysinae Haworth, 1825
Hemimysis sp. A (aff. maderensis)∗
Schistomysis sp. A (aff spiritus)∗
Paramysis arenosa (G.O. Sars, 1977)∗

Subfamilia Heteromysinae Norman, 1892
Heteromysoides cotti (Calman, 1932)

Según Castro (1995), en el contenido estomacal de *Scomber japonicus* colectados en aguas alrededor de Gran Canaria también se citan *A. agilis*, *S. clausii* y *Hemimysis* sp. junto con otras 8 especies:

Subfamilia Mysinae Haworth, 1825
Paramysis spp.
Stilomysis sp. (*S. grandis* ?)

Subfamilia Siriellinae Czerniavsky, 1882
Siriella sp.
S. norvegica (G.O. Sars,1869)
S. thompsoni (Milne-Edwards, 1837)

Subfamilia Gastrosaccinae Norman, 1892
Gastrosaccus normani (G. O. Sars, 1877)

Subfamilia Boreomysinae Holt and Tattersall, 1905
Paramblyops sp.

Subfamilia Leptomysinae Hansen, 1910
Leptomysis sp. (*L. sardica* ?)

En el trabajo de Castro (1995) se estima que la biomasa que consume anualmente *Scomber japonicus* es de 242,000 toneladas de misidáceos y 29,000 toneladas de eufáusidos. Esta estimación se basa en los porcentajes de misidáceos y eufausiáceos encontrados en el contenido estomacal de *Scomber japonicus*, la estimación acústica de abundancia de esta especie en la región y al hecho de que dicha especie consume diariamente un 8 % de su peso corporal en crustáceos y un 2.5 % en peces (anchoa). Este estudio ha puesto de

manifiesto la importancia que tienen los misidáceos como alimento de peces de interés comercial en la zona, a pesar de lo cual hasta la actualidad no se han llevado a cabo investigaciones sobre su abundancia en la isla.

El presente estudio surge en un principio por la necesidad de contar con un organismo marino representativo de la comunidad planctónica, fácil de obtener del medio y de mantener en cautividad para utilizarlo como animal de experimentación y poder llevar a cabo estudios de metabolismo respiratorio. Los misidáceos son organismos muy abundantes y de fácil captura en la costa de Gran Canaria ya que se encuentran a profundidades entre 5 y 15 metros y en zonas de fácil acceso. Por este motivo se planteó la posibilidad de cultivarlos en el laboratorio, ya no solo como organismos de experimentación en estudios de metabolismo respiratorio, sino también como posible alimento para peces en acuicultura.

Los estudios de Woods and Valentino (2003) y Otero-Ferrer (2012) han demostrado que los misidáceos son un excelente alimento para los caballitos de mar, obteniéndose un aumento en las puestas y un mayor crecimiento de las crías. Iglesias et al. (2007) ha obtenido resultados similares en paralarvas de pulpo que habitualmente tienen una supervivencia muy baja, siendo el principal problema en el cultivo de pulpo. Estos estudios han puesto de manifiesto la potencialidad de los misidáceos como alimento vivo, y por lo tanto, la necesidad de investigar con el fin de mejorar el cultivo de estos organismos.

Artemia y rotíferos son las dos presas vivas que tradicionalmente se utilizan en el cultivo de larvas de peces, lo que provoca en muchos casos deficiencias nutricionales (Izquierdo, 1996). Para el normal crecimiento y desarrollo de los peces son esenciales tres ácidos grasos poliinsaturados de cadena larga (PUFA): el ácido docosahexanoico (DHA), el ácido eicosapentaenoico (EPA) y el ácido araquidónico (AA) (Sargent et al., 1999, 1997). Estos ácidos grasos juegan un papel fundamental en el mantenimiento de la estructura y función de las membranas celulares y son precursores de un grupo de hormonas altamente activas conocidas como eicosanoides que se producen como respuesta al estrés. La función de los eicosanoides está determinada por la relación DHA:EPA:AA. Por lo tanto, no solo el contenido de estos ácidos grasos es importante sino también la relación entre ellos. Conocer la relación óptima de estos ácidos grasos es difícil en la práctica, ya que difiere en cada especie

(Sargent et al., 1999).

El estudio del contenido de ácidos grasos de las presas naturales puede proporcionarnos importantes datos acerca del contenido y la relación "óptima" de DHA, EPA y AA para sus predadores, ya que tanto los predadores como sus presas están bien adaptados a las condiciones del medio. Como mencionábamos anteriormente, los misidáceos son uno de los alimentos más importantes para muchos peces costeros, sobre todo juveniles. El estudio de la composición de ácidos grasos y la relación DHA:EPA:AA en misidáceos supone un importante avance en el conocimiento de los requerimientos nutricionales de sus predadores naturales para su normal desarrollo. Este conocimiento puede ser aplicable tanto al desarrollo de piensos como al cultivo de misidáceos para obtener composiciones similares a las que poseen en su medio natural.

La obtención de cultivos de misidáceos económicamente viables supondría un importante paso para la acuicultura, ya que permitiría optimizar el cultivo de peces y cefalópodos, sobre todo en sus etapas más tempranas de desarrollo. Los estudios llevados a cabo por Domingues et al. (Domingues et al., 1999a, 2000, 2001a, 1999b) que investigan el efecto de la temperatura, la densidad y de diferentes alimentos vivos, han representado un avance en el conocimiento del cultivo de misidáceos. Estos cultivos se han llevado a cabo con éxito sobre todo en estudios de ecotoxicología del género *Mysidopsis* (Reitsema, 1980; Ward, 1984; Lussier et al., 1988; Kuhn et al., 1991; Norton et al., 1999; Verslycke et al., 2004). Otros estudios también muestran buenos resultados en el cultivo de *Leptomysis lingvura* (Wittmann, 1981) y *Siriella armata* (Cuzin-Roudy and Tchernigovtze, 1985), dos de las especies presentes en la costa de Gran Canaria.

En nuestro laboratorio hemos diseñado un sistema de recirculación de agua de mar para el cultivo de misidáceos teniendo como referencia el trabajo de Lussier et al. (1988), donde los acuarios presentan sifones que separan los juveniles de los adultos, ya que en misidáceos se han observado casos de canibalismo (Figuras 1.3, 1.4,1.5).

El sistema de recirculación consiste en 8 acuarios de 35 L para alojar a los reproductores, 6 acuarios de 5 L para el crecimiento de las crías y 3 tan-

ques plásticos de 40 L que son utilizados para colocar los misidáceos salvajes recién colectados del medio. En los acuarios de reproductores un sifón con una malla de 2 mm succiona a los juveniles hacia los colectores que poseen una malla de 0.5 mm, éstos pueden extraerse para permitir así separar a las crías de los adultos.

Dos tanques de 1000 L proporcionan el agua de mar necesaria a todo el sistema que posee un volumen total de aproximadamente 500 L. El agua circula desde los acuarios hasta un tanque que funciona como biofiltro que contiene las bacterias nitrificantes necesarias para las transformaciones de amonio a nitrito y finalmente a nitrato. Desde el biofiltro el agua pasa a un espumador de proteínas o Skimmer que retira las proteínas procedentes de la materia orgánica que producirán desechos nitrogenados en su descomposición. A partir de aquí el agua es bombeada a través de un filtro mecánico, luego a un enfirador de agua y de allí vuelve al tanque desde donde se bombea nuevamente hacia los acuarios. El enfriador de agua mantiene la temperatura de los tanques a 18±0.5°C, una temperatura similar a la que se encuentran los misidáceos costeros en Canarias.

Uno de los mayores inconvenientes en el cultivo de misidáceos es la cantidad de desechos nitrogenados generados debido a la excreción y a la descomposición del exceso de alimento, de los organismos muertos y de las heces. La composición total de amonio (TAN) compuesta por amonio ionizado (NH_4^+) y amonio no ionizado (NH_3) es altamente tóxica para los misidáceos. Estos compuestos son transformados por procesos oxidativos a nitrito (NO_2^-) por las bacterias *Nitrosomonas* y luego a nitrato (NO_3^-) por las bacterias *Nitrobacter*, en un proceso llamado nitrificación. El nitrato es mucho menos tóxico para los misidáceos, Lussier et al. (1988) sugieren límites máximos de amonio de 0.1 mg L^{-1} , de nitrito de 0.05 mg L^{-1} y de nitrato de 20 mg L^{-1} . Para mantener estos niveles es muy importante la función del filtro biológico que provee el sustrato para el crecimiento de las bacterias nitrificantes.

Otros aspectos importantes a la hora de cultivar misidáceos son: el pH que debe mantenerse por encima de 7.8 , el oxígeno disuelto cuya concentración recomendada es 7 mg L^{-1} y una salinidad similar a la que poseen los organismos en el medio, que en este caso fue 37 g L^{-1} (PSU) Lussier et al. (1988). El fotoperíodo en el sistema fue de 14 horas de luz: 10 horas de oscuridad.

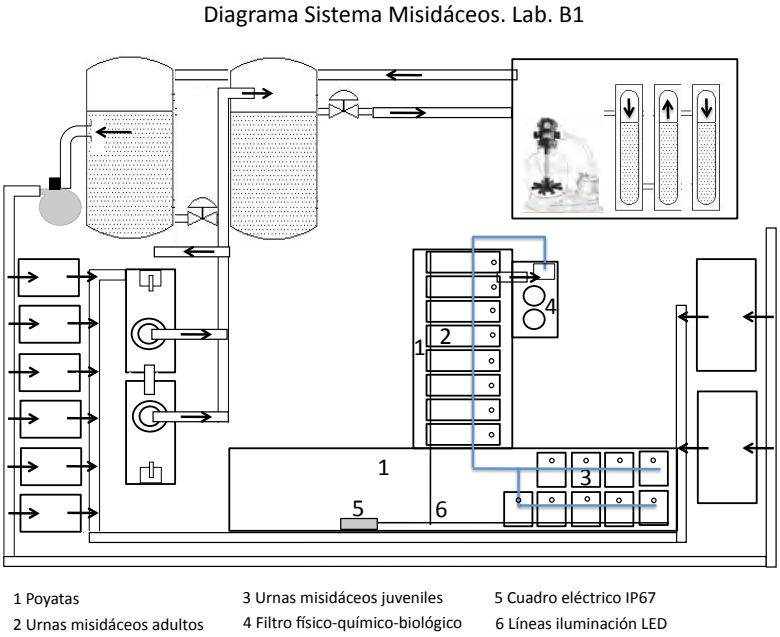


Figura 1.3: Plano del sistema de recirculación de agua de mar para el cultivo de misidáceos.

Figura 1.4: Sistema de recirculación de agua de mar para el cultivo de misidáceos.

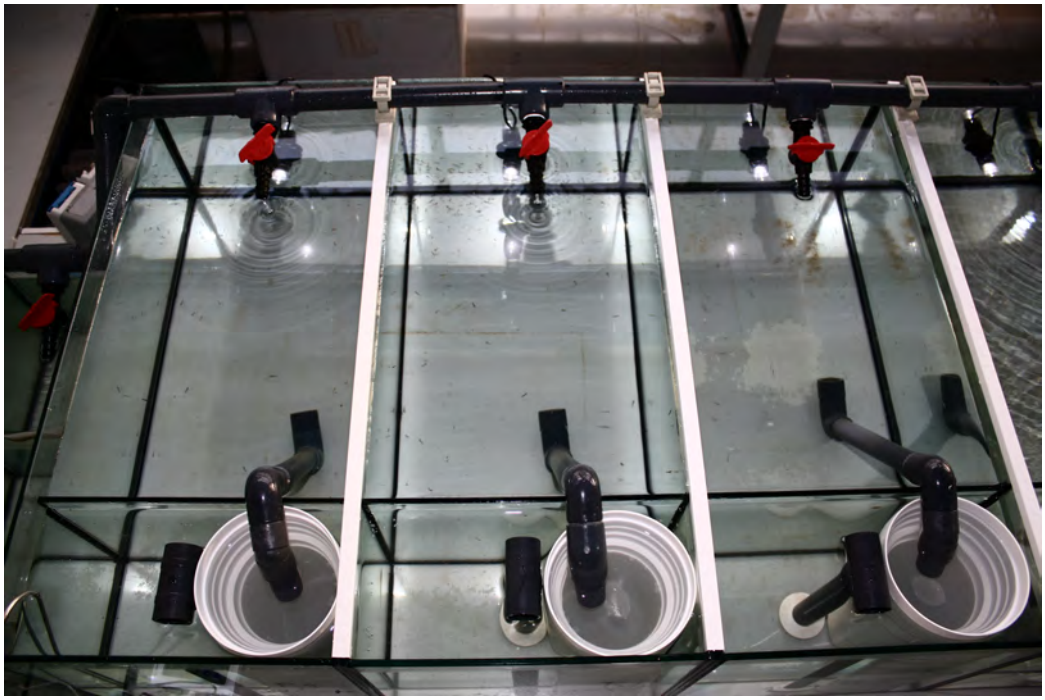


Figura 1.5: Acuarios de misidáceos con un sifón para separar adultos y juveniles.

Debido a la importancia ecológica de este grupo y dado el escaso número de trabajos realizados en Gran Canaria sobre estos organismos se nos planteó la necesidad de profundizar en su estudio. De esta manera el primer paso para ello fue identificar las especies que habitan las costas de Gran Canaria.

En las últimas décadas el número de especies de misidáceos identificados se ha incrementado notablemente. En 1957 Gordon menciona unas 520 especies divididas en 106 géneros, posteriormente 765 especies distribuídas en 120 géneros fueron incluídas en la lista mundial de misidáceos (Mauchline and Murano, 1977) y recientemente Anderson (2010b) presenta un listado de 1106 especies dentro del orden Mysida, 58 dentro del orden Lophogastrida y 16 dentro del orden Stygiomysida.

En la identificación de las especies de misidáceos los caracteres más importantes son el telson, cefalotórax, rostro, ojos, la escama antenal, los apéndices torácicos y los urópodos. La taxonomía de misidáceos es especialmente compleja ya que la distinción entre dos especies del mismo género puede deberse a pequeñas diferencias morfológicas como por ejemplo el número de setas que presenta la escama antenal o la forma de los apéndices torácicos.

En la mayoría de los casos la identificación se llevó a cabo siguiendo los trabajos de Tattersall and Tattersall (1951), Labat (1953), Wittmann (1986) y Wittmann et al. (2010). Sin embargo, para algunas especies fue necesario contar con la opinión de un experto taxónomo de misidáceos, el Dr. Karl Wittmann que amablemente confirmó la identificación previamente realizada.

Dada la dificultad que presentaba la correcta identificación de todas las especies decidimos llevar a cabo la realización de estudios genéticos (secuenciar el gen que codifica la subunidad ribosomal pequeña (18S rRNA)) y compararlos con las secuencias publicadas hasta la actualidad en la base de datos de GenBank utilizando el programa BLAST® (Basic Local Alignment Search Tool). Un ejemplo de las secuencias obtenidas se muestra en la figura 1.6.

Si bien los análisis genéticos son una importante herramienta que permite por medio de la comparación de la secuencia genética determinar una especie, esto solo es posible si el organismo ya ha sido previamente identificado, secuenciado y registrado en la base de datos. Por lo tanto, para facilitar las

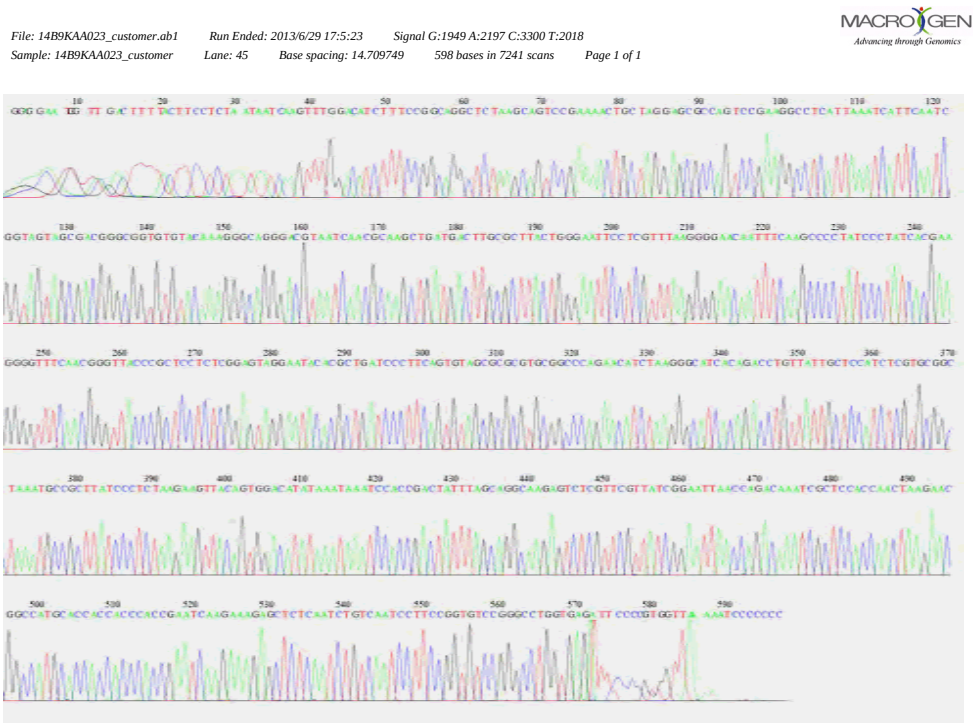


Figura 1.6: Secuencia genética del extremo 3' de *Paramysis arenosa* de 598 pares de bases (bp).

investigaciones futuras en este campo hemos realizado tanto la identificación taxonómica como la secuenciación genética para poder contar con un registro de estas especies en la base de datos.

Uno de los objetivos que se plantean para un futuro a partir de esta tesis, es la recopilación de toda la literatura disponible sobre misidáceos en la región y la publicación de una clave de identificación taxonómica de las especies de misidáceos costeros de Gran Canaria. Asimismo todas las secuenciaras obtenidas serán enviadas para su incorporación en la base de datos en GenBank. Esta clave facilitará futuros estudios ecológicos de esta comunidad en la región y los registros de las secuencias genéticas nos permitirá la identificación en los casos en los que no sea posible mediante el estudio de la morfología.

El siguiente paso en nuestro trabajo fue el estudio de la abundancia de los misidáceos y las variaciones estacionales de la misma. Siendo éste un objetivo demasiado amplio, finalmente se optó por estudiar las especies presentes en las praderas de *Cymodocea nodosa*, uno de los ecosistemas donde los misidáceos son más abundantes. Las praderas de *C. nodosa* son uno de los ecosistemas costeros mas importantes en las Islas Canarias, estas praderas desempeñan importantes funciones como es la regeneración de detritos, servir de hábitat para organismos omnívoros y herbívoros que transfieren carbono a niveles tróficos superiores y ser una importante zona de cría para numerosas especies de peces costeros (Espino et al., 2011a,b).

Se seleccionaron las especies más aptas par el cultivo realizando una serie de experimentos de supervivencia en cautividad. Conocer el ciclo de vida y el comportamiento de los misidáceos fue un paso previo indispensable para el estudio del metabolismo. Fueron necesarios dos años hasta lograr las condiciones óptimas de reproducción y crecimiento, siendo imprescindible construir instalaciones apropiadas para ello. Finalmente *L. lingvura* fue la especie seleccionada debido a la alta supervivencia en cautividad y a su fácil captura durante todo el año.

La tasa de crecimiento de *L. lingvura* se adapta a una función sigmoide (Figura 1.7):

$$W_t = W_0 + \frac{a}{1 + e^{-\left(\frac{t - t_0}{b}\right)}} \tag{1.1}$$

Siendo W_t la biomasa en el tiempo t, W_0 la biomasa en el tiempo cero, t el tiempo en días, a=0.0304 y b=4.2 (r^2=0.91, P<0.0001). A partir del día 30 se observaron las primeras hembras con embriones en el marsupio, por lo que a partir del día 30 los individuos se consideraron sexualmente maduros (adultos). La curva de crecimiento muestra que durante los primeros 20 días el crecimiento en *L. lingvura* es lento, en una segunda etapa entre los 20-35 días sufren un crecimiento rápido y a partir del día 35 el crecimiento es lento otra vez.

Por otra parte, se determinó la tasa de ingestión a diferentes concentraciones de presa para calcular la concentración de *Artemia* necesaria para suministrarle a los cultivos (figura 1.8). *L. lingvura* mostró una respuesta funcional tipo II.

La tasa de ingestion se relaciona con la concentración de *Artemia* L^{-1} según la ecuación:

$$I = \frac{I_{max} \cdot C}{Km + C} \tag{1.2}$$

Donde I= tasa de ingestión, I_{max} (tasa de ingestión máxima)= 20 *Artemia* mysid^{-1} h^{-1}, Km= concentración de (*Artemia* a 0.5 I_{max})=1219 *Artemia* L^{-1}, y C=concentración de *Artemia* L^{-1} (r^2=0.98, P<0.0001).

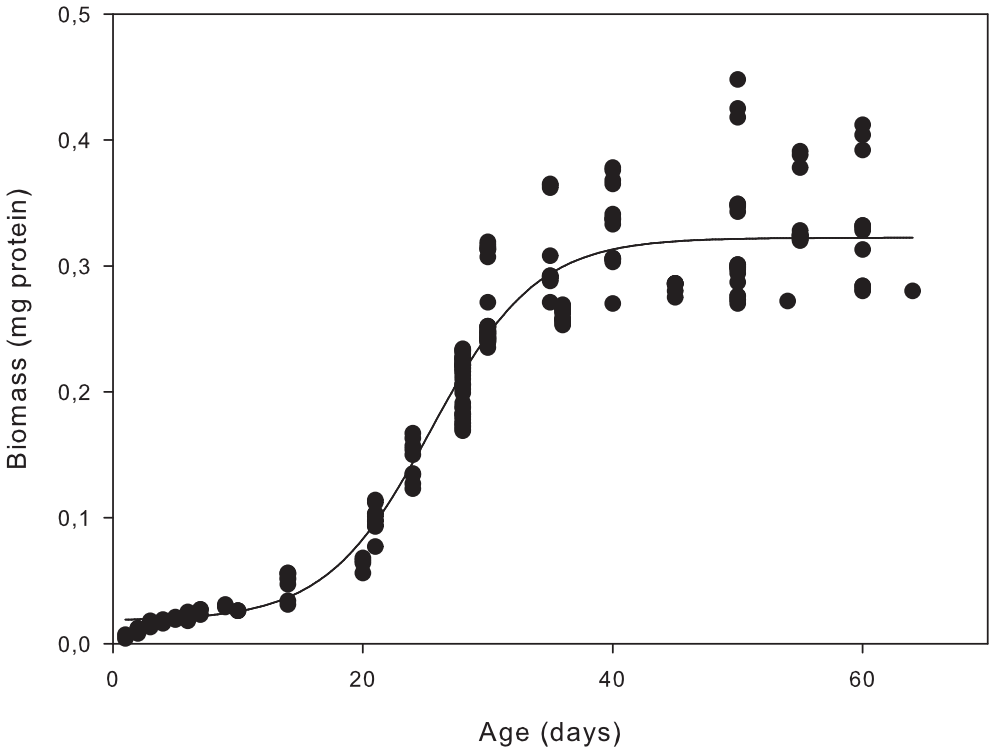


Figura 1.7: Tasa de crecimiento en biomasa proteica en *L. lingvura* hasta el día 60 de vida.

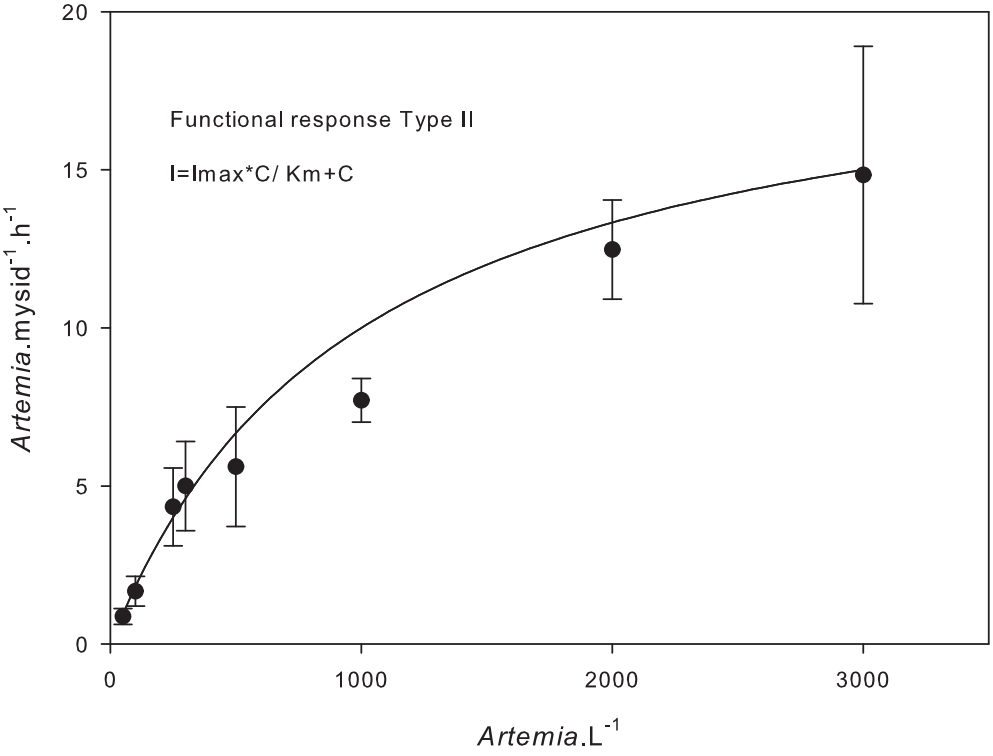


Figura 1.8: Tasa de ingestión en *L. lingvura* en función de la concentración de *Artemia*

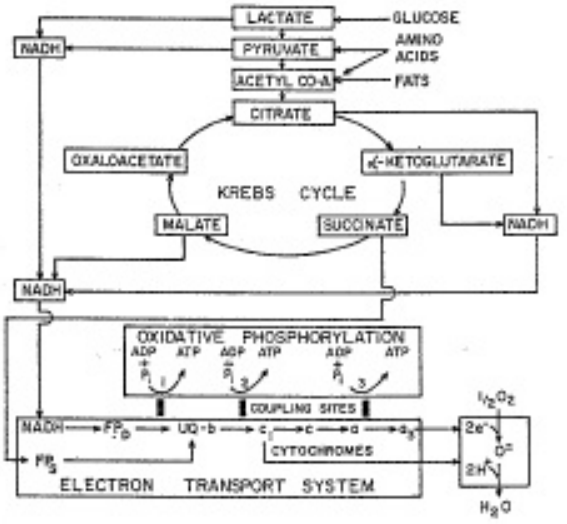


Figura 1.9: Ciclo de Krebs, fosforilación oxidativa y sistema de transporte de electrones. Tomado de Packard (1985).

Nuestro grupo de investigación se ha especializado en el estudio del metabolismo respiratorio en organismos marinos. Uno de sus integrantes, el Dr. Ted Packard ha desarrollado la técnica basada en la actividad enzimática del sistema de transporte de electrones (ETS) que permite estimar el consumo de oxígeno (Packard, 1971; Packard et al., 1971, 1974).

La respiración aerobia puede dividirse en tres etapas, la glucólisis, el ciclo de Krebs y la fosoforilación oxidativa. Durante la glucólisis una molécula de glucosa es descompuesta parcialmente a piruvato con un rendimiento neto de 2 moléculas de piruvato, 2 moléculas de ATP y 2 de NADH; posteriormente en la mitocondria una enzima retira 1 átomo de carbono de cada molécula de piruvato, la coenzima A (CoA) se transforma en acetil coenzima A y este fragmento se transfiere al oxalacetato, que es el compuesto inicial del ciclo de Krebs. Durante el ciclo de Krebs se liberan 6 moléculas de CO_2 y 2 ATP, 6 NADH y 2 $FADH_2$ que pasarán al sistema de transporte de electrones (Figura 1.9.

La etapa final del proceso es la fosforilación oxidativa donde se produce la mayor parte del ATP. En esta etapa la energía libre de la transferencia de electrones desde el NADH y $FADH_2$ al O_2 por medio de los complejos redox unidos a las proteínas está acoplada a la síntesis de ATP por las ATP sintetasas. El sistema de transporte de electrones consta de cuatro complejos de proteínas a través de los cuales los electrones pasan desde los potenciales de reducción estándar mas bajos hacia los mas altos, estos complejos se denominan complejo I (NADH deshidrogenasa), complejo II (Succinato deshidrogenasa), complejo III (Ubiquinona: citocromo c oxidoreductasa y complejo IV (Citocormo oxidasa). El aceptor final de electrones en este proceso es el O_2.

El método ETS (Packard, 1971) consiste en saturar el ETS mitocondrial con NADH y succinato, y el ETS microsomal con NADPH, utilizando Tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloryde (INT) como aceptor artificial de electrones. El INT reducido se transforma en formazan, un compuesto coloreado (Figura 1.10). En este proceso 1 mol de O_2 consumido, es equivalente a 2 moles de INT reducido, y es medido espectrofotométricamente a 490 nm usando 750 nm como línea base; de esta manera se mide la capacidad de mitocondrias y microsomas de transferir electrones desde sustratos fisiológicos (NADH, NADPH y succinato) a un aceptor final de electrones, reduciendo así los problemas asociados con las incubaciones de zooplancton en un ambiente controlado.

Durante años uno de los principales objetivos de nuestro grupo ha sido investigar la bioquímica de estos procesos y experimentar con organismos marinos los procesos fisiológicos que pueden afectar el consumo de oxígeno, con el fin de mejorar esta técnica (Hernández-León and Gómez, 1996; Gómez et al., 1996; Packard and Gómez, 2008; Martínez et al., 2010; Herrera et al., 2011b; Maldonado et al., 2012).

El estudio de la respiración ha sido uno de los principales temas de investigación en oceanografía en las últimas décadas debido principalmente a la importancia que pueda tener el océano en el cambio climático actuando como sumidero de CO_2 (Falkowski and Wilson, 1992). Es por eso que se ha dado tanta importancia a conocer el balance metabólico en los océanos, o lo que es lo mismo conocer la relación entre la producción y el consumo de oxígeno

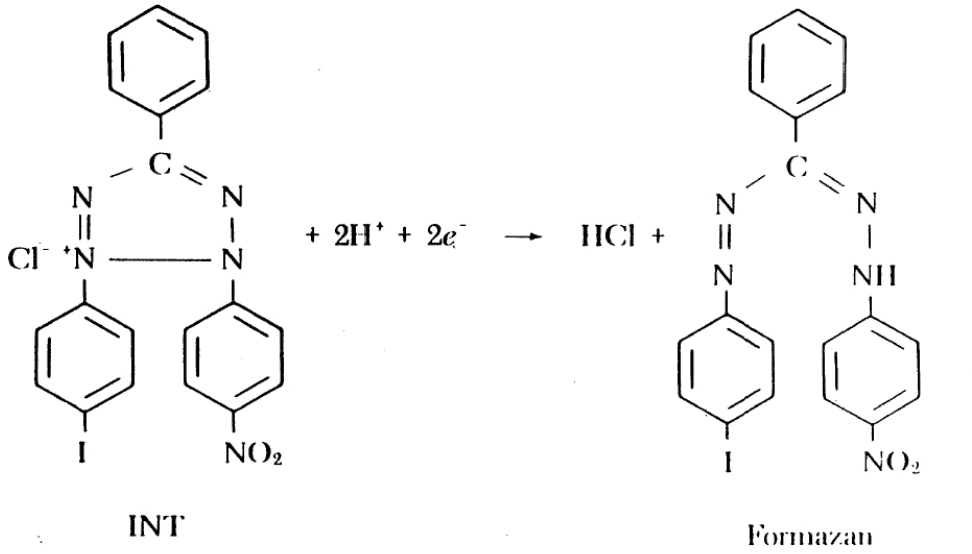


Figura 1.10: Reducción del INT a formazan. Según Packard (1985).

(Falkowski and Wilson, 1992; Del Giorgio and Duarte, 2002; Del Giorgio and Williams, 2005; Duarte et al., 2013). El debate sobre si el océano es autotrófico o heterótrofico continúa hasta hoy día, fundamentalmente debido a la dificultad que presenta la estimación precisa de la respiración de los distintos organismos.

El zooplancton juega un papel fundamental en los flujos de transferencia de carbono, ya que transfiere parte del carbono secuestrado de la atmósfera desde la zona eufótica a zonas más profundas del océano, participando activamente en lo que se conoce como la 'bomba biológica" (Longhurst and Harrison, 1989). Por este motivo la respiración en el zooplancton ha sido estudiada ampliamente (Conover, 1960; Childress, 1968; Ikeda, 1970; Packard, 1971; Packard et al., 1974; King and Packard, 1975; Bämsted, 1980; Hernández-León and Gómez, 1996; Gómez et al., 1996; Del Giorgio and Williams, 2005; Mayzaud et al., 2005; Hernández-León and Ikeda, 2005; Packard and Gómez, 2013).

Hemos llevado a cabo distintos estudios de laboratorio en *L. lingvura* que han permitido determinar como afectan factores como la inanición, las condiciones de alimentación y el crecimiento, a la actividad ETS y a la respiración *in situ* (R). La actividad ETS es utilizada para estimar la respiración potencial (ϕ). Conocer la relación R/ϕ en distintas condiciones nos permite poder estimar con mas exactitud la respiración *in situ* en el zooplancton, y de esta forma facilitar las estimaciones globales de consumo de oxígeno.

Finalmente, a partir de los estudios realizados en misidáceos, se han podido extrapolar los resultados en investigaciones oceanográficas donde se estudió la respiración en organismos suprabentónicos y zooplantónicos colectados durante la campaña IDEADOS 0710 llevada a cabo en julio del 2010 dentro del proyecto multidisciplinar IDEADOS (http://www.ba.ieo.es/ideados) coordinado entre el Instituto Español de Oceanografía, el Consejo Superior de Investigaciónes Científicas (CSIC) y la Universitat de les Illes Balears (UIB). Esta campaña se realizó al oeste y sur de Mallorca, en las subcuencas Balear y Argelina respectivamente que presentan condiciones oceanográficas muy diferentes. Uno de los objetivos fue calcular la demanda 'potencial" de carbono del suprabentos y del zooplancton epipelágico en ambas zonas. Otro de los objetivos del estudio llevado a cabo tanto en suprabentos como en zooplancton fue determinar si existían diferencias en las condiciones fisiológicas en las que se encontraban los organismos en cada región.

1.2. Objetivos

1.2.1. Capítulo 2

Identificar las especies de misidáceos que habitan la costa de Gran Canaria entre los 5 y 15 metros.

Secuenciar los genes que codifican la subunidad ribosomal pequeña (18S rRNA) en las distintas especies identificadas y registrarlos en la base de datos del NCBI (National Center for Biotechnology Information) que se encarga de hacer accesibles las bases de datos de secuencias de ADN de GenBank, con el fin de fomentar el conocimiento de la comunidad de misidáceos en Canarias.

1.2.2. Capítulo 3

Estudiar la abundancia de los principales grupos taxonómicos que componen el suprabenthos asociado a *Cymodocea nodosa*, uno de los ecosistemas más importantes en la costa de Gran Canaria, con especial interés en misidáceos. Determinar si existe variabilidad entre finales de primavera y finales de otoño, ya que durante la primavera las praderas de *C. nodosa* presentan máxima densidad y biomasa foliar, mientras que en otoño e invierno están en un período de senescencia.

1.2.3. Capítulo 4

Estudiar la supervivencia y la producción en cautividad de dos especies de misidáceos presentes en Gran Canaria: *Paramysis nouveli* y *Leptomysis lingvura*. Determinar la composición de ácidos grasos en individuos salvajes e individuos de cultivo alimentados con *Artemia* enriquecida con DHA Selco y compararla con la de *Artemia* y rotíferos.

1.2.4. Capítulo 5

Estudiar en misidáceos los factores que afectan la actividad del sistema de transporte de electrones con el objetivo de mejorar la interpretación de los resultados obtenidos mediante la técnica ETS para estimar respiración.

Evaluar el impacto de la inanición en la respiración *in situ* y en la actividad del sistema de transporte de electrones. Estudiar el efecto de las condiciones de alimentación en la relación respiración-biomasa y en la relación ETS-biomasa.

1.2.5. Capítulo 6

Aplicar los estudios metabólicos llevados a cabo en cultivos de *L. lingvura* al suprabentos de aguas profundas de Mallorca durante la campaña oceanográfica IDEADOS.

Aplicar la técnica ETS para determinar variaciones en la relación ETS-biomasa entre distintas zonas (subcuenca Balear y subcuenca Argelina) y distintas profundidades (250, 650 y 850 m).

Estimar la demanda de carbono de los grupos suprabentónicos representativos (decápodos, misidáceos y eufausiáceos) para determinar su importancia en los ciclos biogeoquímicos en la región.

1.2.6. Capítulo 7

Aplicar los estudios metabólicos llevados a cabo en el laboratorio en *L. lingvura* en el zooplancton epipelágico en aguas de Mallorca durante la campaña oceanográfica IDEADOS.

Estimar la respiración potencial y la demanda de carbono del zooplancton epipelágico mediante el método ETS.

Comparar la pendiente en la relación log ETS-log-biomasa entre las tres fracciones del zooplancton (53-200, 200-500,$>$500μm), las distintas horas de

muestreo (mañana, mediodía, tarde y noche), las dos áreas de muestreo (subcuenca Balear y subcuenca Argelina) y entre dos profundidades (600 y 900 m).

1.3. Principales resultados obtenidos

1.3.1. Capítulo 2: Identificación de misidáceos presentes en la costa de Gran Canaria

La colecta de misidáceos se llevó a cabo en dos zonas principalmente: fondos de arena próximos a las rocas de entre 5 y 15 m de profundidad (Figura 1.11), y praderas de *Cymodocea nodosa* comúnmente llamadas sebadales en Gran Canaria (Figura 1.12). Se utilizó una red de mano para las zonas rocosas (Figura 2.3) y una red tipo chinchorro arrastrada por dos buceadores en los sebadales (Figura 2.2).

Se identificaron 6 especies de misidáceos: *Siriella armata* (Milne-Edwards, 1837), *Leptomysis* sp. aff *heterophila* sensu Wittmann and Wirtz, 1998, *Leptomysis lingvura* ssp. sensu Wittmann and Wirtz, 1998, *Paramysis arenosa* (G.O. Sars, 1877), *Anchialina agilis* (G.O. Sars, 1877) y *Gastrosaccus roscoffensis* (Bacescu, 1970) (Figuras 2.5,2.7, 2.9, 2.11, 2.13 y 2.15).

El estudio de los misidáceos es reciente en el Archipiélago Canario. Wittmann and Wirtz (1998) publicaron el primer inventario de misidáceos costeros en el cual se citan cinco de las seis especies identificadas en el presente estudio:*Siriella armata*, *Anchialina agilis*, *Leptomysis lingvura* ssp., *Leptomysis* sp. (aff. *heterophila*) y *Paramysis arenosa*. Recientemente Wittmann et al. (2010) publican el primer registro de *Gastrosaccus roscoffensis* para las Islas Canarias, otra de las especies identificadas aquí. Esta especie presenta pequeñas diferencias morfológicas con la de Roscoff (Francia), a pesar de lo cual los autores, careciendo de otros estudios como por ejemplo el análisis genético, no han considerado posible establecer un taxon diferente para la especie de Canarias (Wittmann et al., 2010).

El estudio genético de *G. roscoffensis* no ha permitido confirmar la especie, ya que hasta la fecha, no existen genes secuenciados en la base de datos de GenBank para esta especie; la identificación se ha llevado a cabo en base a la características morfologicas descritas por Wittmann et al. (2010) y posteriormente ha sido corroborada por el propio Dr. Karl Wittmann. La secuencia del gen 18S rRNA de *G. roscoffensis* será el primer registro de esta especie en GenBank y de ahora en adelante será posible la comparación con

otras muestras.

En *Siriella armata*, *Paramysis arenosa* y *Anchilina agilis*, el estudio genético proporcionó un 100 % de identidad, confirmando la clasificación previa mediante el estudio taxonómico.

Finalmente, en tres de las muestras (2, 4 y 6) el estudio genético no permitió determinar la especie, obteniéndose en todos los casos un 98 % de identidad con *Leptomysis lingvura lingvura* y *Leptomysis mediterranea*, en estos tres casos es el estudio taxonómico de las muestras lo que permitió la identificación.

Las características morfológicas de la muestra 4 se corresponden con las descritas por Wittmann and Wirtz (1998) para *Leptomysis lingvura* ssp. B., mientras que la descripción de *Leptomysis* sp. aff. *heterophila* concuerda con la de las muestras 2 y 6. Estos dos taxones no han sido todavía descritos por los autores, por lo cual la identificación se basa en las observaciones que aparecen en el trabajo de Wittmann and Wirtz (1998) y en la comprobación por parte del Dr. Karl Wittmann de que estas muestras son las mismas que las previamente mencionadas en su publicación.

Esta claro que aún queda mucho por investigar de la taxocenosis de los misidáceos en las Islas Canarias ya que existen especies y subespecies aún no descritas. El estudio de las muestras colectadas en el presente trabajo y la secuenciación de las mismas permite seguir avanzando en el conocimiento de la comunidad de misidáceos que habitan la costa de Gran Canaria.

Figura 1.11: Fondo de arena y rocas en el veril de Risco Verde en la costa de Gran Canaria.

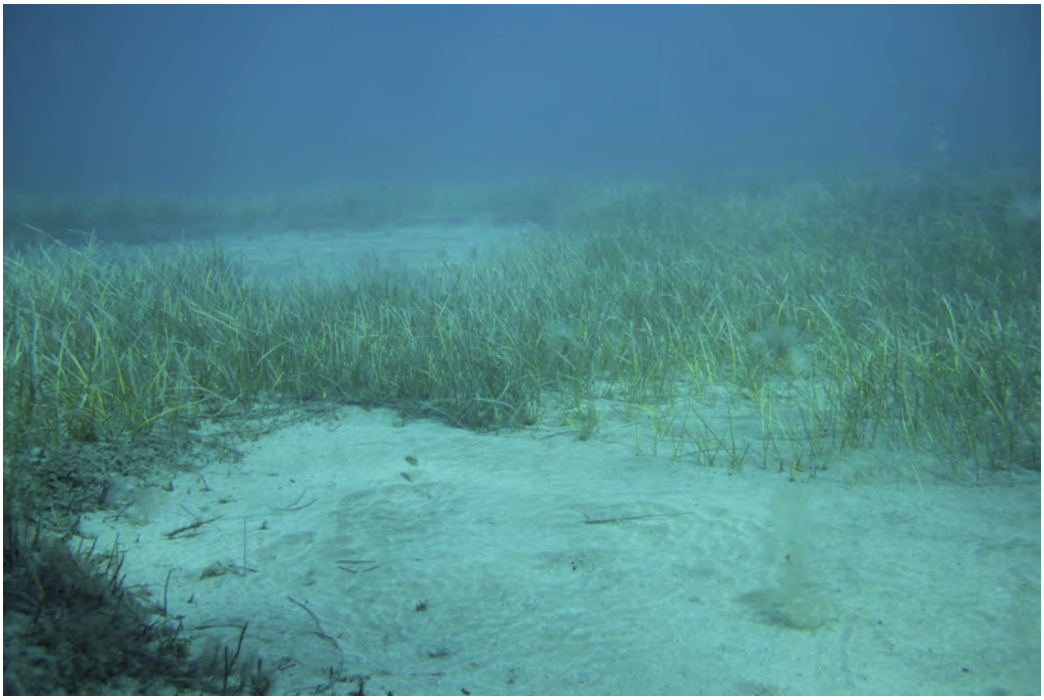


Figura 1.12: Fondo de arena y praderas de *Cymodocea nodosa* en la costa de Gran Canaria.

1.3.2. Capítulo 3: Abundancia de los misidáceos asociados a praderas de *Cymodocea nodosa*

Se seleccionaron 5 praderas de *C. nodosa* en la costa este y oeste de Gran Canaria (Veneguera, Risco Verde, Roque de Arinaga, Faro de Arinaga y Playa del Cabrón),(Figura 2.1) y se realizaron muestreos durante finales de primavera (mayo) y finales de otoño (noviembre) de 2011, con el objetivo de identificar el suprabentos asociado, con especial interés en las especies de misidáceos y determinar la variabilidad temporal en su abundancia.

Las muestras fueron colectadas utilizando una red tipo chinchorro de 6 m de longitud, 4 de ancho y 0.5 de altura, con una abertura de malla de 1 mm. La red fue arrastrada por dos buceadores a 10 cm del sustrato, colectando un volumen de 50 m^{-3} (Figura 2.2). Las muestras fueron fijadas en formol al 4 % para su posterior identificación mediante lupa binocular y microscopio hasta el nivel de grupo, y para los misidáceos hasta el nivel de especie siguiendo los trabajos de Tattersall and Tattersall (1951), Wittmann (1986), Barberá-Cebrián et al. (2001) y Wittmann et al. (2010).

En el presente estudio, la comunidad suprabentónica asociada a praderas de *Cymodocea nodosa* estuvo formada en más de un 95 % por misidáceos, decápodos y anfípodos. Durante la primavera, los misidáceos fueron los organismos más abundantes, mientras que en otoño fue mayor la abundancia de los decápodos (Tabla 3.1). El diagrama MDS para la comunidad de suprabentos muestra un claro patrón de asociación de muestras por estación (Figura 3.2). Los tres grupos representativos (misidáceos, anfípodos y decápodos) mostraron diferencias significativas en la abundancia consistente entre localidades, siendo en todos los casos mayores en mayo que en noviembre (2-way ANOVA: $p<0.05$, Tabla 3.2; Figura 3.3).

Se determinó la abundancia media (± error estándar) de los misidáceos durante mayo y noviembre (Tabla 3.3). Se identificaron seis especies de la familia Mysidae: *Siriella armata* (Milne-Edwards, 1837), *Gastrosaccus roscoffensis*, *Paramysis arenosa* (G.O. Sars, 1877) *Leptomysis* sp., *Anchialina agilis* (G.O. Sars, 1877) y *Leptomysis lingvura* (G. O. Sars, 1866) (Tabla 3.3). Solo *Gastrosaccus roscoffensis* estuvo presente en todas las muestras colectadas durante mayo y noviembre (Tabla 3.3, Figura 3.5). *Siriella armata* y *Gastro-*

saccus roscoffensis mostraron altas abundancias (151.2± 113.9 and 42.3±27.5 ind m^{-3} respectivamente) en Veneguera, y *Paramysis arenosa* también presentó altas abundancias (71.3±52.7 ind $^{-3}$) en Faro de Arinaga a finales de primavera (Tabla 3.3).

El diagrama MDS para la comunidad de misidáceos muestra una separación de las muestras por estación (Figura 3.4). La variabilidad temporal de la abundancia para las especies de misidáceos mostró diferentes patrones. En *L. lingvura* no se observaron diferencias significativas entre mayo y noviembre (2-way ANOVA: "Time" p=0.715, Tabla 3.4). En *P. arenosa*, *Leptomysis* sp. y *G. roscoffensis* la abundancia total fue mayor en mayo que en noviembre, sin embargo las diferencias varían entre localidades, lo que resulta en una interacción significativa "Ti x Lo" (Figura 3.5, Tabla 3.4). Finalmente, *S. armata* solo se encontró durante los muestreos de mayo, sin embargo, no se detectaron diferencias significativas entre estaciones, probablemente debido a la alta variabilidad de la abundancia entre las réplicas de cada localidad (Figura 3.5; 2-way ANOVA: "Time" p=0.446, Tabla 3.4).

A partir de los resultados obtenidos se pone de manifiesto la importancia de los misidáceos en las praderas de *C. nodosa*. Estudios previos, como por ejemplo el llevado a cabo por Castro (1995) ya habían evidenciado la importancia de los misidáceos como alimento de peces en Gran Canaria, siendo más abundantes en el contenido estomacal de *Scomber japonicus* que los eufausiáceos. Es probable que estos peces se alimenten en zonas cercanas a la costa donde los misidáceos son más accesibles. Algunas de las especies identificadas en dicho estudio (*Paramysis sp.*, *Siriella sp.*, *Anchialina agilis* y *Leptomysis sp.*) fueron colectados en las praderas de *Cymodocea* en el presente estudio. Estos resultados son un avance en el conocimiento de los misidáceos costeros y su importancia trófica.

1.3.3. Capítulo 4: Estudio de las técnicas de cultivo y análisis de la calidad nutricional en misidáceos presentes en la costa de Gran Canaria

Se estudió la supervivencia y la producción de *Leptomysis lingvura* y *Paramysis nouveli*, dos de las especies de misidáceos presentes en la costa este de Gran Canaria. Las muestras fueron colectadas en Risco Verde (Figura 2.1). Luego de un período de aclimatación de dos días, 10 machos y 10 hembras de cada especie fueron colocados en tanques de 14 litros en un sistema de recirculación de agua de mar, con triplicados para cada experimento. El pH se mantuvo a 8.2±0.1 y las concentraciones de amonio, nitrato y nitrito por debajo de 0.2, 1 y 0.02 mg L^{-1} respectivamente. El fotoperíodo fue de 14 horas de luz y 10 de oscuridad.

Los misidáceos fueron alimentados dos veces al día con 100 nauplios de *Artemia* por individuo. Adultos y crías fueron contados diariamente. La supervivencia fue expresada como porcentaje del número original de organismos, y la producción relativa fue estimada dividiendo el número de crías por día por el número de hembras vivas. Para el estudio de la calidad nutricional se analizaron proteínas, cenizas y lípidos en porcentaje del peso seco, y se separaron y cuantificaron los ácidos grasos en porcentaje del total de lípidos.

Al final del experimento, la supervivencia de *L. lingvura* fue de 65±8.7 % y la de *P. nouveli* de 16±5.8 % (Figura 4.1). Hasta el día 9 no se observaron diferencias significativas en la supervivencia de ambas especies, a partir de este día, la supervivencia de *L. lingvura* fue significativamente mayor ($P<0.05$). La producción total de crías fue de 166±2 para *L. lingvura* y 45±7 para *P. nouveli*. La longitud estándar de las crías fue de 2.03±0.23 mm y 1.86±0.17 mm en *L. lingvura* y *P. nouveli* respectivamente (Figura 4.2). La producción relativa fue significativamente mayor ($P<0.05$) en *L. lingvura* (18.2±2 crías) que en *P. nouveli* (4.6±0.8 crías) al día 21.

Las proteínas y lípidos fueron en *P. nouveli* el 73.38±1.77 % y el 15.01±1.12 %; y en *L. lingvura* el 74.19±5.22 % y el 14.79±2.66 % del peso seco respectivamente (Tabla 4.1). El porcentaje de ácidos grasos se comparó con los porcentajes obtenidos en misidáceos cultivados, misidáceos salvajes, y en *Artemia* y rotíferos, dos presas utilizadas frecuentemente en acuicultura (Tabla 4.1).

El estudio de los porcentajes de ácidos grasos en individuos salvajes y cultivados de *Paramysis nouveli* y *Leptomysis lingvura* respectivamente (Figuras 4.3 y 4.4) mostró que los ácidos grasos más abundantes para ambas especies en cultivo fueron 16:00, 18:1n9, 20:5n3 (EPA), 22:6n3 (DHA), 18:3n3 y18:2n6 (Tabla 4.1; Figuras 4.3 y 4.4). Los ácidos grasos poliinsaturados (PUFA) n-3 y n-6 totales para *Paramysis nouveli* y *Leptomysis lingvura* en cultivo representaron un 39.44 % y 8.42 %; y 42.4 % y 8.4 % del total respectivamente. El porcentaje de PUFA n-3 fue mayor que el que presentaron *Artemia* (31.14 %) y rotíferos (21.12 %) de acuerdo a los resultados presentados por Roo et al. (2009) (Tabla 4.1).

Los ácidos grasos poliinsaturados DHA, EPA y ácido araquidónico (AA) son necesarios para el normal crecimiento y desarrollo en peces, y no solamente la cantidad sino también la relación entre éstos. Ambas especies poseen un mayor porcentaje de DHA, EPA y AA que la que poseen rotíferos y *Artemia* según datos publicados por Roo et al. (2009). La relación DHA:EPA fue 0.85±0.01 y 0.89±0.01, DHA:AA fue 6.26±0.26 y 4.74±0.14; EPA:AA 7.32±0.26 y 5.32±0.20, en *P. nouveli* y *L. lingvura* respectivamente (Tabla 4.1), estos porcentajes no muestran diferencias significativas con los que presentan *P. nouveli* y *L. lingvura* salvajes (Figuras 4.3 y 4.4).

Sargent et al. (1999), sugieren que la composición que presentan las presas en el medio natural podría ser la "óptima" para sus predadores naturales, lo que puede ser un indicio de por qué los misidáceos han resultado ser un alimento de mejor calidad para sepias y caballitos de mar que *Artemia* o rotíferos (Otero-Ferrer et al., 2007; Woods and Valentino, 2003; Domingues et al., 2001b).

1.3.4. Capítulo 5: Efecto de la inanición y la alimentación en el metabolismo respiratorio de *Leptomysis lingvura* (G.O. Sars, 1866)

Para determinar el efecto de la inanición en el metabolismo respiratorio se colectaron nuevamente misidáceos de la especie *L. lingvura* en la costa este de Gran Canaria. Luego de un período de aclimatación de 7 días alimentados con nauplios de 48 hs de *Artemia* sp. enriquecidos con ácidos grasos (DHA-Selco, INVE, Belgium) se separaron machos de tallas similares en recipientes individuales para evitar el canibalismo y se sometieron a diferentes períodos de inanición: 2, 6, 10, 22, 26, 30, 36, 46, 52 y 74 horas. Finalizado cada período de inanición se separaron 5 individuos para realizarles medidas de respiración in vivo (μl O_2 por hora) con oxímetro (Strathkelvin 928, 6-Channel oxygen system) en celdas individuales de 50 ml en condiciones de oscuridad. Posteriormente los organismos fueron congelados a -196°C en nitrógeno líquido y conservados a -80°C para el estudio de la actividad ETS según Packard (1971) con modificaciones (Owens and King, 1975; Gómez et al., 1996) y estimación de la biomasa (en mg de proteínas) según el método Lowry modificado por Rutter (Lowry et al., 1951; Rutter, 1967).

Para determinar el efecto de las condiciones de alimentación en la relación respiración-biomasa y ETS-biomasa se separaron 100 individuos de distintas tallas y durante una semana se sometieron a diferentes tratamientos de alimentación:
Tratamiento A: dos tomas diarias de 150 nauplios de 48 hs de *Artemia* sp.
Tratamiento B: dos tomas diarias de 75 nauplios de 48 hs de *Artemia* sp.
Tratamiento C: dos tomas diarias de 10 nauplios de 48 hs de *Artemia* sp.

Cada tratamiento se llevó a cabo por triplicado. Luego se escogieron 36 individuos al azar de todo el rango de tallas para realizarles medidas de respiración *in situ* en el oxímetro como se describe anteriormente. Posteriormente fueron fotografiados y medidos; y luego congelados a -196°C y conservados a -80°C para realizar los análisis de la actividad ETS y la estimación de la biomasa.

El estudio de la tasa de respiración *in situ* y la tasa de respiración potencial (ϕ) para cada período de inanición muestra que el estado fisiológico en

que se encuentra *L. lingvura* influye en la tasa respiratoria, que disminuye a medida que aumenta el período de inanición (Figura 5.1), sin embargo, no ocurre lo mismo con la respiración potencial, representada por la actividad enzimática del sistema de transporte de electrones, que en un período de 74 horas no muestra un descenso de su actividad (Figura 5.2).

En el presente estudio, las tasas respiratorias en los individuos recién alimentados son tres veces mayores que las que se observan en los individuos que permanecen en períodos de inanición de 46 horas o superiores, lo que se refleja en cocientes R/ϕ también más altos (Figura 5.3). En los misidáceos que presentan un metabolismo activo y poca reserva de nutrientes, esa falta de sustratos se evidencia en el descenso de la tasa respiratoria en un corto período de tiempo. La variación en los cocientes R/ϕ es consecuencia directa de lo expuesto anteriormente. Otros autores ya han mencionado la importancia del alimento en la variabilidad del cociente R/ϕ y sugieren que la respiración es el factor que produce la alta variabilidad de este cociente (Hernández-León and Gómez, 1996).

Al inicio del periodo de oscuridad, hacia las 20 horas se observa un aumento de la respiración *in situ* (Figura 5.1), por lo cual, si tenemos en cuenta dos variables: horas de inanición y hora del día, el modelo que mejor se ajusta está representado por la ecuación:

$$R = 44{,}49 - 9{,}98\ log\ h + 1{,}82\ t \tag{1.3}$$

Es probable que los ritmos circadianos internos expliquen este comportamiento, muchos misidáceos hiperbentónicos presentan ritmos endógenos de actividad, por ejemplo en el género *Gastrossacus* que descansa sobre el sedimento durante el día y asciende nadando durante la noche, este comportamiento persiste incluso bajo condiciones experimentales de oscuridad durante varios días (Mauchline, 1980). Las especies mediterráneas de *Leptomysis* también presentan este tipo de comportamiento alimentario (Dauby, 1995). Hecq et al. (1984) han realizado estudios sobre la influencia de las condiciones experimentales y ambientales en el consumo de O_2 en *L. lingvura* , observando que durante el día la tasa respiratoria varía entre 20 y 24 μg O_2 h^{-1}mg $prot^{-1}$, mientras que al anochecer la respiración aumenta proporcionalmente, siendo su máximo al final de la noche (48.2 μg O_2 h^{-1} mg $prot^{-1}$).

Respecto a la relación respiración-biomasa y ETS-biomasa con los distintos tratamientos de alimentación, se determinó que la relación entre respiración y biomasa expresada como regresión logarítmica fue para el tratamiento A:

$$log\ R = 2{,}18 + 0{,}84\ log\ W \tag{1.4}$$

(R^2=0.64, n=34), Coeficiente de correlación de Pearson =0.798, p< 0.01; para el tratamiento B:

$$log\ R = 2{,}72 + 0{,}92\ log\ W \tag{1.5}$$

(R^2=0.69, n=36), Coeficiente de correlación de Pearson =0.798 =0.829 p< 0.01; y para el tratamiento C:

$$log\ R = 1{,}87 + 0{,}71\ log\ W \tag{1.6}$$

R^2=0.81, n=35), Coeficiente de correlación de Pearson =0.798 =0.902, p< 0.01 (Figuras 5.4, 5.5, 5.6).

La actividad ETS representa la tasa de respiración potencial (ϕ), es decir, la velocidad máxima de reacción que presentan las enzimas del sistema de transporte de electrones, la relación entre esta actividad y la biomasa fue para el tratamiento A:

$$log\ ETS = 2{,}73 + 0{,}72\ log\ W \tag{1.7}$$

(R^2=0.84, n=34), Coeficiente de correlación de Pearson=0.916, p< 0.01; para B:

$$log\ ETS = 2{,}85 + 0{,}71\ log\ W \tag{1.8}$$

(R^2=0.77, n=36), Coeficiente de correlación de Pearson=0.879, p< 0.01; y para C:

$$log\ ETS = 2{,}44 + 0{,}54\ log\ W \tag{1.9}$$

(R^2=0.85, n=35), Coeficiente de correlación de Pearson =0.924, p< 0.01 (figuras 5.4, 5.5, 5.6).

La relación entre respiración y biomasa se expresa de acuerdo a la ecuación R=a M^b y durante años se ha aceptado que el coeficiente b tiene un valor de 0.75 para todos los organismos, lo que se conoce como "ley de Kleiber" (Hemmingsen, 1960; Kleiber, 1961; Peters, 1983; Calder III, 1983; Schmidt-Nielsen, 1984). De acuerdo a los resultados obtenidos en el presente estudio, en organismos que se sometieron al tratamiento C (con escasez de alimento) los valores para el coeficiente b fueron significativamente inferiores a 0.75, tanto para la relación R-biomasa como para la relación ETS-biomasa.

Respecto a la relación existente entre el metabolismo respiratorio y la biomasa, otros autores (Martínez, 2007; Gómez et al., 2008; Packard and Gómez, 2008; Herrera, 2009) han encontrado correlaciones similares en distintos grupos de zooplancton (Tabla 5.2) y la pendiente b se encuentra en el rango entre 0.5 y 0.9. Este amplio rango en que se sitúa el coeficiente b nos plantea muchos interrogantes respecto a la aplicabilidad de la ley de Kleiber para describir el consumo de oxígeno en un rango pequeño de tallas, corta escala de tiempo o en diferentes estados fisiológicos, tal como plantean Dodds et al. (2001); Lane (2005); Packard and Gómez (2008); Kolokotrones et al. (2010).

1.3.5. Capítulo 6: Aplicación de la técnica ETS en crustáceos suprabentónicos de profundidad: respiración, demanda de carbono y relación ETS-biomasa

El estudio se llevó a cabo durante la campaña oceanográfica IDEADOS en julio de 2010 en dos áreas pesqueras en la costa noroeste y sur de Mallorca (Islas Baleares) a bordo del B/P Punta dés Vent. El área de muestreo al noroeste esta cerca del puerto de Sóller en la subcuenca Balear, mientras que el área sur esta cercana al archipiélago de Cabrera en la subcuenca Argelina (Figura 6.1). Estas subcuencas presentan condiciones oceanográficas muy diferentes (EUROMODEL Group, 1995).

Se colectaron muestras de suprabentos a tres profundidades (250 m, 650 m y 850 m) en las dos localidades (Cabrera y Sóller). En cada localidad y profundidad se colectaron tres muestras de suprabentos. Se separaron entre 3-5 individuos de diferentes especies de decápodos (*Gennadas elegans* (Smith, 1884), *Plesionika heterocarpus* (A. costa, 1871) y *Sergestes arcticus* (Kröyer, 1855)), de eufausiáceos (*Meganyctiphanes norvegica* (M. Sars, 1857) y *Thysanopoda aequalis* (Hansen, 1905)) y de misidáceos (*Boreomysis arctica* (Kröyer, 1855) y *Eucopia unguiculata* (Willemoes-Suhm, 1875)) y se congelaron inmediantamente a -196°C para el posterior análisis de ETS y proteínas. El resto de la muestra fue fijada en formaldehído al 4 % para su identificación taxonómica hasta el nivel de especie y para el estudio de la abundancia.

Se estimó la abundancia en ind 100 m^{-2} de los principales componentes del suprabentos (decápodos, misidáceos y eufausiáceos), (Tabla 6.1). La actividad ETS se estimó según Packard (1971) con modificaciones (Owens and King, 1975; Gómez et al., 1996; Packard and Christensen, 2004) y la biomasa protéica según el método de Lowry (Lowry et al., 1951) con las modificaciones de Rutter (Rutter, 1967).

La abundancia no mostró diferencias significativas entre localidades ni entre profundidades. Sin embargo, en ambas localidades, la abundancia de los decápodos fue menor a 250 m, la de los eufausiáceos fue mayor a 250 m y la de los misidáceos mayor a 650 que a 850 m y en ambos casos mayor que a 250 m (Tabla 6.1, $p<0.001$).

Los valores de ETS por unidad de biomasa expresados como μl O_2 h^{-1} mg $prot^{-1}$ no mostraron diferencias significativas entre especies (ANOVA test $p>0.05$), con valores medios entre 6.54 μl O_2 h^{-1} mg $prot^{-1}$ para *T. aequalis* y 9.76 μl O_2 h^{-1} mg $prot^{-1}$ para *M. norvegica* (Tabla 6.2).

Se estudió la relación log ETS-log biomasa para los organismos suprabentónicos en Cabrera y Sóller respectivamente (Figura 6.4). El coeficiente b de la relación ETS = a W^b fue mayor en Cabrera que en Sóller.

La abundancia de decápodos, eufausiáceos y misidáceos representó un 56 % del total del suprabentos. A partir de los datos de abundancia y actividad ETS se estimó la demanda media anual de carbono debido a la respiración. El total de los tres grupos principales fue en Cabrera 20.48±10.33, 41.84±23.67 y 10.60±2.28 a 250, 650 y 850 m respectivamente, mientras que en Sóller fue 119.70±79.25, 19.10±8.95 y 13.32±7.06 a 250, 650 y 850 m respectivamente (Tabla 6.4). Estos valores representan un 0.03 % de la producción primaria total en Sóller y un 0.015 % en Cabrera según los datos de productividad primaria en la región publicados por Bosc et al. (2004).

La aplicación del método ETS ha permitido llevar a cabo estimaciones de la actividad respiratoria del suprabentos y la detección de cambios fisiológicos entre ambas áreas, así como estimar la demanda de carbono que presenta esta comunidad en la región.

1.3.6. Capítulo 7: Aplicación de la técnica ETS en zooplancton: respiración, demanda de carbono y relación ETS-biomasa

Las muestras fueron colectadas durante la campaña IDEADOS a bordo del buque oceanográfico Sarmiento de Gamboa al noroeste y sur de Mallorca (Sóller y Cabrera). En ambas zonas las muestras se recogieron a profundidades de 200 m y 900 m (Figura 7.1).

El microzooplancton fue capturado con una red Calvet de 53 μm de abertura de malla y el mesozooplancton con una red WP-2 de 200 μm de abertura de malla. Éste último grupo fue separado posteriormente fraccionando las clases de talla en 200-500 μm y >500 μm. Las muestras se colectaron a diferentes horas del día en cada estación (mañana, mediodía, tarde y noche) e inmediatamente congeladas a -196°C para el posterior análisis de la actividad ETS y la biomasa proteica.

Los datos hidrográficos muestran que en ambas zonas la columna de agua esta muy estratificada, con una termoclina entre los 20 y los 40 m de profundidad. La temperatura varía entre 26°C en la superficie y 14°C en profundidad (Figura 7.2). Las imágenes de clorofila superficial indican que ambas regiones son oligotróficas (Figura 7.3).

Se estimó la actividad ETS, la biomasa y la demanda de carbono para las diferentes tallas de zooplancton (53-200, 200-500, >500μm), a diferentes horas del día en cada estación de muestreo (Tablas 7.1, 7.2 and 7.3). La actividad ETS y la biomasa no mostraron diferencias significativas a las distintas horas del día (Figura 7.4). Entre ambas zonas se observaron diferencias significativas en la biomasa y la actividad ETS, siendo significativamente mayores en la región de Cabrera (subcuenca Argelina) (Figura 7.5). Además se observaron diferencias significativas en la actividad ETS en Cabrera entre las estaciones de 200 y 900 metros de profundidad, siendo mayor en las zonas menos profundas (Figura 7.6). La actividad ETS por unidad de biomasa (ETS específico) fue significativamente mayor en las tallas más pequeñas, lo que indica que poseen mayor actividad metabólica (Figura 7.8).

En la figura 7.9 se representa la relación log ETS-log biomasa para las

distintas tallas de zooplancton (53-200, 200-500, >500μm), los coeficientes de las regresiones para cada talla se muestran en la tabla 7.4. En todos los casos el coeficiente b fue menor a 0.75, y en el caso de la fracción 53-200μm significativamente menor que en las otras dos tallas. En estudios previos (Martínez, 2007; Gómez et al., 2008; Packard and Gómez, 2008; Herrera et al., 2011b) estos valores en las pendientes por debajo de 0.75 se observaron en organismos que viven en regiones oligotróficas o en cultivos con limitación de alimento. Aquí, los resultados sugieren que el zooplancton se encuentra en zonas con escasa disponibilidad de nutrientes, esto es consistente con los datos hidrográficos que indican que tanto Sóller como Cabrera son áreas oligotróficas.

En base a las estimaciones de la demanda de carbono a partir de los datos de ETS, el consumo de carbono debido a la respiración del zooplancton epipelágico fue 12.14 g C yr^{-1} m^{-2} en Cabrera, mientras que en Sóller fue 7.17 g C yr^{-1} m^{-2}. De acuerdo a las estimaciones de producción primaria de Bosc et al. (2004), los valores de demanda de carbono representan un 19.7 % de la producción primaria en Cabrera y un 12 % en Sóller.

1.4. Discusión general

Una de las principales aportaciones de esta tesis es la recopilacíon de datos sobre la taxocenosis y abundancia de los misidáceos en la costa de Gran Canaria, datos con los que no se contaba hasta ahora. El estudio taxonómico es aún reciente, por lo que las muestras recogidas y el estudio genético realizado en las mismas permitirá seguir avanzando en este campo.

A partir del estudio de la abundancia se ha puesto de manifiesto la importancia ecológica que tienen los misidáceos en las praderas de *C. nodosa* ya que en algunas zonas las abundancias superaron los 200 ind m^{-2}. Los misidáceos fueron el grupo dominante dentro de la comunidad suprabentónica en primavera. Durante este período en las Islas Canarias *C. nodosa* presenta la máxima densidad de haces y biomasa vegetal (Tuya et al., 2006) y provee de hábitat y "guardería" a numerosas especies de peces, muchas de ellas de interés comercial.

Las praderas de *C. nodosa* son ecosistemas que han mostrado una severa regresión en las últimas décadas (Tuya et al., 2012), a pesar de lo cual, la legislación que protege a estos ecosistemas se ha visto reducida desde 2010. *C. nodosa* ha sido calificada como "sensible a la alteración de su hábitat" por el decreto 151/2001 de 23 de julio de 2001 por el cual se crea el Catálogo de Especies Amenazadas de Canarias; esta categoría fue modificada a "de interés para los ecosistemas canarios" en 2010 por la ley 7L/PPL-0011 del Catálogo Canario de Especies Protegidas, lo que reduce su protección en la región.

Resultados como los obtenidos en el presente trabajo refuerzan la necesidad de plantearse otros estudios en relación con la protección de estos ecosistemas. Se hace necesario evaluar el impacto sobre los organismos suprabentónicos que habitan en los mismos y profundizar en las complejas redes tróficas que no están totalmente comprendidas. En este sentido es probable que *C. nodosa* y los misidáceos asociados jueguen un papel muy importante en el mantenimiento de la productividad costera.

Por otra parte, los resultados obtenidos en el capítulo 4 acerca de la supervivencia y la producción en cautividad plantean la posibilidad del cultivo

de misidáceos aunque no a gran escala. Sin embargo, la optimización de los sistemas de cultivo permitiría por ejemplo, contar con una presa natural para peces ornamentales o como alimento complementario para los primeros estadíos del desarrollo en cefalópodos.

El estudio de la composición bioquímica revela que *L. lingvura* y *P. nouveli* tienen un alto potencial como alimento vivo en acuicultura y los niveles de lípidos, proteínas y ácidos grasos satisfacen los requerimientos alimentarios para el cultivo de peces y crustáceos según las recomendaciones de la FAO (Tacon, 1989). Los misidáceos presentaron una composición de DHA, EPA y AA mayor que la obtenida por otros autores para rotíferos y *Artemia* (Roo et al., 2009; Otero-Ferrer et al., 2010). Es posible que esta diferencia en la composición de ácidos grasos sea la responsable de que los misidáceos sean un mejor alimento para satisfacer los requerimientos nutricionales en acuicultura, sobre todo para las especies de las que son presas naturales en el medio. Asimismo, los datos presentados para *L. lingvura* y *P. nouveli* pueden ser de utilidad para determinar la composición "óptima" del alimento para sus predadores naturales como la caballa, *Sepia officinalis*, *Octopus vulgaris* e *Hippocampus* sp.

Contar con cultivos de misidáceos nos ha permitido el estudio de la respiración en condiciones controladas. Uno de los principales problemas en ecofisiología del zooplancton es poder realizar medidas *in situ* de respiración, en este caso los estudios de laboratorio nos han permitido evaluar la respiración y la actividad ETS en diferentes condiciones de alimentación. Uno de los resultados más importantes fue demostrar el efecto que tiene la inanición en la respiración *in situ* a diferencia de la actividad ETS que no se ve afectada. Este resultado permite establecer diferentes relaciones R/ϕ según las condiciones fisiológicas en que se encuentran los organismos, permitiendo así reducir los errores asociados a la estimación de respiración a partir de la actividad ETS.

Otro resultado importante que se desprende del capítulo 5 es que la relación ETS-biomasa es diferente en los organismos que están en un medio con abundante alimento respecto a la que presentan los misidáceos que tienen poca disponibilidad de alimento a largo plazo. Trabajos previos (Martínez, 2007; Gómez et al., 2008; Packard and Gómez, 2008; Herrera, 2009) ya ha-

bían mostrado que la pendiente en la relación log ETS-log biomasa en el zooplancton varía entre 0.5 y 0.9, y esta variabilidad estaba relacionada con las condiciones en que se encontraban los organismos. Por ejemplo, si asumimos que el zooplancton que vive en las regiones de afloramiento, remolinos y costeros no están limitados por el alimento, se observa que en todos estos casos la pendiente b de la relación log ETS-log biomasa es igual o mayor a 0.75; mientras que si observamos las pendientes b en regiones oceánicas y en cultivos con poca disponibilidad de alimento siempre está por debajo de 0.75; estos resultados son similares a los obtenidos en el capítulo 5.

La actividad ETS está determinada por el complejo I-NADH deshidrogenasa en la mitocondria, y esta concentración varía con el número de células de los misidáceos y por lo tanto con la biomasa. El sistema de transporte de electrones es una parte constituyente de las células y por lo tanto no cambia rápidamente con las condiciones ambientales o con la disponibilidad de sustrato metabolizable, como podría ser el caso de la respiración *in situ*. Sin embargo, si las condiciones se mantienen durante un largo período de tiempo puede que ocurran cambios a nivel estructural, por ejemplo un aumento del número de mitocondrias que permitan una mayor actividad ETS. Para poder corroborar esta hipótesis es necesario llevar a cabo más estudios sobre la relación ETS-biomasa en condiciones controladas y también en el medio.

La campaña IDEADOS 0710, dentro del proyecto multidisciplinar IDEADOS fue la oportunidad para aplicar los estudios realizados previamente en cultivos de misidáceos a suprabentos y zooplancton colectados en el medio. Uno de los principales objetivos en este proyecto era determinar las relaciones entre las condiciones medioambientales y las comunidades nectobentónicas del talud en dos zonas oligotróficas alrededor de Mallorca, la subcuenca Balear y la subcuenca Argelina.

La aplicación de la técnica ETS junto con el estudio de la biomasa y la abundancia permitieron realizar estimaciones de la demanda de carbono en cada una de las regiones. A partir de estas estimaciones se calculó el porcentaje de la producción primaria neta (PP) que consumen en la zona los principales componentes de la comunidad suprabentónica y el zooplancton epipelágico. Estas estimaciones, si bien tienen errores asociados a las variaciones estacionales de la abundancia de estos organismos y a que representan

el valor máximo del consumo de carbono, permiten tener una idea de la importancia que tienen estas comunidades en el ciclo del carbono.

En la comunidad suprabentónica la actividad ETS específica y la relación ETS-biomasa mostraron diferencias significativas entre Cabrera y Sóller, siendo la actividad ETS específica y la pendiente b de la relación log ETS-log biomasa mayor en Cabrera. De acuerdo a los resultados obtenidos previamente en *L. lingvura* estas diferencias podrían deberse a las diferentes condiciones de alimentación en que se encuentran los organismos en la subcuenca Balear y la subcuenca Argelina, sin embargo hasta el momento no hay evidencias concluyentes respecto a la mayor disponibilidad de alimento en una zona u otra.

En el zooplancton epipelágico la biomasa media y la actividad ETS fue significativamente mayor en Cabrera que en Sóller, sin embargo la actividad ETS específica no mostró diferencias significativas entre ambas zonas, lo que podría significar que el zooplancton se encuentra en condiciones fisiológicas similares. En este estudio, la pendiente b de la relación log ETS-log biomasa fue menor a 0.75 en todas las tallas, lo que sugiere que tanto Cabrera como Sóller son regiones oligotróficas, este resultado es consistente con los datos hidrográficos encontrados.

1.5. Futuras líneas de investigación

La presente tesis ha supuesto un primer paso en muchas áreas de investigación, por lo tanto, aún quedan muchos interrogantes por resolver y mucho por estudiar sobre la taxonomía, la abundancia, el cultivo y el metabolismo respiratorio de los misidáceos.

En primer lugar, a partir de este trabajo se ha puesto de manifiesto la necesidad de recopilar todos los datos existentes en la literatura sobre los misidáceos en la región y realizar un catálogo y una clave de las especies costeras de misidáceos de las Islas Canarias. También es necesario continuar con el estudio de la abundancia y de la variabilidad temporal, y de los factores bióticos y antropogénicos que puedan afectarle. Este estudio no se plantea ya solamente en las praderas de *C. nodosa*, sino también en fondos de arena, rocas y cuevas donde estos organismos son muy abundantes.

Otra línea de investigación abierta a partir de esta tesis es la del cultivo. Es necesario contar con instalaciones más amplias que permitan seguir desarrollando las técnicas de cultivo para hacerlos económicamente viables mejorando el rendimiento a pequeña escala. En un futuro se plantea la posibilidad de alimentar cultivos de peces ornamentales, como por ejemplo caballitos de mar, solamente con misidáceos lo que mejoraría considerablemente la supervivencia y producción de este tipo de cultivos.

Por otra parte es necesario continuar con el estudio de la composición en proteínas y acidos grasos de estos organismos, ya que nos puede proporcionar importantes pautas para el desarrollo de piensos con una calidad nutricional "óptima" para muchas especies de peces y cefalópodos.

En cuanto al metabolismo respiratorio, el gran interrogante que queda planteado a partir de este trabajo es: ¿De que manera las condiciones de alimentación podrían afectar a la relación ETS-biomasa?. Una de nuestras hipótesis planteadas es que la disponibilidad de alimento a largo plazo durante el crecimiento permitiría desarrollar mayor número de mitocondrias en las células de estos organismos y junto con éstas mayor cantidad de complejos enzimáticos responsables de la respiración celular y por lo tanto, responsables últimos del consumo de oxígeno.

Evaluar esta hipótesis a través de la correlación entre la cantidad de mitocondrias, la actividad ETS y la biomasa es nuestro siguiente paso en el estudio del metabolismo respiratorio del zooplancton.

Capítulo 2

Identification of mysids in the coast off Gran Canaria

Capítulo 2

Identification of mysids off the coast of Gran Canaria

Abstract: Samples were taken between May and November 2011 to identify the species of mysids present in the east and west coast of Gran Canaria. From samples collected over sandy bottoms near rocks and *Cymodocea nodosa* meadows at depths between 5 and 15 m, 6 species were identified: *Siriella armata* (Milne-Edwards, 1837), *Leptomysis* sp. aff *heterophila* sensu Wittmann and Wirtz, 1998, *Leptomysis lingvura* ssp. B sensu Wittmann and Wirtz, 1998, *Paramysis arenosa* (G.O. Sars, 1877), *Anchialina agilis* (G.O. Sars, 1877) and *Gastrosaccus roscoffensis* (Bacescu, 1970). Two of the mysid species identified (*Leptomysis* sp. aff *heterophila* and *Leptomysis lingvura* ssp.) have never been officially described in the taxonomic literature. *L. lingvura* had never been observed before in the waters off Gran Canaria.
This research describes the first genetic sequencing of the 18S rRNA gene from the mysids, *Leptomysis* sp. aff *heterophila*, *L. lingvura* ssp. and *G. roscoffensis*.

2.1. Introduction

Historically mysids have been classified within the Phylum Arthropoda, Subphylum Crustacea, Class Malacostraca, Superorden Peracarida, Or-

der Mysidacea; containing two sub-orders: Mysida and Lophogastrida. Mysid classification, and generally crustacean classification, is constantly being reviewed (Spears et al., 2005; Martin and Davis, 2001; Meland and Willassen, 2007; Anderson, 2010b; Zhang, 2011) and the development of molecular techniques has raised the need for even more revision. Recent phylogenetic analyzes of "Mysidacea" show that there is not a common ancestor for the group. However, there are three, well supported lineages: Lophogastrida, Mysida and Stygiomysida (Meland and Willassen, 2007). So these authors suggest a revision of the taxa based on monophyletic relationships according to which the "mysids" are classified as follows.

Order Lophogastrida Boas, 1883
Family Lophogastridae G.O. Sars, 1870
Family Gnathophausiidae Udrescu, 1984
Family Eucopiidae G.O. Sars, 1885

Order Stygiomysida Tchindonova, 1981
Family Lepidomysidae Clarke, 1961
Family Stygiomysidae Caroli, 1937

Order Mysida Boas, 1883
Family Petalophthalmidae Czerniavsky, 1882
Subfamily Rhopalophthalminae Hansen, 1910
Subfamily Boreomysinae Holt and Tattersall, 1905
Subfamily Gastrosaccinae Norman, 1892
Subfamily Siriellinae Czerniasky, 1882
Subfamily Erythropinae Hansen, 1910
Subfamily Mancomysinae Bacescu and IliVe, 1986
Subfamily Heteromysinae Norman, 1892
Subfamily Mysidellinae Norman, 1892
Subfamily Mysinae Haworth, 1825
Subfamily Leptomysinae Hansen, 1910
Family Mysidae Haworth, 1825

Martin and Davis (2001) propose to include in the Superorder Peracarida the two orders of former "mysids" treated as the separate orders Lophogastrida and Mysida. These two new orders in the Peracardia would then stand

beside the traditional orders: Thermosbaenacea, Spelaeogriphacea, Mictacea, Amphipoda, Isopoda, Tanaidacea, and Cumacea.

More recently, Anderson (2010b) in the database of Superorder Peracarida, includes orders Cumacea, Lophogastrida, Mysida, Stygiomysida and Tanaidacea. The most recent clasification of crustacea published by Zhang (2011) include the two orders of "mysids" Lophogastrida and Mysida, with the other eight orders: Thermosbaenacea, Spelaeogriphacea, Bochusacea, Mictacea, Amphipoda, Isopoda, Tanaidacea, and Cumacea. In the present work we follow the recent Zhang (2011) classification.

The number of species of mysids has been increasing in recent decades. Gordon (1957) listed about 520 species divided into 106 genera, (Mauchline and Murano, 1977) listed 765 species distributed in 120 genera, and recently Anderson Anderson (2010b) mentions 1106 species with 58 in the order Lophogastrida and 16 in the order Stygiomysida.

The studies that identify mysids in Canary waters are Wittmann and Wirtz (1998) for coastal species and Wittmann et al. (2010); Wittmann et al. (2003) and Soldevilla et al. (2006) for planktonic species. Castro (1995) describes the mysids in the stomach contents of *Scomber japonicus* (Castro, 1995). There are 18 coastal mysids species cited for the Canary Archipelago:

Order Mysida Haworth, 1825
Family Mysidae Haworth, 1825

Subfamily Siriellinae Czerniavsky, 1882
Siriella armata (Milne-Edwards, 1837)∗
Siriella claussi (G.O. Sars, 1877)
Siriella gracilipes (Nouvel, 1942)

Subfamily Gastrosaccinae Norman, 1892
Anchialina agilis (G.O. Sars, 1877)
Gastrosaccus roscoffensis (Bacescu, 1970)∗
Gastrosaccus sanctus (Van Beneden, 1861)∗
Haplostylus bacescui (Hatzakis, 1977)∗
Haplostylus lobatus (Nouvel, 1951)

Subfamily Erythropinae Hansen, 1910
Erythrops elegans (G.O. Sars, 1863)

Subfamily Leptomysinae Hansen, 1910
Leptomysis sp.A (aff heterophila)∗
Leptomysis lingvura
Leptomysis sp.C (aff mediterranea)∗
Paraleptomysis banyulensis (Bacescu, 1966)
Mysidopsis sp. A (aff gibbosa)

Subfamily Mysinae Haworth, 1825
Hemimysis sp. A (aff. maderensis)∗
Schistomysis sp. Ac (aff spiritus)∗
Paramysis arenosa (G.O. Sars, 1977)∗

Subfamily Heteromysinae Norman, 1892
Heteromysoides cotti (Calman, 1932)

Those mysids collected in Gran Canaria are marked with an asterisk (above).

Listed below are other 8 mysids species found in the stomach contents of *Scomber japonicus* sampled off Gran Canaria (Castro, 1995).

Subfamily Mysinae Haworth, 1825
Paramysis spp.
Stilomysis sp. (*S. grandis* ?)

Subfamily Siriellinae Czerniavsky, 1882
Siriella sp.
S. norvegica (G.O. Sars,1869)
S. thompsoni (Milne-Edwards, 1837)

Subfamily Gastrosaccinae Norman, 1892
Gastrosaccus normani (G. O. Sars, 1877)

Subfamily Boreomysinae Holt and Tattersall, 1905
Paramblyops sp.

Subfamily Leptomysinae Hansen, 1910
Leptomysis sp. (*L. sardica* ?)

2.2. Material and Methods

2.2.1. Sampling method

The mysids were collected off the east (Risco Verde, Roque Arinaga, Faro de Arinaga and Playa del Cabron) and west (Veneguera) coasts of Gran Canaria in nearshore areas between 5 and 15 meters deep, mainly in *Cymodocea nodosa* meadows and sandy bottoms near the rocks (Figure 2.1).

Half of each sample was preserved in 4 % formalin for later identification, and the other part was preserved in 90-100 % ethanol for genetic analysis. Individuals of different species were selected and numbered as shown in Table 2.1.

Two methods were used for the collection: in *Cymodocea nodosa* with a 6 m long, 4 m wide, 0.5 m high trawl net with a mesh size of 1 mm towed by two SCUBA divers (Figure 2.2), and in areas near the rocks with a hand net of 1 mm mesh size (Figure 2.3).

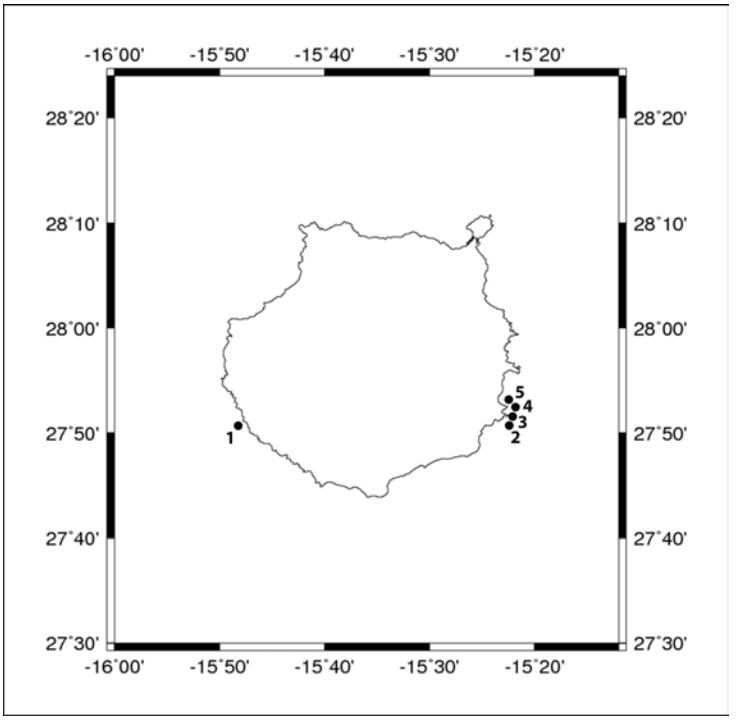


Figura 2.1: Map of Gran Canaria showing sampling areas. 1. Veneguera 2. Risco Verde 3. Roque de Arinaga 4. Faro de Arinaga 5. Playa del Cabron.

Tabla 2.1: Samples ID number and sampling data.

Sample ID	Locations	Substrate	Date
1	Veneguera	*Cymodocea*	25/05/2011
2	Risco Verde	Sandy bottoms	25/05/2011
3	Faro	Sandy bottoms	25/05/2011
4	Faro	*Cymodocea*	17/11/2011
5	Roque	Sandy bottoms	22/05/2011
6	Risco Verde	Sandy bottoms	17/11/2011
7	Cabron	*Cymodocea*	17/11/2011
8	Veneguera	*Cymodocea*	28/11/2011

Figura 2.2: Net towed by two SCUBA divers. This is the method used to collect mysids in *Cymodocea nodosa* meadows. Photo by Enrique Faber.

Figura 2.3: Hand net. This is the method used in sandy bottoms near rocks.

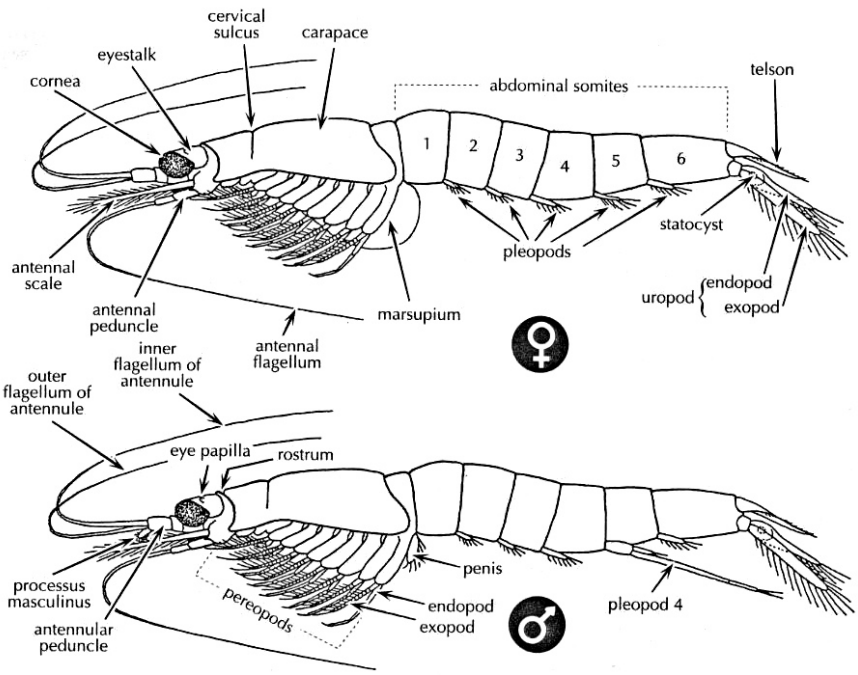


Figura 2.4: Typical mysid in side view. From Murano (1999)

2.2.2. Taxonomic study

The samples were classified to species level with binocular microscope, following keys by Tattersall and Tattersall (1951), Labat (1953), Wittmann (1986), Barberá-Cebrián et al. (2001) and Wittmann et al. (2010). To identify species of mysids one of most important characters is the telson, also the carapace, rostrum, eyes, antennal scale, pereopods and uropods (Figure 2.4).

2.2.3. Genetic study

DNA extraction, PCR amplification and subsequent sequencing of nuclear small-subunit ribosomal DNA (18S rRNA) gene was carried out in the Instituto Universitario de Sanidad Animal y Seguridad Alimentaria (IUSA). DNA extraction was performed following the phenol-chloroform method described by Sambrook et al. (1989). The extracted DNA was stored at 4°C in TE (Tris

0.01 mM and 0.001 mM EDTA, pH 8.0). The quality and quantity of DNA was determined using a spectrophotometer Nanodrop 1000 v.3.7 (Thermo Fisher Scientific, Wilmington, USA). DNA integrity was checked by gel electrophoresis on 1 % agarose (8 v cm^{-1}) by staining with ethidium bromide (0.5 μg μl^{-1}) and by analysing with the Quantity One (Bio-Rad Laboratories, Hemel Hempstead Hertfordshire, United Kingdom), using Lambda Hind III, as a molecular weight marker (Navarro et al., 2008; Lee-Montero et al., 2013). PCR conditions consisted of an initial denaturation at 95°C for 3 min, followed by 35 cycles of 1 min at 95°C, 30 sec at 52.4°C and 2 min at 72°C, with a final extension of 5 min at 72°C. The reaction volume was 25 μl with the following concentrations shown in the table 2.2.

Tabla 2.2: Concentration of reagents in the PCR anaysis.

Reagents	[Initial]	Volume per sample
$MgCl_2$	25mM	2.5 μl
Buffer	10X	2.5 μl
Mix (dATP, dCTP, dGTP, dTTP)	10mM	0.5 μl
PrimerF	10 pmol μl^{-1}	2 μl
PrimerR	10 pmol μl^{-1}	2 μl
H_2O MilliQ		12.3 μl
DNA	80 ng μl^{-1}	1 μl
TAQ-polymerase		0.2 μl

Redesign of primers were performed in the IUSA laboratory where they were optimized to the maximum consecutive amplification product and to the length of the sequence obtained. This, in all cases exceeded 300 base pairs (bp). The primers redesigned were:
5'-GCCAGTAGTCATATGCTTG-3'
5'-GTGGTAGCCGTTTCTCAG-3'for sample 8; and
5'-AACACGGGAAATCTCACCAG-3'
5'-TGATCCTTCCGCAGGTTCACCT-3' for the other samples.

Amplicons obtained by PCR were purified and sequenced using the BigDye®

Terminator v3.1 Cycle Sequencing Kit following the recommendations of the manufacturer to ensure the correct amplification of the target genes. The sequencing products were run on an ABI Prism 3100 Genetic Analyzer (Applied Biosystem®) and electropherograms were analysed with MEGA v.4 software. BLAST® (Basic Local Alignment Search Tool) was carried out to search for homology. The alignment with the sequences registered in the NCBI (National Center of Biotechnology Information) database was used for construction of the matrix of genetic distances and for the graphical representation of phylogenetic tree of each sample.

2.3. Results

Six species were identified: *Siriella armata* (Milne-Edwards, 1837) from samples 1 and 3, *Leptomysis* sp. aff *heterophila* sensu Wittmann and Wirtz, 1998 from samples 2 and 6, *Leptomysis lingvura* ssp. B sensu Wittmann and Wirtz, 1998 from sample 4, *Paramysis arenosa* (G.O. Sars, 1877) from samples 5, *Anchialina agilis* (G.O. Sars, 1877) from sample 7 and *Gastrosaccus roscoffensis* (Bacescu, 1970) from sample 8.

2.3.1. *Siriella armata* (Milne-Edwards, 1837)

Scientific synonyms and common names:
Cynthis armata Milne-Edwards, 1837
Cynthia flemingii Goodsir, 1842
Mysis rostrata Guerin-Meneville, 1844
Cynthilia flemengii (Goodsir, 1842)
Mysis griffithsiae Bell, 1853
Mysis productus Gosse, 1853
Siriella armata (Milne-Edwards, 1837)
Siriella flemingi (Goodsir, 1842)
Pseudosiriella frontalis Norman, 1886
Siriella intermedia Gourret, 1888
Cynthilia armata (Milne-Edwards, 1837)
Cynthilia frontalis (Norman, 1886)
Siriella frontalis (Norman, 1886)

Description: (Abstracted directly from Tattersall and Tattersall (1951))
General form very long and slender. Rostrum very long and acutely pointed, reaching almost to the distal end of the second segment of the antenal peduncule.
Carapace small.
Antennular peduncle very long and slender.
Antennal scale long and narrow with the sides almost parallel and a small distal suture present, outer margin naked and slightly concave terminating in a strong tooth, inner margin slightly convex. Eyes with long cylindrical stalks projecting laterally beyond the sides of the carapace.
Telson long and slightly tapering, entire; proximal marginal spines subequal, distally the large prominent spines have small ones between in series of six to ten; apex narrowly rounded, with two large spines, and between them from three to five (usually four) small equal spinules and two small setae.
Uropod very long and slender; exopod from six to six and a half times as long as broad, with a distal suture. Outer margin, armed with a continuous row of about thirty spines.
Endopod shorter than, and about half as broad as, the exopod.

Size: Length 21 mm (Tattersall and Tattersall, 1951). In collected samples average length was 15 mm.

Colour: Almost completely transparent, lacking all trace of chromatophores on the posterior part of the thoracic somites.

Habitat: Hyperbenthic; shallow waters: 0-30 metres. Rarely taken at a depth of more than 20 metres.

World distribution: North-East Atlantic: 33-56°N; Mediterranean; coastal, shelf.
Distribution in Gran Canaria: *S. armata* was recorded by Wittmann and Wirtz (1998) in Muelle de Arinaga, 27.85N/15.40W in *Cymodocea* meadow at 7 m. In the present study was collected in *Cymodocea nodosa* meadows in Veneguera, Risco Verde, Roque de Aringa, Faro de Arinaga and Playa del Cabron at depths between 10 and 15 m.

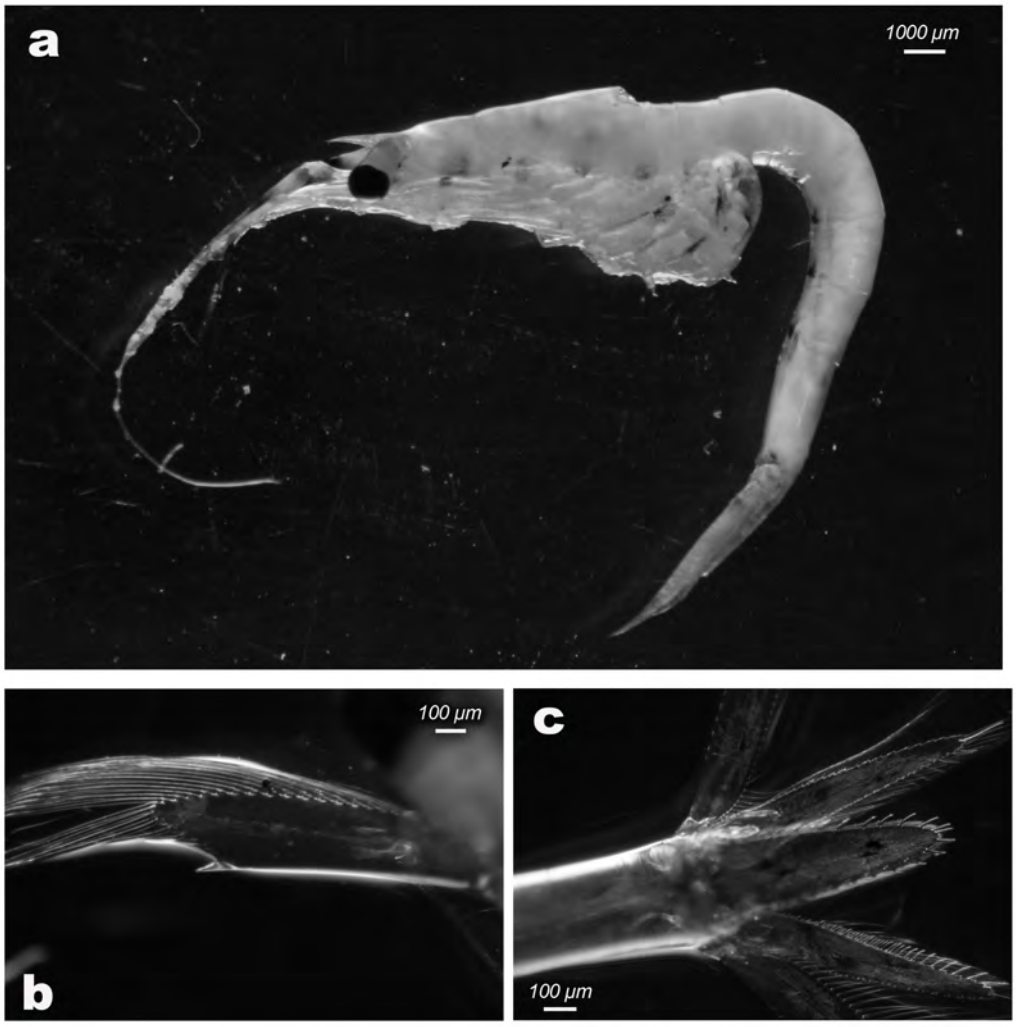


Figura 2.5: *Siriella armata*. a. Adult female b. Antennal scale c. Telson

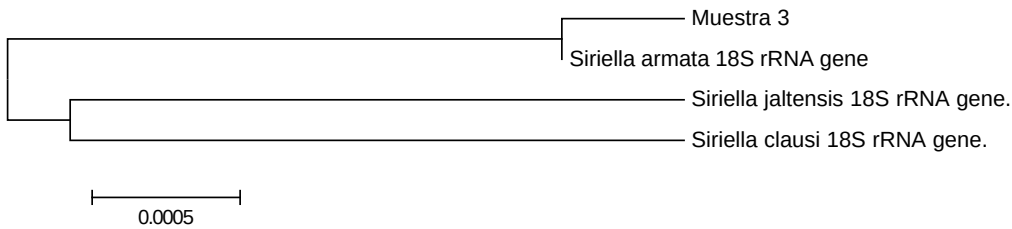


Figura 2.6: Phylogenetic tree representation of sample 3.

Genetic study: After amplification and subsequent sequencing, sample 3 achieved a sequence of 587 pairs of bases (bp). Using BLAST® the alignment with the species of the database provided 100 % identity with *Siriella armata*. The genetic distances between species are shown in the table 2.3, and figure 2.6 shows the corresponding phylogenetic tree representation.

Tabla 2.3: Genetic distances between species in sample 3. For more information, GenBank accession numbers are given.

Sample 3	Accession GenBank			
Siriella armata	AJ566105.1	0.000		
Siriella clausi	AJ566107.1	0.005	0.004	
Siriella jaltensis	AJ566106.1	0.005	0.004	0.004

2.3.2. *Leptomysis* sp. aff *heterophila* sensu Wittmann and Wirtz, 1998

Description: (Abstracted directly from Wittmann and Wirtz (1998)) This new species resembles *L. mediterranea* (G.O. Sars, 1877) by the form of the antennal scale and the large rostrum, however, the terminal joint of the antennal scale shows only 11-16 setae. The species differs from *L. buergii* (Bacescu, 1966) by having the posterior pore group not integrated into the posterior margin of the carapace.

Size: In collected samples, the average length was 12 mm.

Colour: Transparent.

Habitat: Bentic. Shallow waters.

Distribution in Gran Canaria: This new species was sampled for the first time off Gran Canaria over rocks, sand and *Cymodocea* meadows between 7 and 22 m by Wittmann and Wirtz (1998). For the present work it was sampled in *Cymodocea* meadows between 10 and 15 m in Veneguera, Risco Verde, Roque de Arinaga and Faro de Arinaga.

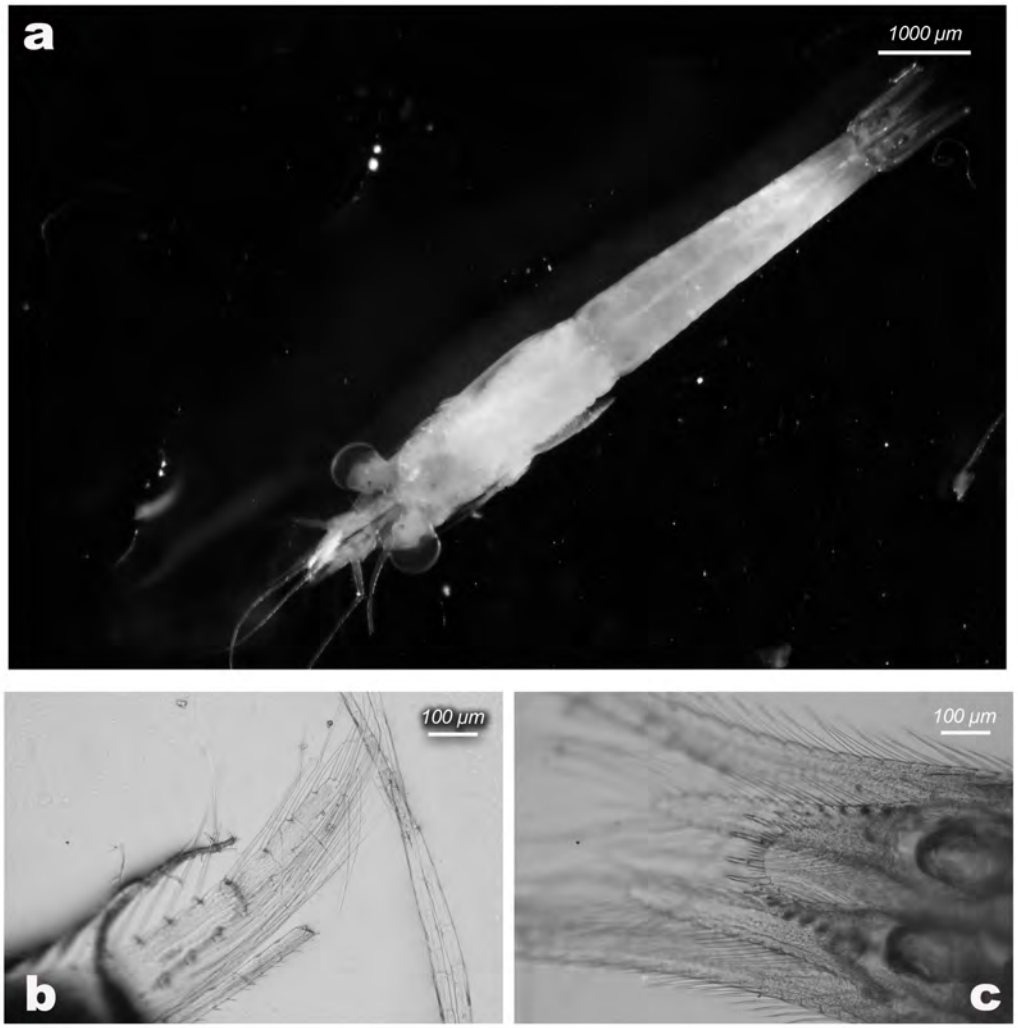


Figura 2.7: *Leptomysis* sp. aff *heterophila*. a. Adult b. Antenal scale c. Telson

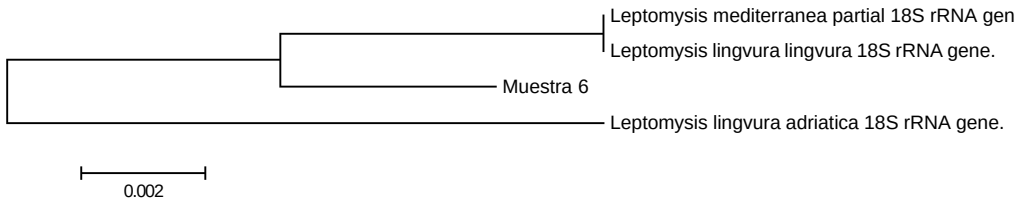


Figura 2.8: Phylogenetic tree representation of sample 6.

Genetic study: For sample 6 was obtained a sequence of 640 bp. The alignment with the species of the NCBI database provided 98 % identity with *Leptomysis lingvura lingvura* and *Leptomysis mediterranea*. Table 2.4 shows the genetic distances and figure 2.8 shows the corresponding phylogenetic tree representation.

Tabla 2.4: Genetic distances between species in sample 6. For more information, GenBank accession numbers are given.

Sample 6	Accession GenBank			
Leptomysis mediterranea	AM422503.1	0.007		
Leptomysis lingvura lingvura	AJ566099.1	0.007	0.000	
Leptomysis lingvura adriatica	AJ566098.1	0.021	0.025	0.025

2.3.3. *Leptomysis lingvura* ssp. sensu Wittmann and Wirtz, 1998.

Scientific synonyms and common names of *Leptomysis lingvura*:
Mysis lingvura G.O. Sars, 1866
Leptomysis sardica G.O. Sars, 1877
Leptomysis pontica Czerniavsky, 1882
Leptomysis marioni Gourret, 1888
Leptomysis linguura Zimmer, 1933

Subspecies:
Leptomysis lingvura lingvura Wittmann, 1986
Leptomysis lingvura adriatica G.O. Sars, 1877
Leptomysis lingvura marioni Gourret, 1888

Description of species: (Abstracted directly from Tattersall and Tattersall (1951))
General form more shorter, more compact and robust than *L. mediterranea*. Rostrum short, triangular, acutely pointed. The form of the rostrum is the most reliable character by which the species may be recognized.
Antennal scale shorter than in *L. mediterranea*, less than twice the length of the antennular peduncle; divided in two segments, distal segment occupies one-fourth to one-third of the total length and has from four to five setae on each margin.
Eyes more closely set than in the other species, with characteristic pigments.
Uropod shorter and considerably broader than in the other species. Exopod is longer and curved outward, and the endopod has a big statocyst and spines in their inner margin.
Telson entire, linguiform, shorter and broader than in the other species, apex broadly rounded, normally armed with two long spines flanking two much smaller median spinules.

Description of the new subspecies: (Abstracted directly from Wittmann and Wirtz (1998))
The local population of Madeira and the Canaries correspond to *L. lingvura lingvura* (G.O. Sars 1866) in most aspects, but are different by showing 8-segmented exopod of fourth male pleopod (as in *Leptomysis lingvura adria-*

tica (Wittmann1986) and *Leptomysis lingvura marioni* (Gourret, 1888)), in contrast to 10 to 11-segmented one in other Atlantic populations.

Size: Length 17 mm (Tattersall and Tattersall, 1951). In the present work the average length for *L. lingvura* ssp. was 12 mm.

Colour: Less transparent than other species, with brown pigmentation.

Habitat: Littoral; usually found in swarms near the coast in water of 10 meters in depth.

Distribution in Gran Canaria: *L. lingvura* ssp. was not previously cited for Gran Canaria, but was sampled in El Hierro island (Wittmann and Wirtz, 1998). For the present work it was sampled in swarms resting in sandy bottoms near the rocks in Risco Verde at 5 m; and in *Cymodocea* meadows between 10 and 15 m in Veneguera, Risco Verde, Roque de Arinaga and Playa del Cabron.

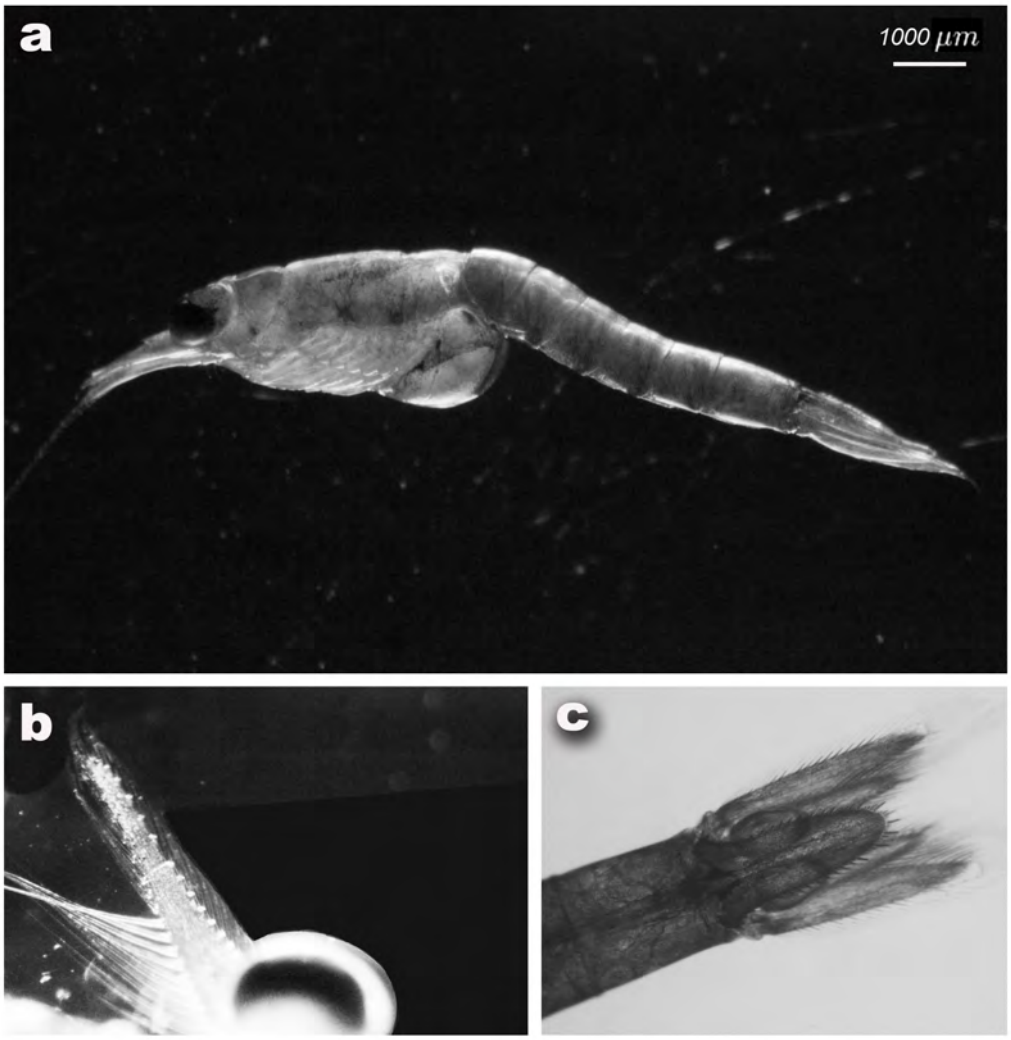


Figura 2.9: *Leptomysis lingvura*. a. Adult b. Antennal scale c. Telson

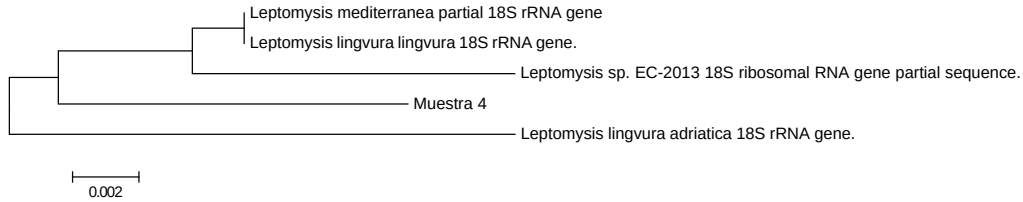


Figura 2.10: Phylogenetic tree representation of sample 4.

Genetic study: For sample 4 after amplification and subsequent sequencing achieved a sequence of 506 bp. The alignment with the species of the NCBI database provided 98 % identity with *Leptomysis lingvura lingvura* and *Leptomysis mediterranea*. The genetic distances between species are shown in the table 2.5, and figure 2.10 shows the corresponding phylogenetic tree representation.

Tabla 2.5: Genetic distances between species in sample 4. For more information, GenBank accession numbers are given.

Sample 4	Accession GenBank				
Leptomysis mediterranea	AM422503.1	0.007			
Leptomysis lingvura lingvura	AJ566099.1	0.007	0.000		
Leptomysis lingvura adriatica	AJ566098.1	0.021	0.025	0.025	
Leptomysis sp. EC-2013	KC763182.1	0.025	0.011	0.011	0.030

2.3.4. *Paramysis arenosa* (G.O. Sars, 1877)

Scientific synonyms and common names:
Mysis arenosa G.O. Sars, 1877
Austromysis arenosa (G.O. Sars, 1877)
Schistomysis arenosa (G.O. Sars, 1877)

Description: (Abstracted directly from Tattersall and Tattersall (1951))
General form small and robust, with closely branching chromatophores, especially in the dorsal region of the carapace. Abdomen robust.
Carapace short and emarginate posteriorly, anterior margin rounded produced into a very short, obtusely triangular rostrum.
Antennule with short, robust peduncle, first segment nearly equal in length to the sum of the second and third segments.
Antennal scale oval, short and about twice as long as broad, outer margin unarmed and ending in a strong tooth beyond which the apex extends considerably, a small distal suture present.
Eyes short and thick, cornea occupying about one half of the whole organ, pigment black.
Uropod short, endopod only slightly longer than the telson, statocyst small, inner margin armed along the whole of its length with 28-30 spines which are arranged in an irregular row of large spines with small graduated spines between them. Exopod oval, about one-fifth as long again as the endopod.
Telson longer and narrower than the last abdominal somite, tapering distally; lateral margins armed with 19-22 small spines on each side, apex deeply cleft for about one-fourth of the total length of the telson, armed with even, closely-set teeth.

Size: Length 7 mm (Tattersall and Tattersall, 1951). In samples collected for the present study the average length was 8 mm.

Colour: Transparent with arborescent yellow and brown and rose markings.

Habitat: Coastal; shallow waters of 10 metres in depth.

World distribution: Eastern- North Atlantic, 48-57°N; Mediterranean;

coastal, shelf.

Distribution in Gran Canaria: *P. arenosa* was collected in Gran Canaria in Puerto de Pasitos Blancos, 26.75°N, 15.62°W, in *Cymodocea* meadow at 9 m.; and in Muelle de Arinaga, 27.85°N, 15.40°W in *Cymodocea* meadow at 7 m Wittmann and Wirtz (1998).
In the present study *P.arenosa* was collect in shallow sandy bottoms in Risco Verde at 5 m; and in *C.nodosa* meadow between 10 and 15 m in Veneguera, Roque de Arinaga, Faro de Arinaga and Playa del Cabron.

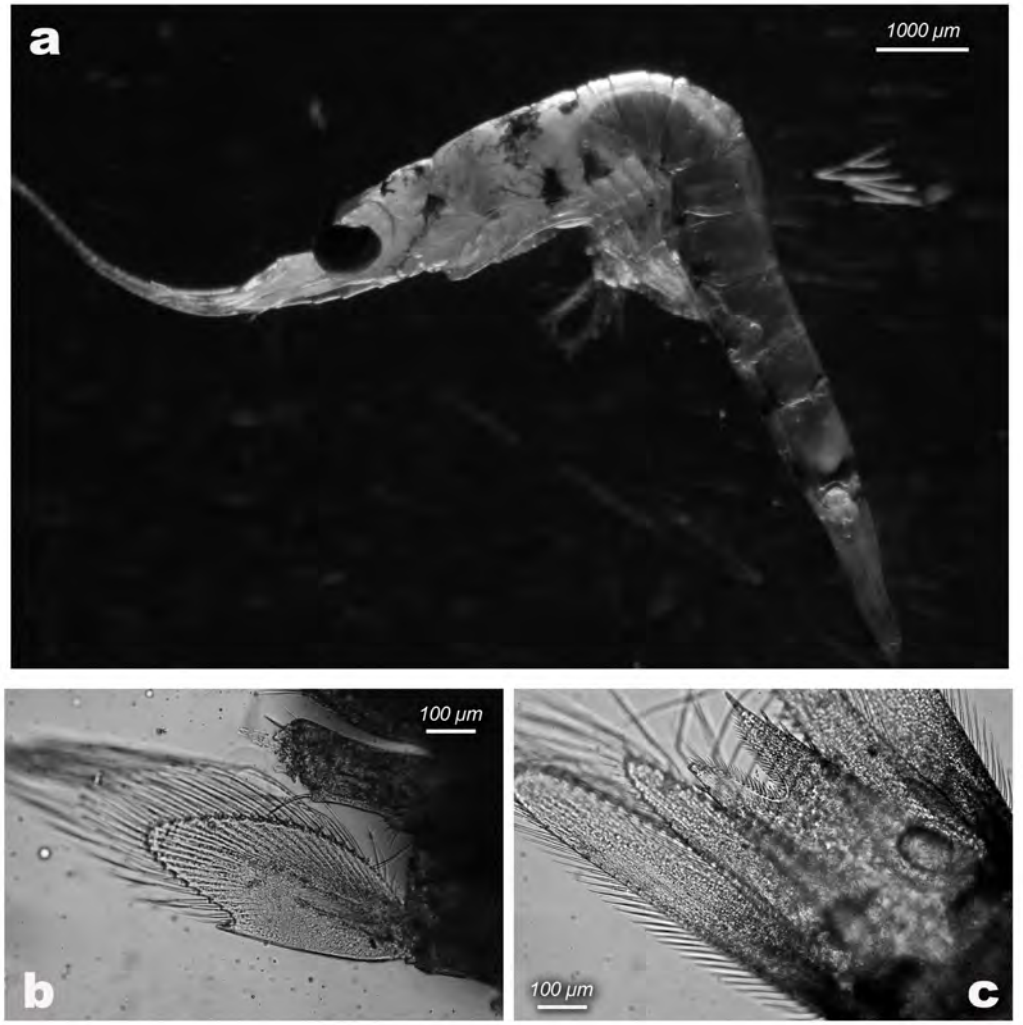


Figura 2.11: *Paramysis arenosa*. a. Adult b. Antennal scale c. Telson

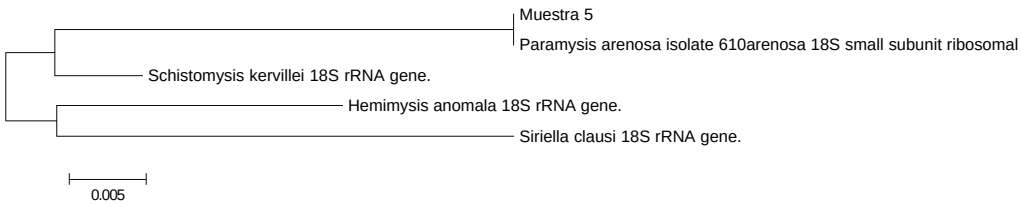


Figura 2.12: Phylogenetic tree representation of sample 5.

Genetic study: For sample 5 was obtained a sequence of 600 bp. Using BLAST®, the alignment with the species of the database provided 100 % identity with *P. arenosa*. Table 2.6 shows genetic distances between species and figure 2.12 shows the phylogenetic tree representation.

Tabla 2.6: Genetic distances between species in sample 5. For more information GenBank accession numbers are given.

Sample 5	Accession GenBank				
Paramysis arenosa	EU233513.1	0.000			
Schistomysis kervillei	AJ566103.1	0.007	0.000		
Hemimysis anomala	AJ566104.1	0.021	0.025	0.025	
Siriella clausi	AJ566107.1	0.025	0.011	0.011	0.030

2.3.5. *Anchialina agilis* (G.O. Sars, 1877)

Scientific synonyms and common names:
Anchialina mediterranea Colosi, 1922

Description: (Abstracted directly from Tattersall and Tattersall (1951))
General form very compact and robust, whole animal unusually broad in proportion to its length.
Carapace large, covering the whole of the thorax and more than half the first abdominal somite, in dorsal view, widening considerably posteriorly, posterior margin straight and transverse.
Antennal scale very small, extending only slightly beyond the first segment of the antennular peduncle, outer margin naked, terminating in a small spine beyond which the apex extends considerably, small distal suture present.
Uropods, endopod longer than exopod. In the endopods the two strong spines arming the distal end are very characteristic of the species, statocyst small.
Telson very long, nearly half as long as the whole abdomen, three times as long as broad at the base; lateral margins straight, armed with 25-30 closely set, plumose spines, terminal one much larger than the rest; cleft small, about one-seventh of the length of the telson, armed with a close row of about 25 teeth on each side. Apex of each lobe armed with a strong spine.

Size: 9 mm in males, 7-8 mm in females Tattersall and Tattersall (1951). In the present work the average length was 6 mm.

Colour: Transparent.

Habitat: Hyperbenthic, coastal to upper slope. *A. agilis* is found near to the bottom during day but it is capable of very rapid movement from one layer of water to another and can also be taken at the surface and in varying depths during the day. It has not been taken in depths of more than 85 m, but has been collected at the surface and in mid-water over 150 m in Irish waters. It has been most frequently taken at the surface at midnight when it occurs in large numbers (http://species-identification.org/index.php).

World distribution: Eastern- North Atlantic: <26 - 56°N; Mediterranean.

Distribution in Gran Canaria: *A. agilis* was previously cited in the stomach content of *Scomber japonicus* sampled in waters off Gran Canaria (Castro, 1995); and for Tenerife island in *Cymodocea* meadows at 18 m Wittmann and Wirtz (1998).
In the present work it was sampled in *Cymodocea* meadows between 10 and 15 m in Veneguera, Risco Verde, Roque de Arinaga, Faro de Arinaga and Playa del Cabron.

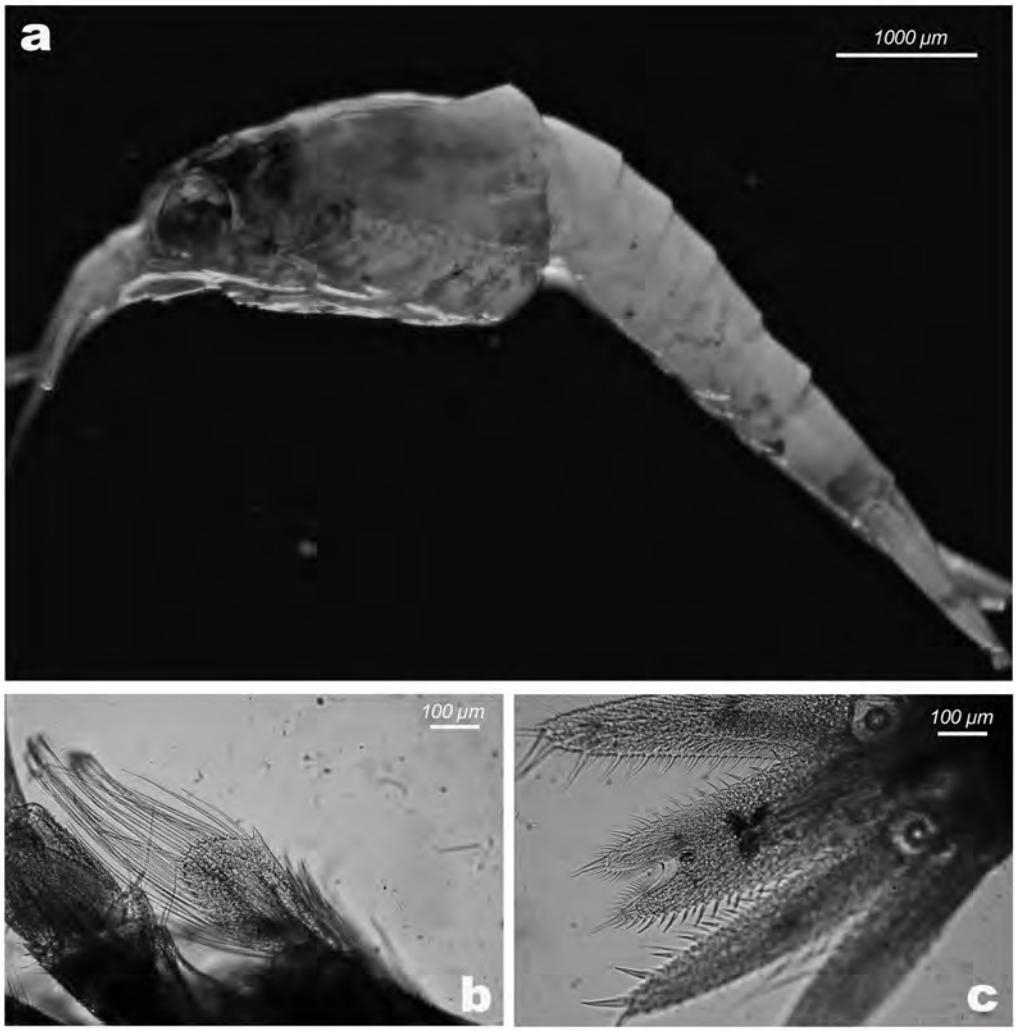


Figura 2.13: *Anchialina agilis* a. Adult b. Antennal scale c. Telson

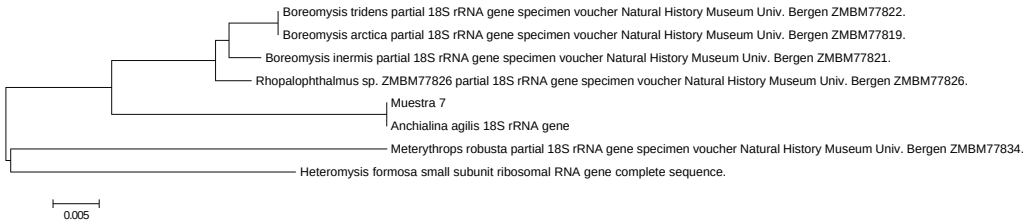


Figura 2.14: Phylogenetic tree representation of sample 7.

Genetic study: After amplification and subsequent sequencing , sample 7 achieved a sequence of 575 bp. The alignment with the species of the NCBI database provided 100 % identity with *Anchialina agilis*. The genetic distances between species and corresponding phylogenetic tree representation are shown in the table 2.7 and figure 2.14 respectively.

Tabla 2.7: Genetic distances between species in sample 7. GenBank accession numbers are given.

Sample 7	Accession GenBank					
Anchialina agilis	AM422487.1	0.000				
Boreomysis inermis	AM422482.1	0.047	0.047			
Boreomysis tridens	AM422484.1	0.047	0.047	0.009		
Boreomysis arctica	AM422481.1	0.047	0.047	0.009	0.000	
Rhopalophthalmus sp.	AM422488.1	0.047	0.047	0.009	0.011	0.011

2.3.6. *Gastrosaccus roscoffensis* (Bacescu, 1970)

Description: (Abstracted directly from Tattersall and Tattersall (1951) and Wittmann et al. (2010))
General form slender and transparent, somewhat laterally compressed. Antennal scale short, outer margin without setae and terminating in a strong spine. Small eyes. Telson long, quadrangular, with apical cleft armed with spines, lateral margin also armed with spines.
G. roscoffensis differs from other species of *Gastrosaccus* by the form of the lobes on the caudal side of carapace. Differs from close genus *Haplostylus* Kissmann, 1880, by less number of spines, usually five, rarely six, on the lateral margin of the telson, and the multi-segmented endopod of the third male pleopod.

Size: Length 6.1-11.1 mm in females and 6.5-8.7 in males (Wittmann et al., 2010). Here, length ranged from 4 to 8 mm.

Colour: transparent

Habitat: Benthic. Littoral shallow waters.

World distribution: Atlantic coast of Spain and France. Spanish mediterranean coast. South coast of Portugal and Canary Islands.

Distribution in Gran Canaria: *Gastrosaccus roscoffensis* has recently been cited for the Canary Islands. In Gran Canaria samples were collected between 7 and 15 m depth at night from Pasito Blanco to Faro de Maspalomas (Wittmann et al., 2010). Here, samples were collected in Veneguera, Risco Verde, Roque de Arinaga, Faro de Arinaga and Cabron between 7 and 15 m depth in *Cymodocea* meadows.

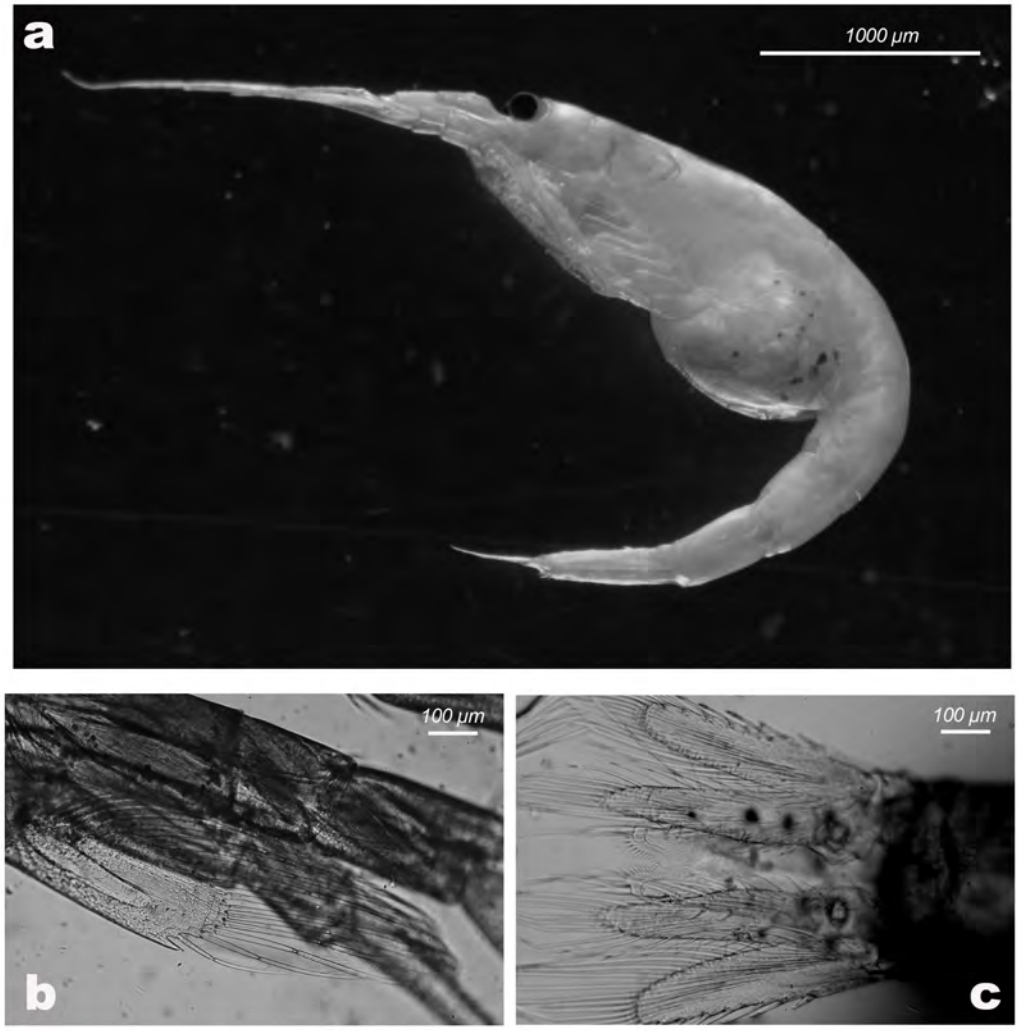


Figura 2.15: *Gastrosaccus roscoffensis*. a. Adult b. Antennal scale c. Telson

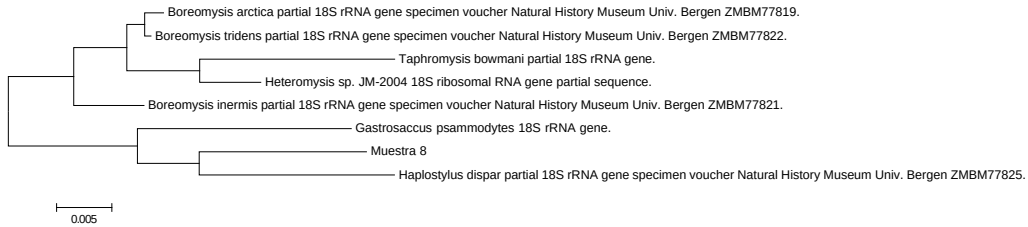


Figura 2.16: Phylogenetic tree representation of sample 8.

Genetic study: For sample 8 after amplification and subsequent sequencing achieved a sequence of 310 bp. The alignment with the species of the NCBI database provided a 95 % identity with *Haplostylus dispar*. The genetic distances between species and corresponding phylogenetic tree representation are shown in the table 2.8 and figure 2.16 respectively.

Tabla 2.8: Genetic distances between species in sample 8. GenBank accession numbers are given.

Sample 8	Accession GenBank						
Haplostylus dispar	AM422487.1	0.033					
Boreomysis arctica	AM422481.1	0.045	0.048				
Boreomysis tridens	AM422484.1	0.047	0.050	0.002			
Taphromysis bowmani	AM422514.1	0.056	0.064	0.030	0.028		
Gastrosaccus psammodytes	AJ566087.1	0.042	0.040	0.045	0.048	0.056	
Boreomysis inermis	AM422482.1	0.045	0.048	0.014	0.011	0.040	0.043

2.4. Discussion

The study of mysids is recent in the Canary archipelago. Wittmann and Wirtz (1998) published the first inventory of coastal mysids which cited five of the six species identified in this study: *Siriella armata*, *Anchialina agilis*, *Leptomysis lingvura* spp., *Leptomysis* sp. (aff. *heterophila*) and *Paramysis arenosa*. Wittmann et al. (2010) recently published the first record of *Gastrosaccus roscoffensis* for the Canary Islands, one of the species identified here. This species has small morphological differences with the Roscoff (France) species, consequently the authors, lacking other studies such as genetic analysis, have not considered the possible establishment of a different taxon for the Canary Islands species (Wittmann et al., 2010). Genetic studies of *G. roscoffensis* have not confirmed the species because to date there are no genes sequenced in the GenBank database for this species. Identification has been carried out based on morphological characteristics described by Wittmann et al. (2010) and has been corroborated by Dr. Karl Wittmann. The 18S rRNA gene sequence of *Gastrosaccus roscoffensis* will be the first record of this species in GenBank, and will facilitate the comparison with the samples collected in other areas.

In *Siriella armata*, *Paramysis arenosa* and *Anchilina agilis* the genetic study provided a 100 % identity, confirming the previous taxonomic classification. However, in samples 2, 4 and 6 the genetic study did not permit a determination of the species. It only provided 98 % identity with *L. mediterranea* and *L. lingvura lingvura*. In these cases the identification was only possible by taxonomic examination of the samples.

The morphological characteristics of sample 4 corresponded to those described by Wittmann and Wirtz (1998) for *Leptomysis lingvura* ssp. B., while the description of *Leptomysis* sp. aff. *heterophila* matched the description of samples 2 and 6. These two taxa have not yet been described by the authors, therefore the identification is based on the observations that appear in the work of Wittmann and Wirtz (1998). This was corroborated by one of the authors, Dr. Karl Wittmann. He personally confirmed that the species are the same as the previously mentioned ones in his publication with Wirtz (above). *L. lingvura* ssp. had never been observed before in waters off Gran Canaria.

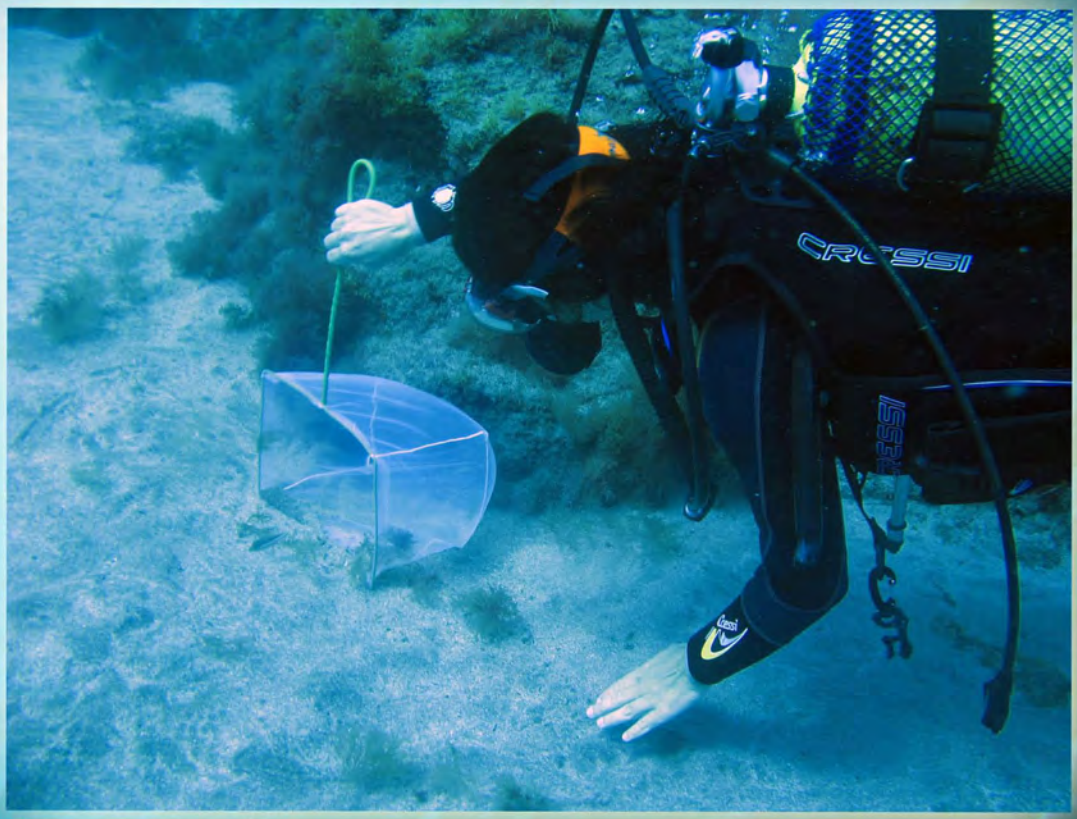
It is clear that much remains to be investigated in the taxocenosis of mysids in the Canary Islands, as there are species and subspecies described yet. The study and sequencing of the samples collected in this work will foster advances in the knowledge of the community of mysids inhabiting the coast of Gran Canaria.

Acknowledgements

I would like to thank Tony Sánchez, Fernando Espino and Fernando Tuya for their cooperation during the sampling. I am gratefull to Dr. Karl Wittmann and José María Espinosa for his collaboration in the identification. This work was supported by Project BIOMBA project (CTM2012-32729) granted to M. Gómez, and PhD scholarship from University of Las Palmas de Gran Canaria granted to A. Herrera.

Capítulo 3

Abundance of mysids (Crustacea Mysidacea) asociated with Cymodocea nodosa seagrass



Capítulo 3

Abundance of mysids (Crustacea Mysidacea) associated with *Cymodocea nodosa* seagrass meadows.

Abstract: Marine phanerogam meadows are important ecosystems in shallow coastal waters maintaining a high diversity of species. Mysids are the dominant taxa in suprabenthic organisms associated with seagrass meadows in temperate coastal waters, where they are an important food resource for the coastal fishes. Five meadows of *C. nodosa* were sampled off the east and west of Gran Canaria Island in spring and autumm to describe associated suprabenthos and to determine seasonal changes in the abundance of suprabenthos community. Mysids, decapods and amphipods made up 95 % of total suprabenthos abundance, which was more abundant in spring than in autumm. Six species (in order of abundance) of mysids were identified: *Siriella armata* (Milne-Edwards, 1837), *Gastrosaccus roscoffensis*, *Paramysis arenosa* (G.O. Sars, 1877) *Leptomysis* sp. aff. *heterophila* sensu Wittmann and Wirtz (1998), *Anchialina agilis* (G.O. Sars, 1877), and *Leptomysis lingvura* (G. O. Sars, 1866). *Leptomysis lingvura* did not show seasonal differences, while *Anchialina agilis* showed greater abundance in May at all localities. For the other mysid species abundances were higher in May than November. However, significant differences varied among

localities, but was probably masked by high variability among replicates. From these results, it was clear that there is an overlap between the natural life cycle of *Cymodocea* and the associated suprabenthos in Gran Canaria Island.

3.1. Introduction

Marine phanerogam meadows are important ecosystems that contribute to ocean primary and secondary production, and have important ecological and physical functions, being responsible for about 15 % of the carbon storage in the ocean (Duarte and Cebrian, 1996; Duarte and Chiscano, 1999). Conservation of seagrass meadows is important specially because these ecosystems are declining worldwide mainly due to human disturbances (Duarte et al., 2002).

Suprabenthos, also called "hyperbenthos", are an important community in coastal ecosystems, exploiting a diversity of food resources: organic particles, detritus, zooplankton, and have an important trophic role as food for juveniles and adults of several commercially important fish species (Mees and Jones, 1997; Cunha et al., 1999). Suprabenthos include mysids, amphipods, cumaceans, isopods, decapods and polichaetes bottom-dependent, which perform with regular vertical migrations above the bottom (Sainte-Marie and Brunel, 1985). Mysids are a dominant motile macrofauna in temperate coastal seagrass ecosystems and as suprabenthos, they are important as food in both juvenile and adult commercial fish (Mauchline, 1980; Murano, 1999; Yamada and Kumagai, 2012). For example, in Gran Canaria Island, Castro (1995), highlighted the importance of mysids as food, based on the stomach contents of *Scomber japonicus*. He estimated that this species consumes annually about 242,000 tons of mysids and 29,000 tons of euphausiids.

In Gran Canaria Island, the seagrass *Cymodocea nodosa* is the dominant vegetal specie on soft bottoms in coastal waters along the eastern and southern coasts (Reyes et al., 1995; Pavón-Salas et al., 2000; Barberá et al., 2005). There they serve important ecological functions such as: generation

of detritus, habitat creation for omnivorous and herbivorous organisms that transfer carbon to high trophic levels, recruitment and nursery habitat for numerous coastal fish species (Espino et al., 2011a,b). *C. nodosa* was legislated as an endangered species by the autonomous government of the Canary Islands (Decreto 151/2001, de 23 de julio, Catálogo de Especies Amenazadas de Canarias), however, despite *C.nodosa*'s serious decline in the past 17 years (Tuya et al., 2012), environmental protection has been reduced since 2010 by law 7L/PPL-0011 Del Catálogo Canario de Especies Protegidas (BOPC núm. 167, de 14/5/10).

For this study we hypothesized that patterns in the abundance of suprabenthos in the Canary Islands would follow a similar seasonal trend of *C. nodosa* that show a maximum production period during summer and spring (Reyes et al., 1995; Tuya et al., 2006). We sampled in May (late spring) and November (late autumm) to determine the main taxonomic groups of suprabenthos associated with *C. nodosa* and their temporal variability, with special interest in mysids. Mysids were chosen because of the higher abundance in seagrass meadows and their trophic importance in coastal ecosystems. While there are several studies about *C. nodosa* ecosystems and associated ichtyofauna and macrofauna in the Canaries (Tuya et al., 2001; Barberá et al., 2005; Tuya et al., 2006; Espino et al., 2011b,a), there is no study on diversity and seasonality of seagrass associated suprabenthos.

3.2. Material and Methods

3.2.1. Sampling method

We selected five meadows of *C. nodosa* in the east and west coast of Gran Canaria: Veneguera, Risco Verde, Roque de Arinaga, Faro de Arinaga and Playa del Cabron (Figure 3.1). Surveys were conduced in late spring on May and late autumm on November of 2011.

Samples (n=4) were collected using a 6 m-long, 4 m-wide, 0.5 m-high trawl net with a mesh size of 1mm. The net was towed by two SCUBA divers along 25 m transect, collecting a volume of 50 m^{-3}. This net captures small organisms and has been previously used by Espino et al. (2011a) to collect

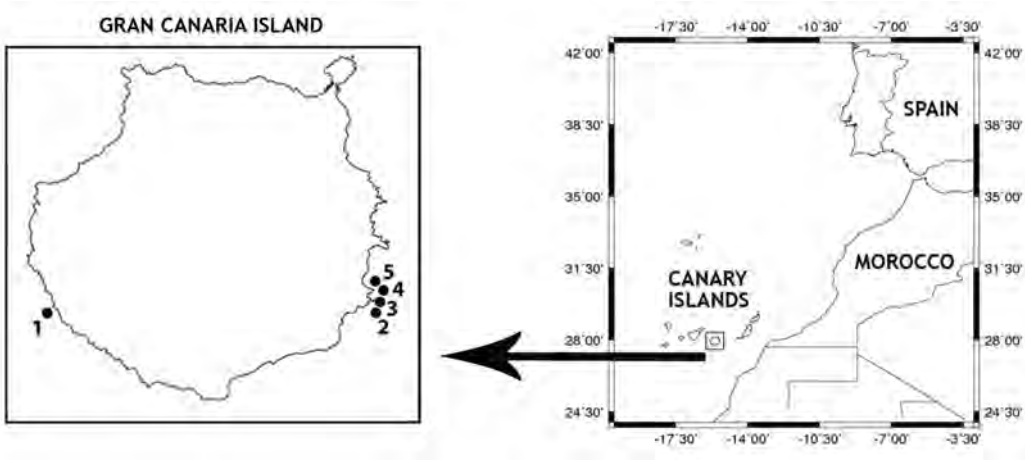


Figura 3.1: Map of Gran Canaria showing the location of *Cymodocea nodosa* meadows surveyed. 1. Veneguera 2. Risco Verde 3. Roque de Arinaga 4. Faro de Arinaga 5. Playa del Cabron.

ichthyofauna in *C. nodosa* meadows. Samples were fixed in 4 % formaldehide and have been identified by group with a binocular microscope. Mysids were classify to species level, according to Tattersall and Tattersall (1951), Wittmann (1986), Barberá-Cebrián et al. (2001) and Wittmann et al. (2010).

3.2.2. Statistical Analysis

Differences in abundance of suprabenthos and mysid species at different periods (May 2011 vs. November 2011) were tested by means of a 2-way, permutational ANOVA (PERMANOVA) (Anderson, 2001), based on Euclidian distances calculated from square root-transformed data. ANOVA model included the factors: "Locality" (random factor) and "Time" (fixed factor). P-values were calculated from 999 unrestricted permutations of the raw data. Pairwise comparisons (using 999 permutations) were used, when appropriate, to resolve differences among levels of significant factors.

Multivariate analysis of assemblage structure using multi-dimensional scaling (MDS) was applied to visualize differences in the structure of the entire suprabenthos and mysid assemblages using PRIMER 6.1 software. The similarity matrix used was calculated by the Bray-Curtis index with a double square transformation of data.

3.3. Results

3.3.1. Suprabenthos assemblage

Mysids, decapods and amphipods are the main constituents of suprabenthos in the seagrases of *C. nodosa* (65 %, 17 % and 16 % of total abundance respectively), which represent up to 95 % of the total suprabenthos abundance (Table 3.1). During late spring at most locations, mysids constituted the largest fraction, followed by amphipods and decapods. In late autumn, decapods assumed this position, followed by mysids and amphipods (Figure 3.3), Table 3.1).

The MDS bidimentional representation showed a pattern of sample aggregation by season (Figure 3.2). In the three most abundant groups of suprabenthic crustaceans (mysid, decapod and amphipods) mean abundances showed seasonal differences consistent among localities, being higher in May than in November (2-way ANOVA: $p<0.05$, Table 3.2; Figure 3.3).

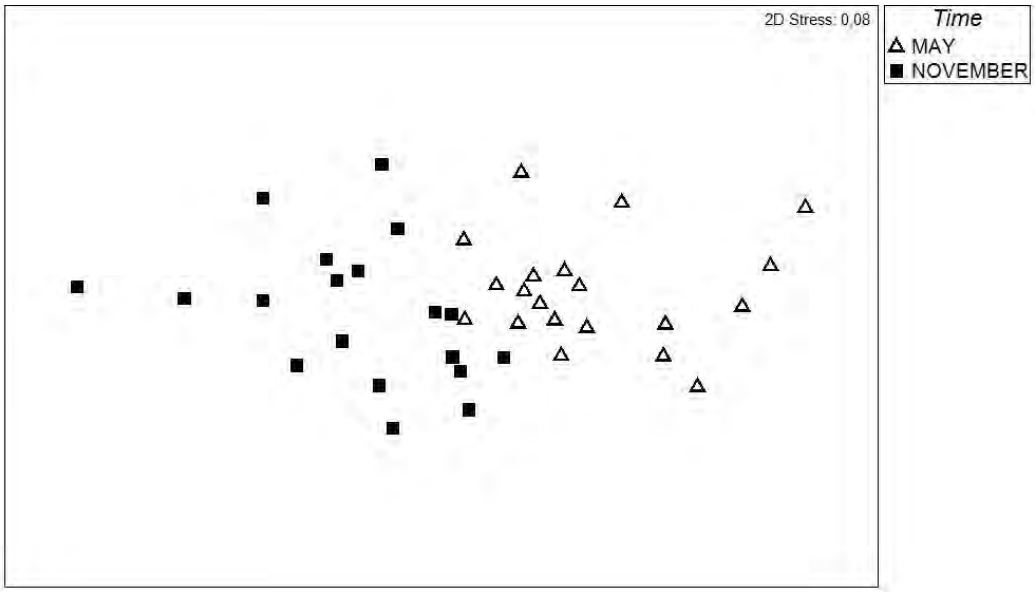


Figura 3.2: Bidimensional representation of MDS (stress=0.08) showing similarities in suprabenthos assemblage structure at different sampling time. Triangles: May 2011; squares: November 2011.

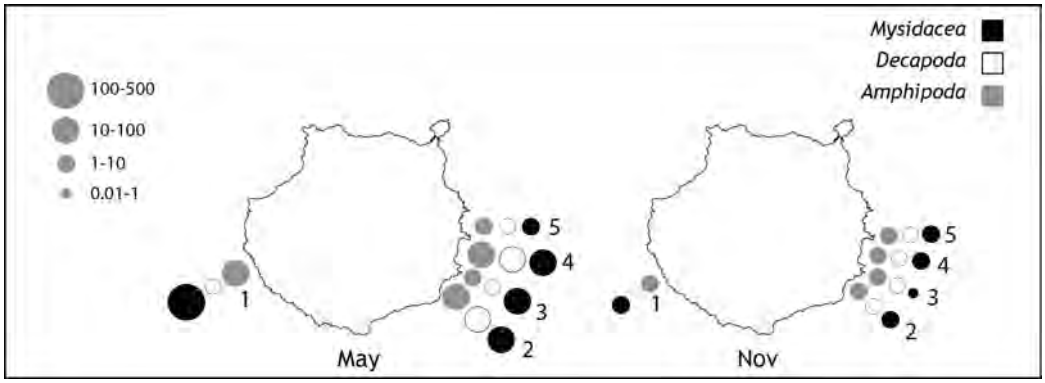


Figura 3.3: Mysid, decapod and amphipod abundance (individuals m^{-3}) in May and November. Sampling areas: 1. Veneguera, 2. Risco Verde, 3. Roque de Arinaga, 4. Faro de Arinaga and 5. Playa del Cabron.

Tabla 3.1: Abundance (individuals m^{-3} ± SE) of taxonomic groups of suprabenthos collected in *Cymodocea nodosa* seagrass during late spring (May) and late autumm (November).

		Mysidacea	Decapoda	Amphipoda	Copepoda	Cumacea	Isopoda	Tanaidacea
VENEGUERA	May	211.88 ± 120.70	3.53 ± 0.99	17.16 ± 7.17	4.38 ± 3.29	0.16 ± 0.16	0.00 ± 0.00	0.00 ± 0.00
	November	3.35 ± 1.35	0.71 ± 0.34	0.97 ± 0.16	0.00 ± 0.00	0.14 ± 0.05	0.00 ± 0.00	0.12 ± 0.05
RISCO VERDE	May	22.99 ± 7.60	25.77 ± 7.77	22.97 ± 6.29	2.99 ± 2.99	0.00 ± 0.00	0.30 ± 0.18	0.00 ± 0.00
	November	2.36 ± 0.88	9.19 ± 2.32	2.75 ± 0.90	0.02 ± 0.02	0.17 ± 0.08	0.01 ± 0.01	0.02 ± 0.00
ROQUE	May	13.44 ± 2.64	9.56 ± 0.73	8.22 ± 2.21	0.34 ± 0.34	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00
	November	0.83 ± 0.43	6.115 ± 2.32	1.34 ± 0.94	0.00 ± 0.00	0.20 ± 0.13	0.01 ± 0.01	0.03 ± 0.02
FARO	May	74.40 ± 52.36	14.56 ± 2.76	20.00 ± 3.48	0.32 ± 0.19	0.42 ± 0.25	0.00 ± 0.00	0.00 ± 0.00
	November	1.25 ± 0.65	1.225 ± 0.42	1.74 ± 0.76	0.03 ± 0.03	0.22 ± 0.14	0.02 ± 0.01	0.01 ± 0.01
CABRON	May	8.24 ± 4.62	8.18 ± 2.11	7.50 ± 1.50	0.76 ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	November	1.65 ± 0.91	10.195 ± 5.73	2.56 ± 1.41	0.00 ± 0.00	0.34 ± 0.23	0.00 ± 0.00	0.04 ± 0.03
TOTAL	May	330.95 ± 187.92	61.60 ± 14.35	75.85 ± 20.65	8.79 ± 6.96	0.58 ± 0.41	0.34 ± 0.22	0.00 ± 0.00
	November	9.42 ± 4.20	27.435 ± 11.13	9.34 ± 4.18	0.05 ± 0.05	1.05 ± 0.63	0.04 ± 0.03	0.22 ± 0.10

Tabla 3.2: Results of 2-way ANOVA testing differences in mysid, amphipod and decapod abundance between times (Ti) and seagrass meadows (Lo).

Mysidacea	Factor	df	MS	F	P
Ti=time	fixed	1	240.18	10.15	0.006
Lo=location	random	4	35.36	2.54	0.049
TixLo		4	23.66	1.70	0.163
Residual		30	13.93		
Total		39			
Amphipoda	Factor	df	MS	F	P
Ti=time	fixed	1	70.30	60.88	0.004
Lo=location	random	4	2.70	2.61	0.061
TixLo		4	1.16	1.12	0.371
Residual		30	1.03		
Total		39			
Decapoda	Factor	df	MS	F	P
Ti=time	fixed	1	22.30	9.48	0.034
Lo=location	random	4	9.14	6.72	0.002
TixLo		4	2.35	1.73	0.172
Residual		30	1.36		
Total		39			

3.3.2. Mysids assemblage

A total of six species (in order of abundance), from the family Mysidae, were identified: *Siriella armata* (Milne-Edwards, 1837), *Gastrosaccus roscoffensis*, *Paramysis arenosa* (G.O. Sars, 1877) *Leptomysis* sp., *Anchialina agilis* (G.O. Sars, 1877), and *Leptomysis lingvura* (G. O. Sars, 1866) (Table 3.3). Only *G. roscoffensis* were present at all sampled seagrass of *C. nodosa* in both sampled periods (Table 3.3; Figure 3.5). *S. armata* and *G. roscoffensis* showed higher abundances (151.2±113.9 and 42.3±27.5 ind m^{-3} respectively) in Veneguera, and *Leptomysis* sp. showed higher abundance (71.3±52.7 ind $^{-3}$) in Faro de Arinaga during late spring (Table 3.3; Figure 3.5).

The MDS bidimensional representation showed a separation of mysid assemblages by seasons (Figure 3.4). Different patterns were observed in temporal variability in mysid abundance. In *L. lingvura* differences between May and November were not significant (2-way ANOVA: "Time" p=0.715, Table 3.4). In *P. arenosa*, *Leptomysis* sp. and *G. roscoffensis* total abundance was higher in May than November. However, significant differences varied among localities, resulting in significative "Ti x Lo" interaction (Figure 3.5, Table 3.4). Abundance of *A. agilis* was greater in May than November (Figure 3.5; 2-way ANOVA: "Time" p=0.042, Table 3.4), independently of the location (Table 3.4, 2-way ANOVA: "Ti x Lo" p>0.05). Finally, *S. armata* were found only during the spring sampling, but no significant differences were detected between seasons, probably they were masked by the high variability between replicates at each locality (Figure 3.5; 2-way ANOVA: "Time" p=0.446, Table 3.4).

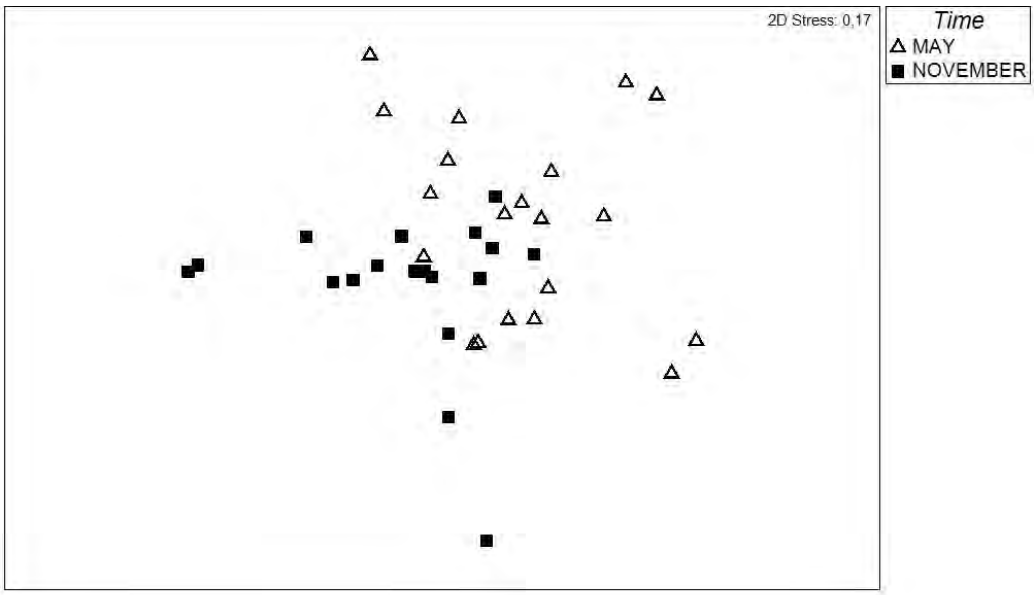


Figura 3.4: Bidimensional representation of MDS (stress=0.17) for mysids species abundance. Triangles: May 2011; squares: November 2011.

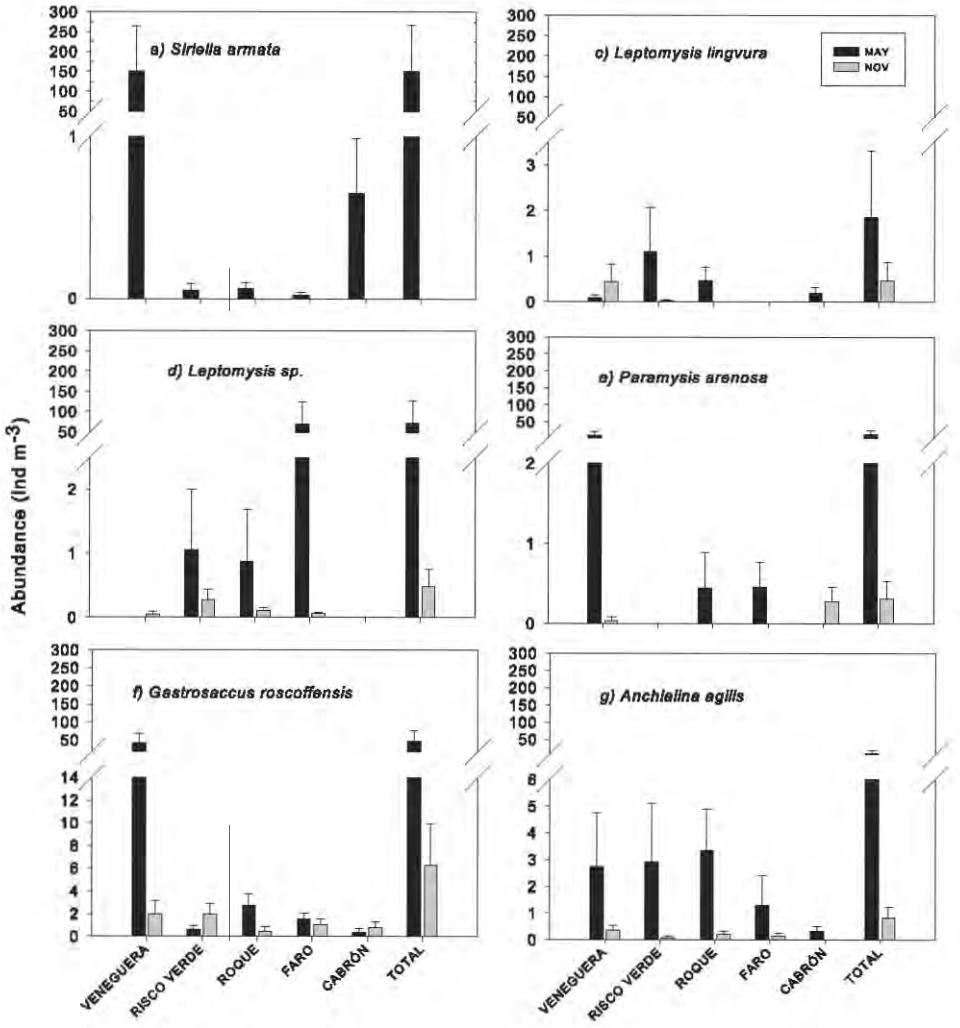


Figura 3.5: Abundance (ind m⁻3) of mysids, in spring (black bars) and autumm (gray bars). Error bars are ± SE of means.

Tabla 3.3: Abundance (individuals m^{-3} ± SE) of mysids associated with *Cymodocea nodosa* seagrass meadows

		S. armata	*L. lingvura*	*Leptomysis* sp.	*P. arenosa*	*G. roscoffensis*	*A. agilis*
VENEGUERA	May	151.20 ± 113.87	0.10 ± 0.06	0.00 ± 0.00	11.78 ± 10.80	42.34 ± 27.48	2.72 ± 2.04
	November	0.00 ± 0.00	0.45 ± 0.37	0.05 ± 0.05	0.04 ± 0.04	2.02 ± 1.18	0.35 ± 0.18
RISCO VERDE	May	0.05 ± 0.04	1.10 ± 0.97	1.05 ± 0.95	0.00 ± 0.00	0.64 ± 0.35	2.92 ± 2.20
	November	0.00 ± 0.00	0.02 ± 0.02	0.28 ± 0.16	0.00 ± 0.00	1.95 ± 0.97	0.08 ± 0.05
ROQUE	May	0.06 ± 0.04	0.46 ± 0.30	0.88 ± 0.80	0.44 ± 0.44	2.70 ± 1.08	3.34 ± 1.55
	November	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.04	0.00 ± 0.00	0.49 ± 0.37	0.23 ± 0.08
FARO	May	0.02 ± 0.02	0.00 ± 0.00	71.34 ± 52.72	0.46 ± 0.30	1.54 ± 0.56	1.31 ± 1.10
	November	0.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.02	0.00 ± 0.00	1.01 ± 0.59	0.16 ± 0.10
CABRON	May	0.65 ± 0.34	0.20 ± 0.12	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.30	0.34 ± 0.15
	November	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.27 ± 0.18	0.81 ± 0.52	0.00 ± 0.00
TOTAL	May	151.98 ± 114.31	1.86 ± 1.45	73.27 ± 54.47	12.68 ± 11.54	47.62 ± 29.76	10.63 ± 7.04
	November	0.00 ± 0.00	0.47 ± 0.39	0.49 ± 0.27	0.31 ± 0.22	6.27 ± 3.63	0.82 ± 0.41

Tabla 3.4: Results of 2-wayANOVA testing differences in mysid species abundances between times (Ti) and seagrass meadows (Lo).

S. armata	Factor	df	MS	F	P
Ti=time	fixed	1	28.86	1.08	0.446
Lo=location	random	4	26.80	2.42	0.091
TixLo		4	26.80	2.42	0.066
Residual		30	11.06		
Total		39			
L. lingvura	Factor	df	MS	F	P
Ti=time	fixed	1	0.10	0.62	0.715
Lo=location	random	4	0.09	0.58	1.000
TixLo		4	0.16	1.08	0.588
Residual		30			
Total		39			
Leptomyis sp.	Factor	df	MS	F	P
Ti=time	fixed	1	17.25	1.42	0.171
Lo=location	random	4	12.12	2.28	0.081
TixLo		4	12.12	2.28	0.073
Residual		30	5.31		
Total		39			
P. arenosa	Factor	df	MS	F	P
Ti=time	fixed	1	2.82	1.68	0.165
Lo=location	random	4	1.69	1.75	0.023
TixLo		4	1.69	1.75	0.022
Residual		30	0.96		
Total		39			
G. roscoffensis	Factor	df	MS	F	P
Ti=time	fixed	1	9.60	1.15	0.338
Lo=location	random	4	10.31	4.34	0.002
TixLo		4	8.37	3.52	0.006
Residual		30	2.38		
Total		39			
A. agilis	Factor	df	MS	F	P
Ti=time	fixed	1	5.89	11.51	0.042
Lo=location	random	4	0.51	0.76	0.577
TixLo		4	0.51	0.76	0.546
Residual		30	0.67		
Total		39			

3.4. Discussion

The suprabenthic community associated with *C. nodosa* seagrass in Gran Canaria were composed almost entirely of crustaceans of the taxonomic groups Mysidacea, Decapoda and Amphipoda. These taxonomic groups were also dominant in others suprabenthic studies (Mees et al., 1995, 1993; San Vicente and Sorbe, 1999, 2001). Seasonal changes in the total abundance of mysids, decapods and amphipods follow the same pattern that density of ichthyofauna and macrofauna associated with *C. nodosa*. It is characterized by maxima in spring and summer, and minima in winter (Tuya et al., 2001; Espino et al., 2011b).

Mysidacea are a major taxa in suprabenthic communities associated with seagrass meadows in temperate coastal waters (Mauchline, 1980; Yamada et al., 2007). Furthermore, it has been known that mysids are the most important food for small fish because they are easier to catch than other species (Yamada et al., 2010). Here, during late spring mysids were the most abundant organism, with abundances up to 200 ind $^{-3}$, this highlights the importance of mysids in *C. nodosa* ecosystems. In the Canary Islands during summer and spring the shoot density and biomass of *C. nodosa* reaches its maxima (Tuya et al., 2006) and provides a nursery habitat for fishes, many of them with commercial interest (Espino et al., 2011a). This period coincides with the maximum abundance of mysids, which could indicate a trophic relationship between juvenile fish and mysids.

The study of Castro (1995) provide evidence of the importance of mysids as fish food in Gran Canaria; they were more abundant in the stomach content of *Scomber japonicus* than euphausiids. It is probable that chub mackerel feed closer to the shore where swarms of mysids are more accessible. Some of the mysids identified in the stomach content of chub mackerel (*Paramysis sp.*, *Siriella sp.*, *Anchialina agilis* and *Leptomysis sp.*) were found in *Cymodocea* meadows in the present study.

In recent years there has been an increase in the interest in suprabenthic studies because of their possible importance in trophic chains (Wang and Dauvin, 1994; Ansell et al., 1997; Cartes et al., 2008, 2011; Madurell et al., 2008). Most mysids feed on organic detritus and can be responsible

for the remineralization of an important part of refractile detritus (Ansell et al., 1997). Other studies shown the importance of suprabenthic mysids in nitrogen regeneration in the surf zone (Cockcroft et al., 1988). Many mysid species are vertical migrators, living near the surface of the sediment during the day and migrating to the water surface at night (Mauchline, 1980). Here the samples were collected at 10 cm above the bottom only during the day to avoid underestimating mysid abundance.

In this study, a total of six mysids species were sampled, a low value, similar to the number captured in seagrasses meadows in the Mediterranean Sea by Barberá-Cebrián et al. (2002) and Sánchez-Jerez and Esplá (1996). *S. armata*, *A. agilis*, *Leptomysis* sp., *L. lingvura* and *P. arenosa* have been previously recorded in *Cymodocea* meadows by Barberá-Cebrián et al. (2002) and Wittmann and Wirtz (1998). The first record of *G. roscoffensis* in the Canary Archipelago was only recently published by Wittmann et al. (2010).

Several mysid species swarm in close spatially arranged groups (Clutter, 1969; Mauchline and Murano, 1977; Wittmann et al., 1977). Wittmann et al. (1977) describes several types of relationships that mysids have with their substrates. These range from substrate specialist to swarm specialist. Within the substrate specialists are *A. agilis* and *S. armata* which are inactive, do not react to predators and cling to the leaves of *Zoostera*. The second group, including *L. lingvura*, swarm and swim near *Cystoceira*, rocks and recesses rocks. *L. mediterranea* forms a third group that swarms in groups above sandy bottoms.

The present study based on in situ observations has shown that many mysids, such as *S. armata*, *L. lingvura*, *Leptomysis* sp. and *P. arenosa* aggregate in swarms. This behavior helps to explain the high variability between replicates at each locality. It is likely that the level of replication was insufficient to determine seasonal changes in abundance in these species. *A. agilis* was not observed forming swarms as reported by Wittmann et al. (1977) in the northern Adriatic Sea. This was the only species that showed significant differences in abundance between seasons. It peaked in abundance in late spring in all localities.

The present work is the first step in understanding the distribution of

coastal mysids off Gran Canaria and their importance as food for commercially important fish. More studies are needed to reveal the trophic relationships, but *C. nodosa* and associated mysids are likely to play an important role in maintaining coastal productivity. The *C. nodosa* meadows have declined severely in Gran Canaria over the last two decades (Tuya et al., 2012) and this loss of habitat must have reduced the mysid abundance in the coastal waters of Gran Canaria. Even though the processes that caused this decline have not been identified, it would be prudent to protect these habitats.

Acknowledgements

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Capítulo 4

Rearing techniques and nutritional quality of two mysids from Gran Canaria (Spain)

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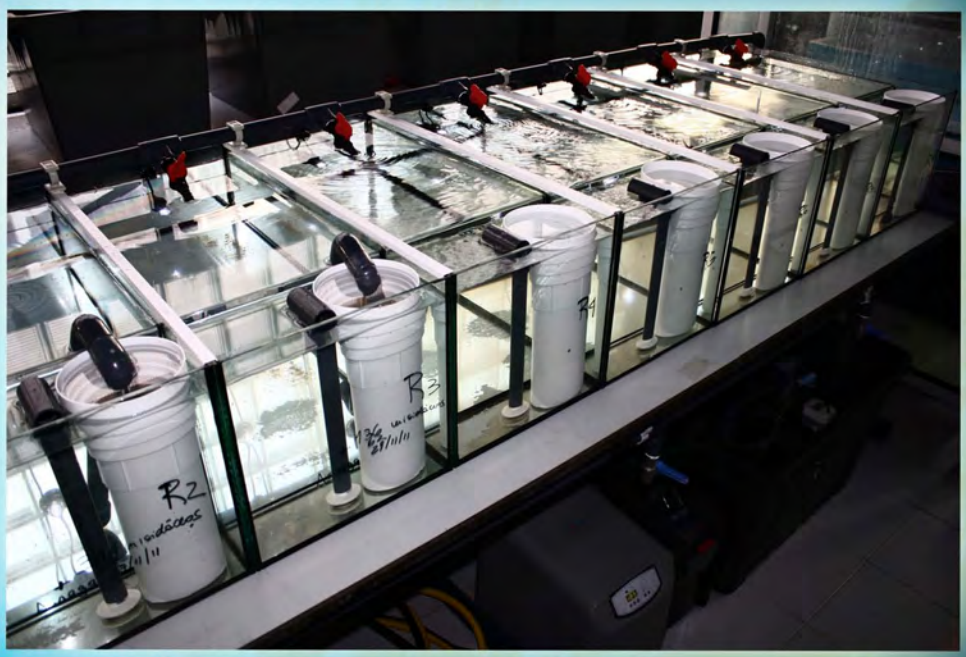

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Rearing techniques and nutritional quality of two mysids from Gran Canaria (Spain)

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Abstract

This paper presents the preliminary results of different trials carried out with two species of mysids from Gran Canaria: Leptomysis lingvura (G.O. Sars, 1866) and *Paramysis nouvel*. Experiments lasting 21 days showed significantly higher fecundity and survival in *L. lingvura* than in *P. nouveli* ($P < 0.05$). We also report the biochemical profile of both species fed 48-h-*Artemia* nauplii enriched with Easy-DHA-Selco® for 7 days. A comparison of our results with those of for *Artemia* and rotifers, organisms frequently used as live food in aquaculture, showed that mysids have a high percentage of protein per dry mass (73.38% in *P. nouveli*, and 74.19% in *L. lingvura*). Furthermore, the percentage of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) in total fatty acids was higher in both species than that reported by Roo and colleagues for rotifers and *Artemia*. In addition to the content of these fatty acids, their ratios between them are also important for normal growth and larval development. We found that the ratio, DHA:EPA, was 0.85 0.02 and 0.89 0.01; the ratio, DHA: AA, 6.25 0.26 and 4.74 0.14; and the ratio, EPA:AA, 7.32 0.26 and 5.32 0.2, respectively, for *P. nouveli* and *L. lingvura* in cultures and these ratios do not significantly differ ($P > 0.05$) from organisms in the wild. Here, we argue that as mysids are prey for many commercially important fish, cephalopods and rays, it is likely that the biochemical composition of mysids in their natural environment is "optimal" for these predators. Therefore, we studied the lipid profile of both species as they naturally occur in their environment. The results indicate that these mysids could be used to develop high quality live fish food.

Keywords: mysids, *Leptomysis lingvura*, *Paramysis nouveli*, live prey, nutritional quality, production

Introduction

The order Mysidacea comprises 780 species in about 120 genera, all included in the superorder Peracarida (Mauchline 1980; Bowman & Abele 1982). Mysids are omnivorous. The stomachs of mysids collected near the coast contain detritus, bodies and appendages of small crustaceans, and small amounts of diatom shells (Murano 1999).

Studies on the relationships between fish and mysids indicate that mysids are a keystone food for fish, especially in coastal environments, where they are abundant (Mauchline 1980; Murano 1999).

The stomach contents of chub mackerel (*Scomber japonicus*) indicate that mysids have a trophic importance even greater than euphausiids in the waters around the island of Gran Canaria (Castro 1995). This mackerel represents 52% of mid-sized pelagic fish in the region. It daily consumes 8% of its body mass in crustaceans and 2.5% in fish (anchovy). Accordingly, Castro (1995) estimated that annually, this mackerel consumes about 242 000 tonnes of mysids and 29 000 tonnes of euphausiids. These data give us an idea of the trophic importance of mysids as food in the region.

In aquaculture, mysids have proven to be a high-quality food for the juvenile stages of cuttlefish *Sepia officinalis* (Domingues, Sykes & Andrade 2001) and adult seahorse *Hippocampus abdominalis* (Woods & Valentino 2003) and *Hippocampus hippocampus*

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wild. Here, we argue that as mysids are prey for many commercially important fish, cephalopods and rays, it is likely that the biochemical composition of mysids in their natural environment is "optimal" for these predators. Therefore, we studied the lipid profile of both species as they naturally occur in their environment. The results indicate that these mysids could be used to develop high quality live fish food.

4.1. Introduction

The order Mysidacea comprises 780 species in about 120 genera, all included in the superorder Peracarida (Mauchline, 1980). Mysids are omnivorous. The stomachs of mysids collected near the coast contain detritus, bodies and appendages of small crustaceans, and small amounts of diatom shells (Murano, 1999).

Studies on the relationships between fish and mysids indicate that mysids are a keystone food for fish, especially in coastal environments where they are abundant (Murano, 1999; Mauchline, 1980). The stomach contents of chub mackerel (*Scomber japonicus*) indicate that mysids have a trophic importance even greater than euphausiids in the waters around the island of Gran Canaria (Castro, 1995). This mackerel represents 52 % of mid-sized pelagic fish in the region. It daily consumes 8 % of its body mass in crustaceans and 2.5 % in fish (anchovy). Accordingly, Castro (1995) estimated that annually this mackerel consumes about 242,000 tonnes of mysids and 29,000 tonnes of euphausiids. These data give us an idea of the trophic importance of mysids as food in the region. In aquaculture, mysids have proven to be a high quality food for the juvenile stages of cuttlefish, *Sepia officinalis* (Domingues et al., 2001b) and adult seahorse, *Hippocampus abdominalis* (Woods and Valentino, 2003) and *H. hippocampus* (Otero-Ferrer et al., 2010).

In culturing fish larvae, only *Artemia* and rotifers are used traditionally as food and this poverty of choice can lead to nutritional imbalances (Izquierdo, 1996), and other foods are needed to improve this situation.Three fatty acids are essential for normal development of marine fish: DHA, EPA, AA.

They fill a fundamental role in developing both the structure and function of integral cell-membranes. Furthermore, they and the EPA:AA ratio, serve as precursors or are otherwise important for the development of a group of highly active hormones known as eicosanoids (Izquierdo, 1996; Sargent et al., 1999; Roo et al., 2009). However, not only is the content of these fatty acids important, but their inter-relationships: DHA: EPA: AA are also important. Knowing the optimal ratios is difficult in practice because it is likely to differ in each species (Sargent et al., 1999). Consequently, we suggest analyzing the prey of each species in its natural environment, as predator and prey are well adapted to the same environment conditions.

This paper is a pilot study of the survival and production of *L. lingvura* and *P. nouveli* in captivity. Here, we analyze the nutritional quality (lipid and protein profiles) of both species to determine their suitability as live prey in aquaculture. We present the protein and fatty acid profiles of both species in their natural environment in order to determine if the diet used during cultivation changes their natural biochemical composition. Other investigators have cultivated mysids, mainly from the genus *Mysidopsis*, and used them for laboratory experimentation and for water toxicity testing (Reitsema, 1980; Ward, 1984; Lussier et al., 1988; Domingues et al., 1999a; Verslycke et al., 2004). We intend to use our results to facilitate the development of fish food for cultivating ornamental fish as well as commercially important fish.

4.2. Material and Methods

4.2.1. Survival and production experiments

On the east coast of Gran Canaria, in Risco Verde bay (27°51N and 15°23 W), samples were taken weekly from August to October 2008. Sampling took place at depths between 5 and 15 meters in areas near the rocks using SCUBA equipment and a hand net of 500-μm mesh. Species identification was performed with a binocular microscope (Wild M8, Heerbrugg, Switzerland), following the work of Tattersall and Tattersall (1951), Labat (1953), Wittmann (1986), Barberá-Cebrián et al. (2001).

To study the survival and production, samples of *L. lingvura* and *P. nou-*

veli, two of the most abundant species in our samples, were taken in October 2008. After acclimatization for 2 days, 10 males and 10 females of each species were then placed in small 1L farrowing containers that in turn, were placed in larger 14 L open flow tanks of filtered seawater with a salinity of 37 g L^{-1} (PSU). The seawater, common to the farrowing containers and the 14 L tanks, was maintained at 18.2 ± 0.4°C, renewed every 12 hours, and monitored for pH, oxygen, ammonium, nitrate and nitrite. The pH was maintained at 8.2 ± 0.1, the O_2 at 7.1 ± 0.1 mg L^{-1}, and NH_4^+, NO_3^-, and NO_2^-, at concentrations below 0.2, 1 and 0.02 mg L^{-1} respectively. The photoperiod was 14h:10h light and dark. Mysids were fed twice daily using 100 *Artemia* nauplii per mysid. The *Artemia* (EG type) were enriched with Easy-DHA Selco®; INVE aquaculture, Dendermonde, Belgium).

Mysids were counted daily. Survival of adults was expressed as a percentage of the original number. Relative production was estimated by dividing the number of hatchlings per day by the number of females alive. Production rates were expressed as young per female. The experiments were carried out in three replicates. To measure the standard length (from the rostrum in between the eye stalks to the end of the last abdominal segment) of young we used a binocular microscope with a reflex digital camera of 10 megapixels (Canon EOS 1000D, Tokyo, Japan) and the software Image J 1.40g (National Institutes of Health, USA) to estimate the length from the megapixels in the photograph.

4.2.2. Nutritional quality experiments

Samples for lipid and protein analysis were also collected in Risco Verde between March and April 2009. Samples of *P. nouveli* and *L. lingvura* were separated immediately after capture using a binocular microscope and kept frozen at -80°C for further analysis. For culture experiments the mysids were separated by species and after an acclimatization period of 2 days, were maintained for 7 days, fed twice daily using 100 *Artemia* nauplii per mysid (as above). The culture conditions were identical to those used in the survival and production experiments. At day 7 the organisms were placed on filters, washed with distilled water, and stored at -80°C until analysis was made.

Moisture was determined in the samples by drying them to constant weight in an oven at 110°C (AOAC, 1995). The ash content was determined by incinerating the samples to constant weight in a muffle furnace at 600°C (AOAC, 1995).

Protein was calculated from total nitrogen in the samples as determined by the Kjeldhal technique (AOAC, 1995). Crude lipids (% wet mass) were extracted following the method of Folch et al. (1957). Fatty acid methyl esters from total lipids were prepared by transmethylation as described by Christie (1982), separated and quantified by Gas-Liquid chromatography as described by Izquierdo et al. (1990). Proteins, lipids, ash and moisture were expressed as % dry mass. Fatty acids are expressed as % of total.

4.2.3. Statistical analysis

Mann-Whitney non-parametric test with significance $P<0.05$ was used to determine statistical differences in the survival and production of each species and Kruskal Wallis one-way ANOVA with significance $P<0.05$ was performed for the three replicates. All the biochemical data were expressed as means ± SD. To evaluate the homogeneity of variances between wild and cultured mysids we applied Levene's test, and to study differences between them we applied the Student t-test with significance level, $P<0.05$. These statistical analyses were done using SPSS Statistical Software version 14.0 (SPSS Chicago, Illinois, 1999).

4.3. Results

4.3.1. Survival and production experiment

At the end of the experiment the average survival for *L. lingvura* was 65 ± 8.7 % (mean ± S.D.) and for *P. nouveli* 16 ± 5.8 % (Figure 4.1). The cultures of the two mysids showed no significant differences in survival until day 9, since then values were higher ($P<0.05$) in *L. lingvura* (Figure 4.1). The total hatchling production was 166 ± 2 and 45 ± 7 for *L. lingvura* and *P. nouveli* (Figure 4.2) and the hatchling average standard length was 2.03 ± 0.23 mm

and 1.86 ± 0.17 mm, respectively, showing significant differences between species ($P<0.05$). The relative production (young female^{-1}) was significantly higher ($P<0.05$) in *L. lingvura* (18.2±2) that *P. nouveli* (4.6±0.8), at day 21. No hatchlings of *P. nouveli* were found from day 12 of experiment.

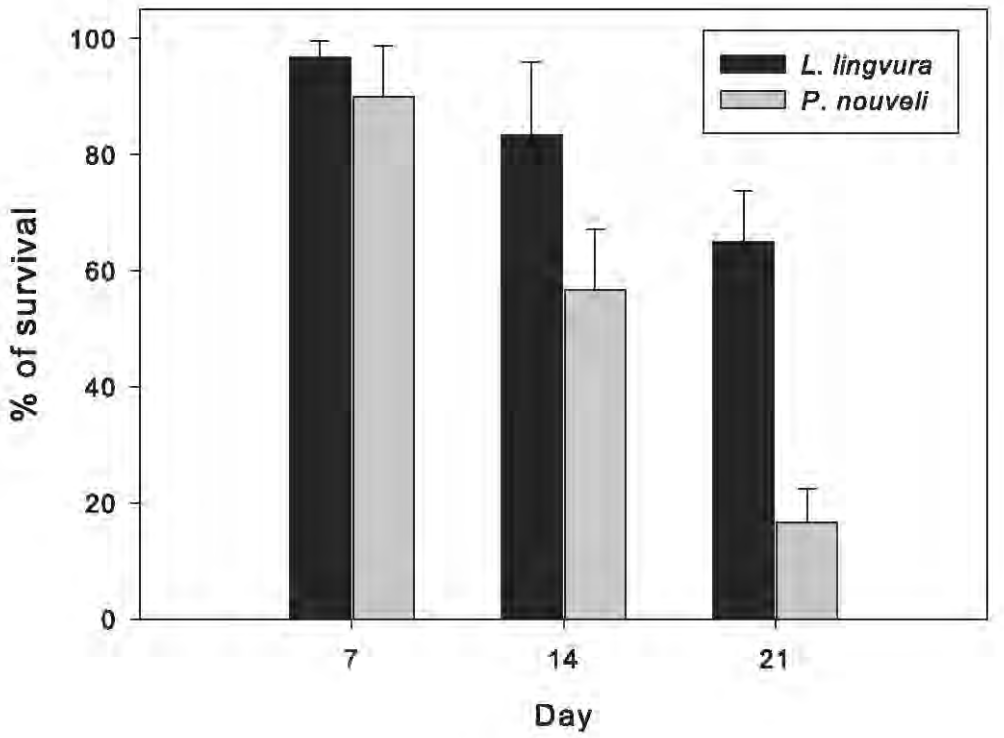

Figura 4.1: Survival in percentage of *L. lingvura* and *P. nouveli* at day 7, 14 and 21 of the experiment.

4.3.2. Nutritional quality experiments

Lipid and protein analysis was the first step in determining the nutritional quality of the cultured mysids. The proteins and lipids as a percentage of dry mass, for *P. nouveli* were 73.38 ± 1.77 % and 15.01 ± 1.12 % and for *L. lingvura*, 74.19 ± 5.22 % and 14.79 ± 2.66 % (Table 4.1). The most abundant fatty acids in both species were oleic acid 18:1 n-9, palmitic acid 16:0, eicosapentaenoic acid (EPA) 20:5 n-3, docosahexaenoic acid (DHA) 22:6 n-3, α linoleic acid (ALA) 18:3 n-3 and linolenic acid (LA) 18:2 n-6 (Table 4.1; Figures 4.3 and 4.4).

The omega-3 (n-3) and the omega-6 (n-6) polyunsaturated fatty acids (PUFA), in *P. nouveli* and *L. lingvura* accounted for 39.45 ± 0.73 % and 8.43 ± 0.22 %, and 42.4 ± 0.36 % and 8.34 ± 0.06 % of the total lipids, respectively (Table 4.1). The ratio DHA:EPA was 0.85 ± 0.02 and 0.89 ± 0.01, DHA: arachidonic acid (AA) 6.25 ± 0.26 and 4.74 ± 0.14 and EPA:AA 7.32 ± 0.26 and 5.32 ± 0.2 %, (Table 4.1).

In mysids collected in the wild; lipids, protein and ash as a percentage of dry mass were for *P. nouveli*: 17.83 ± 0.12 %; 74.24 ± 1.28 % and 2.69 ± 0.2 % respectively; and for *L. lingvura*: 16.25 ± 4.96 %; 77.34 ± 1.24 % and 3.72 ± 0.31 %, respectively (Table 4.1). Fatty acids as a percent of total are presented in table 4.1 and represented with the percentages obtained for mysids fed *Artemia* in culture in figure 4.3 for *L. lingvura* and figure 4.4 for *P. nouveli*.

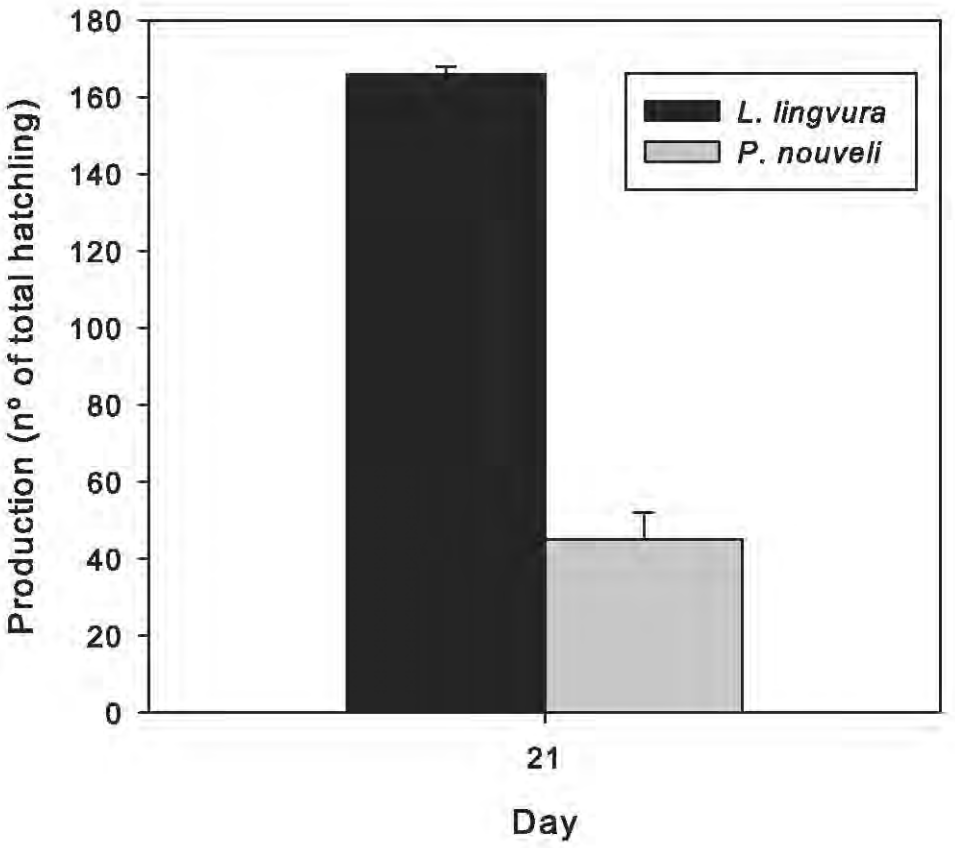

Figura 4.2: Total hatchling production of *L. lingvura* and *P. nouveli* at day 21 of the experiment.

Tabla 4.1: Lipids, proteins and ash composition (% dry mass) and fatty acids (% total fatty acids) of wild and cultured *P. nouveli* and *L. lingvura*; and two live prey used frequently in aquaculture (rotifers and *Artemia*) enriched with Selco® reported by Roo et al. (2009). Values (mean±SD). ∗Significant differences between wild and cultivated.

		Wild *P. nouveli*	Cultured *P. nouveli*	Wild *L. lingvura*	Cultured *L. lingvura*	Enriched Rotifers	Enriched *Artemia*
% Lipids (dw)		17.83±0.12	15.01±1.12	16.25±4.96	14.79±2.66	22.05±3.84	26.04±0.41
% Proteins (dw)		74.24±1.28	73.38±1.77	77.34±1.24	74.19±5.22	54.28±4.57	56.39±4.84
% Ash (dw)		2.69±0.2	2.99±0.07	3.72±0.31	3.63±0.21	1.48±0.50	0.75±0.02
16:00	Palmitic acid	22.88±0.34∗	16.94±0.62∗	21.71±0.07∗	15.48±0.23∗	13.0±2.48	15.22±3.8
18:00	Stearic acid	4.28±0.14	4.01±0.1	4.48±0.25	3.64±0.05	4.73±1.21	4.42±0.37
18:1n9	Oleic acid	12.85±0.46∗	19.11±0.38∗	12.53±0.93	17.9±0.24	20.1±1.72	20.36±7.38
18:2n6	Linolenic acid	3.15±1.07	4.79±0.24	2.86±0.63	4.76±0.02	8.14±1.31	3.78±2.61
18:3n3	α linoleic acid	0.78±0.12∗	8.22±0.19∗	0.67±0.08∗	14.18±0.26∗	1.62±0.11	10.81±4.23
20:5n3 EPA	Eicosapentaenoic acid	19.39±0.68	14.77±0.2 1	7.89±1.85	12.45±0.15	6.51±0.62	11.10±4.27
22:6n3 DHA	Docosahexaenoic acid	16.98±1.22	12.63±0.37	16.62±0.86	11.10±0.20	9.68±0.93	4.47±1.43
20:4n6 AA	Arachidonic acid	2.92±0.01∗	2.02±0.06∗	3.57±0.96	2.34±0.09	1.46±0.73	1.49±0.37
·PUFA n-3		40.31±0.38	39.45±0.73	38.04±2.95	42.4±0.36	21.12±0.48	31.14±11.43
·PUFA n-6		7.99±1.18	8.43±0.22	8.53±0.31	8.34±0.06	10.77±2.11	7.03±3.73
DHA/EPA		0.88±0.09	0.85±0.02	0.93±0.05	0.89±0.01	1.49±0.01	0.4±0.34
DHA/AA		5.81±0.44	6.25±0.26	4.8±1.06	4.74±0.14	8.1±4.45	2.99±3.87
EPA/AA		6.64±0.21	7.32±0.26	5.13±0.86	5.32±0.20	5.45±2.99	7.43±11.53

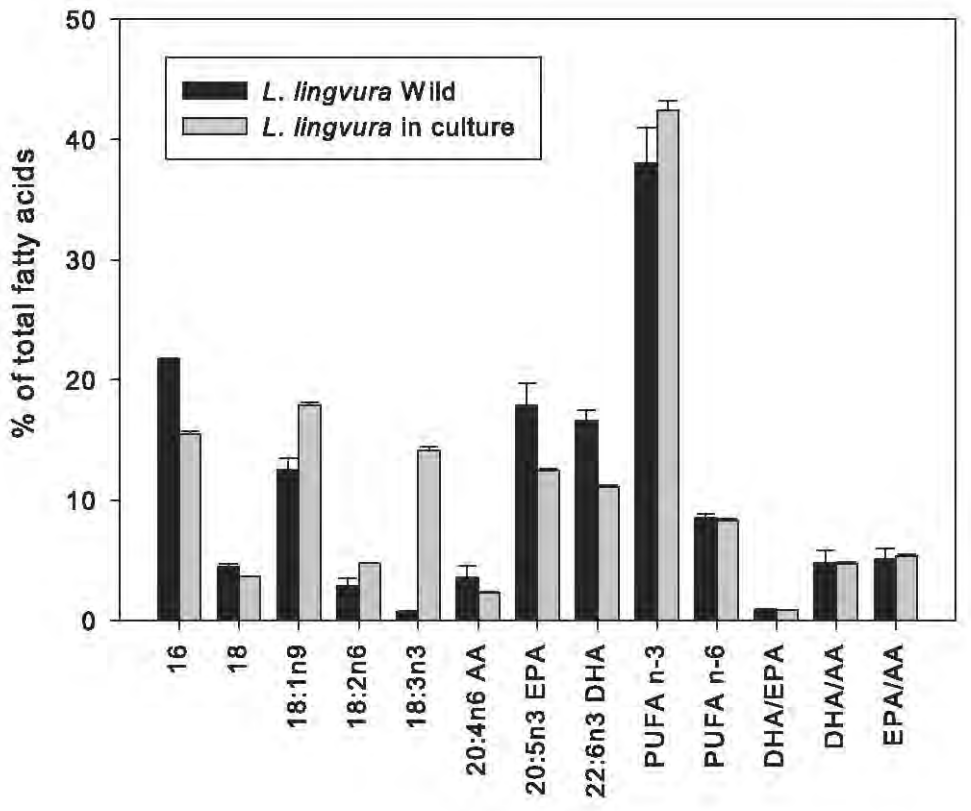

Figura 4.3: The most abundant fatty acids as a percentage of total for wild *L. lingvura* and for the same species cultured fed for 7 days on *Artemia* nauplii enriched for 48 h with EasyÐDHA Selco® , INVE, Belgium. The fatty acids are identifiable by the following key, 16:0 (Palmitic acid), 18:0 (Stearic acid), 18:1 n-9 (Oleic acid), 18:2 n-6 (Linolenic acid), 18:3 n-3 (αlinoleic acid), 20:5 n-3 (EPA), 22:6 n-3 (DHA), and 20:4n-6 (AA).

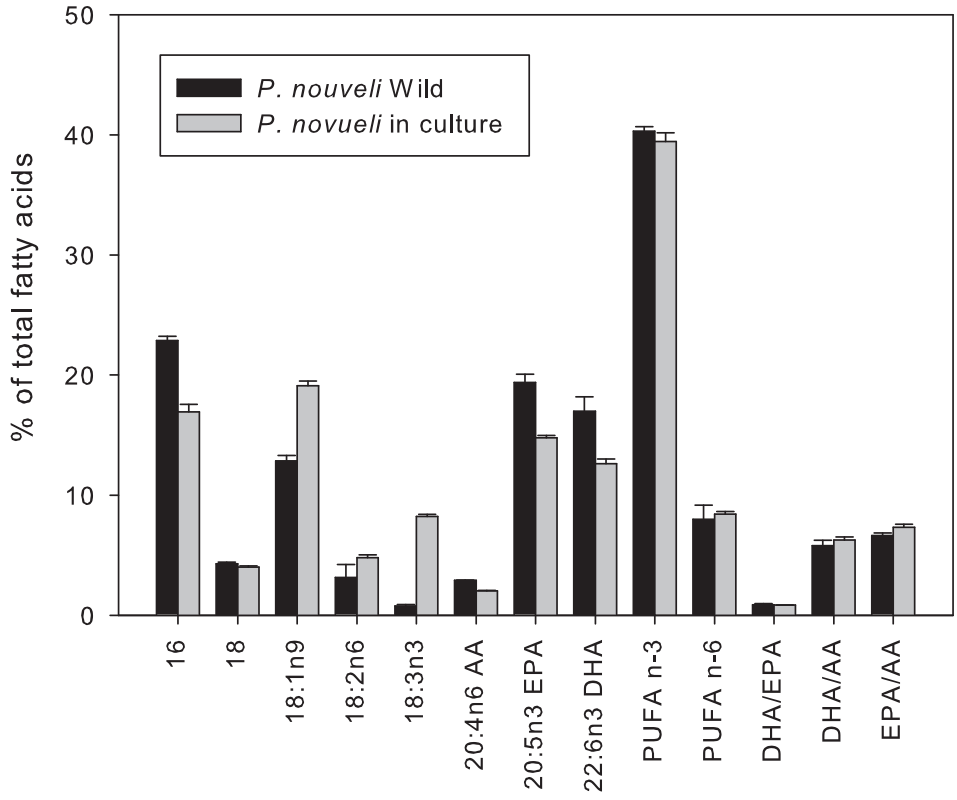

Figura 4.4: The most abundant fatty acids as a percentage of total for wild *P. nouveli* and for cultured *P. nouveli* fed for 7 days on *Artemia* nauplii enriched for 48 h with EasyÐDHA Selco®, INVE, Belgium. The fatty acids are identifiable by the following key, 16:0 (Palmitic acid), 18:0 (Stearic acid), 18:1 n-9 (Oleic acid), 18:2 n-6 (Linolenic acid), 18:3 n-3 (αlinoleic acid), 20:5 n-3 (EPA), 22:6 n-3 (DHA), and 20:4n-6 (AA).

4.4. Discussion

From the results obtained in the preliminary experiments with survival and production, we determined that *L. lingvura* is the more suitable of the two species for culture in our facilities. These results could vary if we changed the culture conditions and feeding treatment because the mysids are omnivorous and in the natural environment feed on copepods, rotifers, diatoms and organic detritus (Mauchline, 1980; Murano, 1999; Domingues et al., 1999a, 2000), and in cultures may not be receiving adequate food. As previously reported by Domingues et al. (2000) the complete replacement of *Artemia* nauplii by rotifers caused decreased production and survival of juvenile and adult *Leptomysis* sp., however, the partial replacement of *Artemia* by rotifers (1/3 *Artemia* + 2/3 rotifers) showed no significant differences in production and survival of offspring and adults as compared to being fed 100 % *Artemia* nauplii. In general, our results with *L. lingvura* especially around day 20, were similar to those of Domingues et al. (2000).

To optimize the culture conditions further experiments with different types of prey, for example, different algae, rotifers as well as *Artemia* must be carried out. In addition, one should experiment with environmental conditions by modifying temperature, density and salinity, as they directly affect survival and growth production (Mauchline, 1980; Murano, 1999; Domingues et al., 1999a).

The study of lipid and protein composition revealed that both species have a high potential as live food in aquaculture. The levels of proteins and lipids and fatty acids in *P. nouveli* and *L. lingvura* meet nutritional requirements for fish according to FAO (Tacon, 1989). Both mysids species in culture showed higher levels of PUFA (polyunsaturated fatty acids) n-3; *P. nouveli* (39.45 %) and *L. lingvura* (42.4 %) in comparison with *Artemia* (31.14 %) and rotifers (21.12 %) according to Roo et al. (2009) (Table 4.1).

PUFA, DHA, EPA and AA are required, by themselves and in specific dietary ratios, for normal growth and development of fish. Both mysids have a composition of DHA, EPA and AA, higher than that reported by Roo et al. (2009) for rotifers and *Artemia* enriched with, DHA Protein Selco® (INVE, Belgium) and Selco® (INVE, Belgium) respectively (Table 4.1). Otero-Ferrer

et al. (2010) reported results of DHA (6.6 %), EPA (5.5 %) and AA (1.3 %) close to Roo et al. (2009) (4.47 %; 11.5 % and 1.46 respectively) for the same type of *Artemia* sp. enrichment under similar conditions; the results for rotifers (2.2 %; 1.8 % and 0.6 % respectively) are lower than those obtained by Roo et al. (2009) (9.68 %; 6.5 % and 1.49 % respectively). The results of DHA, EPA and AA obtained for *L. lingvura* (11.10 %; 12.45 %; 2.34 % respectively) and *P. nouveli* (12.63 %; 14.77 %; and 2.02 % respectively) are higher than those obtained by both authors for rotifers and *Artemia* (Table 4.1). We suspect that these differences in fatty acid composition could make mysid food more likely, than rotifer or *Artemia* food, to satisfy the nutritional requirements of aquaculture, especially the aquaculture of those species that in the wild prey naturally of mysids.

Domingues et al. (2001b) made experiments with survival and growth in cuttlefish (*Sepia officinalis*), fed at an early stage of growth with two different treatments: *Artemia* and mysids (*P. nouveli*). In both experiments, the hatchlings, fed mysids, reached larger sizes and survival were higher. These results support our hypothesis that mysids are a higher quality food for the cultivation of the commercially important species that prey on mysids in nature. However, the preliminary results do not show a high production, which argues against using the mysids for cultivation on a commercial level. It is clear that mysid cultivation is more expensive and less productive than that of *Artemia* and rotifers. Nevertheless, they may serve as food for ornamental fish or as supplementary food for cultures suffering high mortality at certain stages of development. This is the case in cultured paralarvae of *Octopus vulgaris* where high mortality and low growth have been observed (Iglesias et al., 2007). In this situation, the mysids could complement other cheaper food since the mysid hatchlings have a size appropriate for the *O. vulgaris* paralarvae (1.8-2 mm). Furthermore, the data presented for *P. nouveli* and *L. lingvura* can be useful in determining the composition of "optimal" food for natural predators such as mackerel, *Sepia officinalis*, *Octopus vulgaris*, *Hippocampus* sp.

The study of lipids in wild mysids and in their natural food show differences between the wild and cultured mysids. In the wild, palmitic acid (16:0) in both *P. nouveli* and *L. lingvura* was present at higher percentages (P±0.05) of total lipids than it was in cultures; however, in both mysids αlinoleic acid

(18:3 n-3) was significantly higher (P±0.05) in culture than in the wild (Figures 4.3 and 4.4; Table 4.1). *P. nouveli* also showed significant differences in the percentages of oleic acid (18:1n9) and arachidonic acid (20:4n6) (P±0.05). These differences are likely due to the wide variety of foods the mysids consume in the wild. However, the ratios DHA: EPA, DHA: AA and EPA: AA do not show significant differences ($p>0.05$) between wild and cultured organisms. Research in mysid cultures growing on different prey suggest ways in which the diet could be modified to attain optimum lipid ratios in the mysids, themselves.

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Capítulo 5

Effect of starvation and feeding on respiratory metabolism in Leptomysis lingvura (G.O. Sars, 1866)

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Effect of starvation and feeding on respiratory metabolism in *Leptomysis lingvura* (G.O. Sars, 1866)

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ABSTRACT

The mysid, *Leptomysis lingvura*, is found along east coast of Gran Canaria (Spain) swimming in the plankton above sandy bottoms at depths between 5 and 15 m. As with many mysids around the world, it is an important component in the food chain for many coastal fish and could be a potential live prey for use in aquaculture (Herrera et al., 2011; Jumars, 2007). We studied *L. lingvura*'s survival and reproduction in captivity and determined its suitability for physiological and biochemical research in the laboratory. This mysid proved to adapt well to aquarium life and to be highly suitable for studying respiratory metabolism. This investigation documents the effect of feeding and starvation on the enzymology and physiology of respiration. The research strategy was to follow a simple time course of both the oxygen consumption rate of whole mysids and the activity of their respiratory electron transport system (ETS). Respiration (R) decreased logarithmically during starvation whereas the ETS activity remained constant. As a consequence, the ratio of R to ETS activity decreased along with the respiration. Superimposed on the declining respiration rate was an unforeseen diel rhythm that elevated R during the light and depressed it during the dark. The slope in the R-biomass log–log Kleiber plot in well fed mysids is close to 0.75 while for starved mysids it was lower than 0.75.

1. Introduction

Mysids are peracaridan crustaceans that inhabit many varied aquatic habitats. They are abundant in coastal regions, some benthic, others planktonic, but they also occur in the pelagic waters throughout the oceanic water column far from land. (Jumars, 2007; Mauchline, 1980). They are omnivorous filter feeders eating small planktonic organisms such as copepods, tinntinids, and diatoms as well as organic detritus (Mauchline, 1980; Murano, 1999; Tattersall and Tattersall, 1951). In cultures, cannibalism occurs if not enough food is provided (Domingues et al., 1999; Lussier et al., 1988). Some species exhibit daily feeding rhythms. Dauby (1995) has studied the behavior of Mediterranean *Leptomysis* species and finds that during the day they form swarms and rest just above the bottom while during the night they swim to feed on sediments and particulate organic matter (phytoplankton, seagrasses, macro and micro algae). Females carry the embryos in a marsupium where larval development occurs. Juvenile mysids emerge morphologically similar to adults.

Leptomysis lingvura inhabits the east coast of Gran Canaria (2751 N; 1523 W) and grows well in the laboratory. Not only does it survive in culture, but it can complete its life cycle in captivity. These characteristics enabled us to document its growth and respiration under controlled conditions (Herrera, 2009; Herrera et al., 2011).

Respiration is a good index of physiological activity and energy production in zooplankton (Gómez et al., 1996). The direct measurement of zooplankton respiration is difficult in practice, because it is difficult to simulate natural conditions in laboratory cultures. Differences between laboratory conditions and those of the natural environment include predation stress, food limitation, schooling, omnidirectional migration tendencies, and variability in temperature, light, and ocean currents. As a result a laboratory measurement of respiration is not equivalent to *in situ* respiration and proxies, models, or some combination of the two are needed to calculate in situ oceanic respiration accurately. Hence we investigate the biochemical basis of respiration. ETS (electron transport system) activity is the biochemical foundation of respiration and energy production (Lane, 2005). We use the term, electron transport system as a synonym for the electron transport chain. It is measured in plankton to estimate the "potential" respiration (ϕ) (Packard and Gómez, 2008). This technique uses the cytoplasmic reduction of an artificial electron acceptor: tetrazolium-salt (INT), to stoichiometrically measure the capacity of the cytoplasm to consume O_2. This can be done because the reduction of 2 mol of INT by the ETS is equivalent of the ETS-driven reduction of 2 atoms of oxygen (or 1 molecule of O_2). The relationship between the ETS activity and the respiration rate is complicated. Respiration is likely to be depressed during starvation and

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Capítulo 5

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The relationship between metabolic rates (R) and biomass (W), can be expressed by the equation:

$$R = W^b \tag{5.1}$$

or in logarithmic form :

$$R = log\ a + b\ log\ W \tag{5.2}$$

It is known as the Kleiber's law (Kleiber, 1961) and more recently has been used to develop a theory that combines the effect of two variables: temperature and body size. Reputed to be based on chemical, physical and biological principles (Gillooly et al., 2001, 2006; Brown et al., 2007), this theory is known as the Metabolic Theory of Ecology (MTE). This theory applies a single equation for metabolic rates of all organisms,

$$Y = i_0\ W^{3/4} e^{-E/k.T} \tag{5.3}$$

where i_0 is the normalization constant, W is the biomass, and $e^{-E/k.T}$ is the Boltzmann-Arrhenius factor, where E is the activation energy, k is the Boltzmann constant and T is the absolute temperature in degrees K (Gillooly et al., 2001).

The regression coefficient b=3/4, as established by Kleiber's law, is a general average, but many authors have found variations in it (Glazier, 2005,

2006; Atanasov, 2010). Lane (2005) discusses the reliability of this exponent and concludes that based on detailed examination it is not a constant for all species and sizes of organisms as is claimed. In this study, we obtained the values of coefficient b for *L. lingvura* across a range of sizes and ages (1 day-adult) with three different feeding treatments, trying to determine how this factor affects Kleiber's coefficient b. The present investigation of respiratory metabolism in *L. lingvura*, aims to examine:
1. How the periods of starvation affect respiration and ETS activity.
2. How different food conditions affect the respiration-biomass ratio and ETS-biomass relationship.

5.2. Materials and Methods

5.2.1. Collection and laboratory maintenance

Adults of *L. lingvura* were captured in coastal waters off Risco Verde in Gran Canaria, at depths of between 5 and 15 m with SCUBA equipment and a hand net of 500 μm mesh size, cultured as described in Herrera et al. (2011a), and identified microscopically following the keys of Tattersall and Tattersall (1951) and Wittmann (1986).

5.2.2. Effect of starvation on respiratory metabolism

After acclimation for 7 days, males of similar sizes were separated in individual containers to avoid cannibalism and subjected to different periods of starvation: 2, 6, 10, 22, 26, 30, 36, 46, 52 and 74 h. The experiment began at 10 am, the starvation period of 2 h occurred at 12 am, and the 74 h starvation period, 3 days later at 14 pm. At the end of each starvation period, five individuals were separated for measurements of *in vivo* respiration (μl O_2 per h) with an oxymeter (Strathkelvin 928 6-Channel oxygen system) in dark individual 50 ml chambers at 20.5℃. Afterwards, the mysids were frozen at -196℃ in liquid nitrogen and then stored at -80℃ for ETS activity (Gómez et al., 1996) and for protein measurements according to Lowry method (Lowry et al., 1951), as modified by Rutter (1967).

5.2.3. Effect of feeding conditions on the respiratory metabolism-biomass relationship

For this experiment, 100 different sized mysids were put into each of 3 different tanks and fed 3 different treatments for a week:
Treatment A: twice-daily ration of 150 *Artemia* sp. (artemia) 48 hours- nauplii.
Treatment B: twice-daily ration of 75 artemia 48 hours- nauplii.
Treatment C: twice-daily ration of 10 artemia 48 hours- nauplii.

After one week 36 mysids from each of the different treatments were separated for measurements of in vivo respiration (μl O_2 h^{-1}) with an oximeter. Individual darkened cells of 50 ml were used. The mysids were subsequently photographed, sized, frozen at -196℃, and stored at -80℃ for ETS activity (Gómez et al., 1996) and for protein measurements.

5.2.4. ETS activity determination

Samples were homogenized by sonication for 45 s with an ultrasonic probe (Cole Parmer) in 1.5 ml of Milli-Q distilled water, then centrifuged for 10 minutes at 4000 rpm at 0℃. A 0.5 ml aliquot of the supernatant was added to 1.5 ml of a solution containing (0.2 (v / v) Triton X-100, 50 mM sodium phosphate (Sorenson's) buffer pH 8, 0.133M disodium succinate, 0.835mM NADH, and 0.24mM NADPH) and 0.5 ml of 4mM INT (Sigma Lab). For each sample a blank was performed without ETS substrates. Samples were incubated at 20.5℃ for 20 minutes after which the reaction was stopped with a quench solution consisting of 50 % phosphoric acid 0.1M and 50 % formaldehyde to 36 %. Absorbance was read spectrophotometrically (Beckman DU 650, USA) at 490nm (INT-formazan) and 750nm (turbidity). Potential respiration was calculated from ETS activity according to Packard and Christensen (2004). Respiration rates (R) and potential respiration rates (Φ) were normalized by biomass (protein) resulting in units of μl O_2 h^{-1} mg $protein^{-1}$.

5.2.5. Statistical analysis

The starvation experiment data were analyzed using the program R Development Core Team 2010 (R Foundation for Statistical Computing, Vienna, Austria). To confirm normality, the respiration (R), ETS activity and R/ETS

by the Shapiro Wilk test and the homoscedasticity of the residuals was assessed graphically. To study the correlation between respiration-biomass and ETS-biomass in different feeding conditions we use the program PASW Statistical Software version 18.0 to obtain the regression equations, using a confidence limits of 95 % and the Pearson correlation coefficient.

5.3. Results

5.3.1. Effect of starvation on respiratory metabolism

The correlation in the relationship between *in vivo* R and starvation time (h) (figure 5.1) is represented by the equation:

$$R = 71{,}78 - 24{,}36\ log\ h \tag{5.4}$$

The Shapiro-Wilk normality test yields W=0.9551, p-value=0.07953; R^2=0.44, n=45, p< 0.001. Figure 5.1 depicts the decrease in R with starvation time in mysids.
Peaks in the respiration are observed at the beginning of the periods of darkness, if we take into account two variables: starvation time and time of day (t) (figure 5.1), the model that fits the data best is:

$$R = 44{,}49 - 9{,}98\ log\ h + 1{,}82\ t \tag{5.5}$$

The Shapiro-Wilk normality test yields W=0.9643, p-value=0.1778; R^2=0.65, n=45, p< 0.001.

Although the ETS data are scattered, ϕ shows no correlation with the diel periodicity. Furthermore, it does not decrease with increasing starvation-time in *L. lingvura* (R^2 =-0.02, p = 0.754) (figure 5.2) reflecting the constitutive nature of the ETS in the mysidÕs mitochondria. Since ϕ is constant, it is the decreasing R that forces the R/ϕ ratio to decrease with starvation time as in figure 5.3. The regression equation is:

$$R/ETS = 2{,}03 - 0{,}29\ log\ h \tag{5.6}$$

The Shapiro-Wilk normality test yields W=0.9687, p-value=0.3116; R^2= 0.44, n=41, p< 0.001.

If we consider both variables starvation time and time of day, the model that fits the data best is:

$$R/ETS = 1{,}16 - 0{,}27\ log\ h + 0{,}06\ t \tag{5.7}$$

The Shapiro-Wilk normality test yields W=0.9893, p-value=0.9614; R^2= 0.72, n=41, p< 0.001.

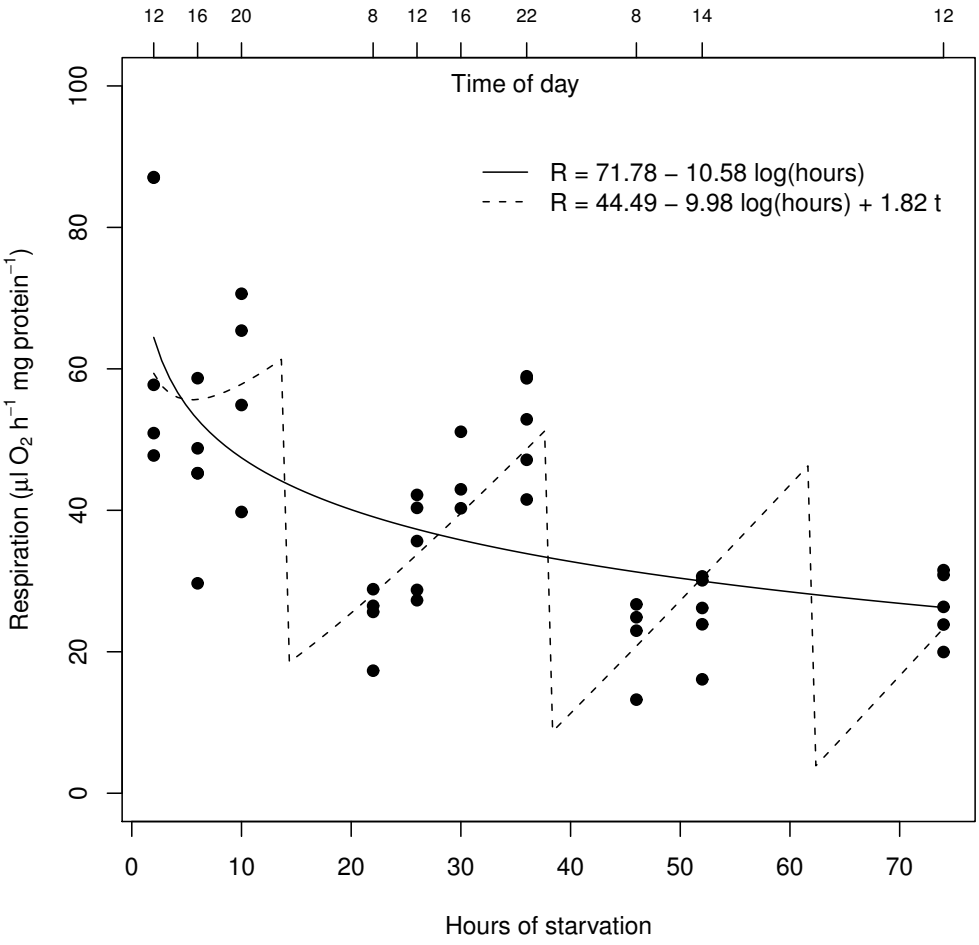

Figura 5.1: Relationship between R (μl O_2 h^{-1} mg prot $^{-1}$) and starvation period (h), R^2=0.44, n=45 (solid line); and relationship between R, starvation period and time of day (t), R^2=0.64, n=45 (dotted line). The dark period started at 20:00 hs.

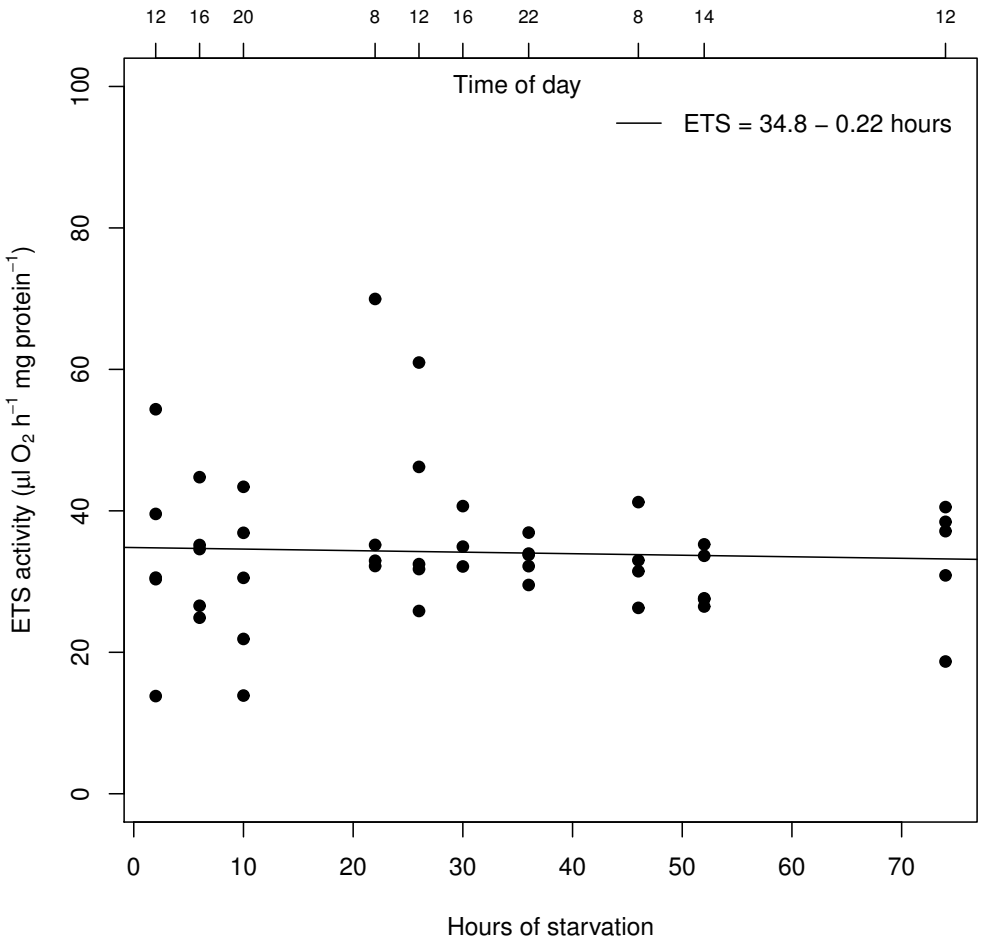

Figura 5.2: Relationship between ϕ (μl O_2 h^{-1} mg prot $^{-1}$) and starvation period (h), R^2=0.021, n=45.

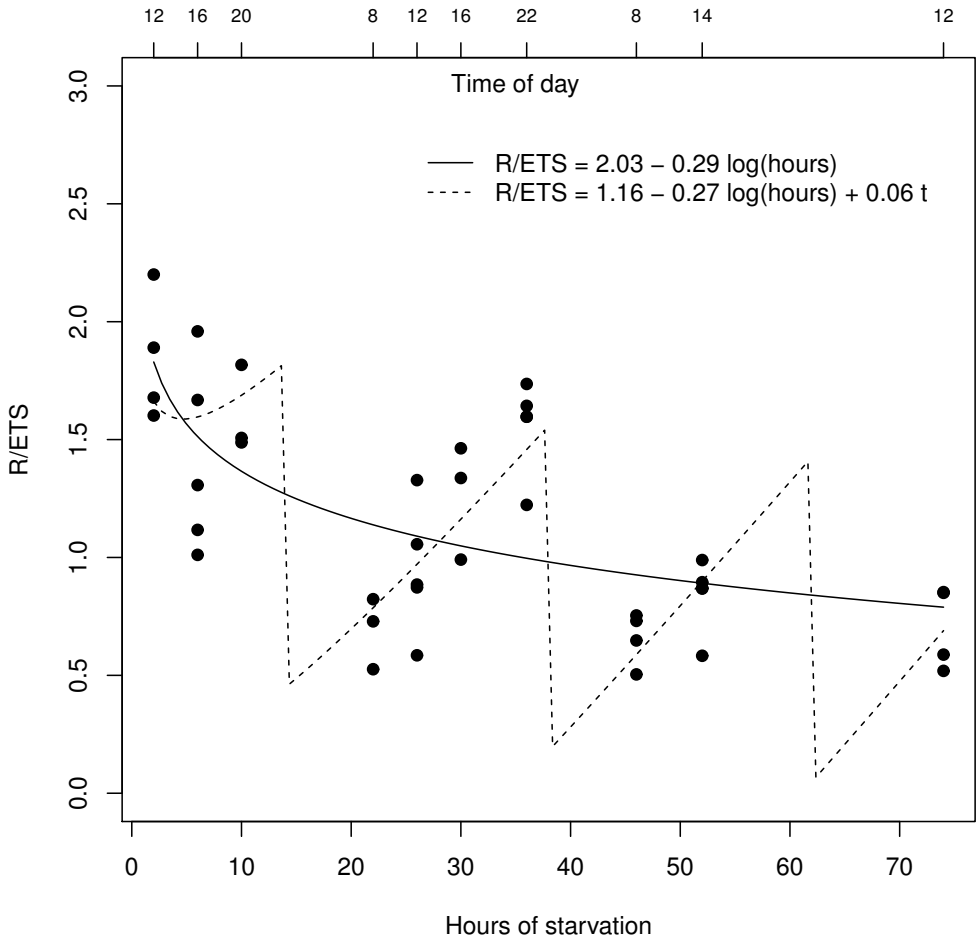

Figura 5.3: Relationship between R/ϕ ratio and starvation period (h), R^2=0.44, n=41 (solid line); and relationship between R/ETS, starvation period and time of day (t), R^2=0.72, n=41 (dotted line). The dark period started at 20:00 hs.

The biomass range of male mysids studied was between 0.129 and 0.319 mg of protein. The mean *in vivo* R and ϕ for each period of starvation are shown in table 5.1.

Tabla 5.1: Values of respiration rate (R) and potential respiration rate (ϕ) (μl O_2 h^{-1} mg $protein^{-1}$) (mean ± SD) for different periods of starvation. Ratio R/ϕ (mean ± SD) for each period of starvation.

Hours of starvation	R ± SD	n	ϕ ± SD	n	R/ϕ ± SD
2	66.1±19.5	5	33.7±14.8	5	2.17±0.76
6	45.5±10.4	5	33.2±7.9	5	1.41± 0.40
10	57.7±13.6	4	29.3±11.7	4	1.60±0.18
22	24.6±5.0	4	42.6±18.3	3	0.69±0.15
26	34.8±6.7	5	39.5±14.2	5	0.94±0.27
30	44.8±5.6	3	35.9±4.4	3	1.26±0.24
36	51.8±7.5	5	33.3±2.7	5	1.56±0.20
46	22.8±6.0	4	33.0±6.2	4	0.66±0.11
52	25.4±6.9	5	30.1±4.0	5	0.84±0.15
74	26.5±4.9	5	33.1±8.8	5	0.70±0.17

5.3.2. Effect of feeding conditions on the respiratory metabolism-biomass relationship

The relationship between R and biomass expressed in logarithms for treatment A was:

$$log\ R = 2{,}18 + 0{,}84\ log\ W \tag{5.8}$$

(R^2=0.64, n=34) with a Pearson correlation coefficient =0.798, p< 0.01; for treatment B:

$$log\ R = 2{,}72 + 0{,}92\ log\ W \tag{5.9}$$

(R^2=0.69, n=36) with a Pearson correlation coefficient =0.829 p< 0.01; and treatment C:

$$log\ R = 1{,}87 + 0{,}71\ log\ W \tag{5.10}$$

R^2=0.81, n=35) with a Pearson correlation coefficient =0.902, p< 0.01 (figures 5.4, 5.5, 5.6).

ETS activity represents the ϕ , the maximum reaction rate of Complex I, the NADH dehydrogenase iron-sulfur protein flavin mononucleotide conglomerate, that controls the electron flux through the mitochondrial. The relationships between this activity and biomass was observed in the 3 treatments are as follows:
Treatment A:

$$log\ ETS = 2{,}73 + 0{,}72\ log\ W \tag{5.11}$$

(R^2=0.84, n=34) with a Pearson correlation coefficient =0.916, p< 0.01; for B:

$$log\ ETS = 2{,}85 + 0{,}71\ log\ W \tag{5.12}$$

(R^2=0.77, n=36) with a Pearson correlation coefficient =0.879, p< 0.01; and C:

$$log\ ETS = 2{,}44 + 0{,}54\ log\ W \tag{5.13}$$

(R^2=0.85, n=35) with a Pearson correlation coefficient =0.924, p< 0.01 (figures 5.4, 5.5, 5.6).
The exponent, b, in Kleiber plots is the coefficient in these log-log equations. For both R and ϕ , b is lower in poorly fed mysids than in the well fed ones (table 5.2).

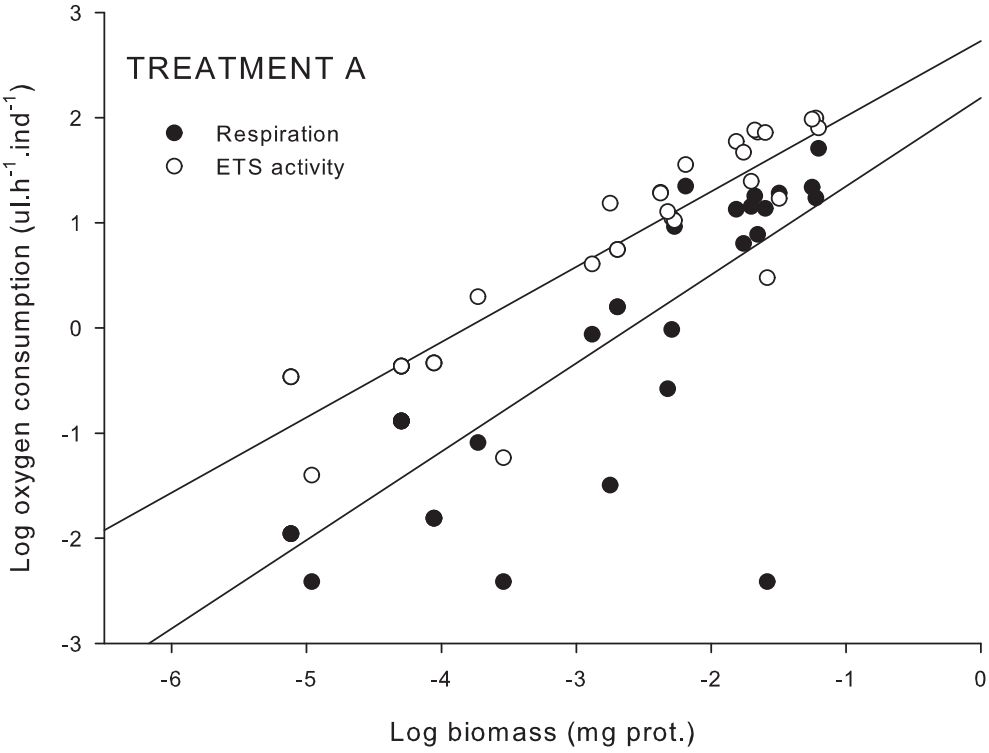

Figura 5.4: Relationship between R-biomass; and ϕ-biomass for mysids with treatment A.

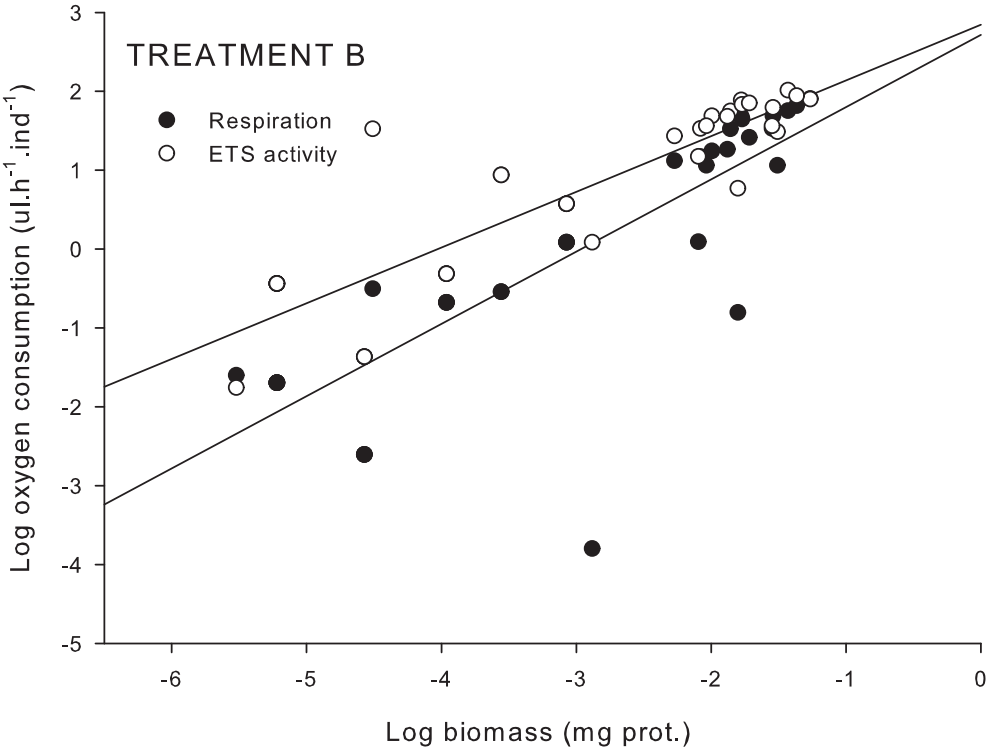

Figura 5.5: Relationship between R-biomass; and ϕ-biomass for mysids with treatment B.

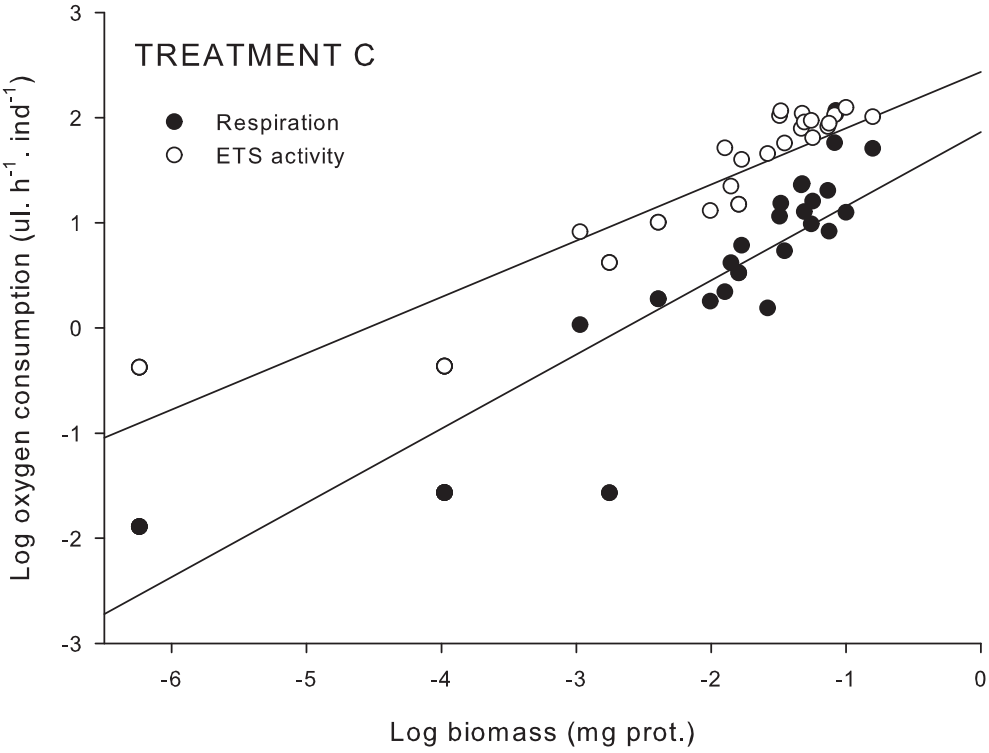

Figura 5.6: Relationship between R-biomass; and ϕ-biomass for mysids with treatment C.

Tabla 5.2: Regression constants of the relationship between potential respiration and biomass represented by the equation: log ETS= a logW^b. Mean scaling exponent ± 95 % confidence limits (C.L.). *Significantly different from 0.75.

Organism	Food conditions or colect conditions	a	b ± 95 % C.L.	n	R^2	Reference
Artemia salina	5000 *Nanoclorosis* sp. ind $^{-1}$	-0.05	0.59±0.39	10	0.60	Martínez et al. (2010)
Artemia salina	1000 *Dunaliella* sp. ind $^{-1}$	-4.40	0.50 ±0.18*	14	0.73	Martínez et al. (2010)
Zooplankton mix	upwelling areas	0.14	0.89 ±0.11*	248	0.53	Gómez et al. (2008)
Zooplankton mix	eddies areas	-0.12	0.98±0.40	30	0.48	Gómez et al. (2008)
Zooplankton mix	oceanic areas	-0.03	0.64±0.11	220	0.38	Gómez et al. (2008)
Zooplankton mix	coastal areas	0.08	0.79±0.15	64	0.64	Gómez et al. (2008)
Zooplankton mix	incubated for 1 day	0.52	0.79±0.12	14	0.94	Packard and Gómez (2008)
L.lingvura	200 *Artemia* nauplii.d^{-1}.ind^{-1}	4.15	0.92±0.07*	32	0.96	Herrera (2009)
L.lingvura	300 *Artemia* nauplii.d^{-1}.ind^{-1}	2.73	0.72±0.11	34	0.84	present work
L.lingvura	150 *Artemia* nauplii.d^{-1}.ind^{-1}	2.85	0.71±0.13	36	0.77	present work
L.lingvura	20 *Artemia* nauplii.d^{-1}.ind^{-1}	2.44	0.54±0.08*	35	0.85	present work

5.4. Discussion

According to the results, when *L. lingvura* is starving its respiratory rate decreases, other authors found similar results in the bathypelagic mysid *Gnathophausia ingens* (Hiller-Adams and Childress, 1983). This is not the case with ϕ, represented by the enzymatic activity of the ETS. Over a period of 74 h it does not decrease. The enzymes of the ETS are responsible for 90 % of all biological O_2 consumption (Nelson and Cox, 2005) and unless the number of mitochondria or their size changes, their capacity (V_{max}) should not change, at least during short-term changes in physiological state. In *L. lingvura* other metabolic rates, such as the ammonia excretion rate also show similar behavior. It decreases logarithmically with the starvation time, while the activity of GDH, the enzyme that controls this process, is not affected (Fernández-Urruzola et al., 2011).

In our method for monitoring the ETS Vmax we saturate the enzyme assay with ETS substrates (NADH, NADPH and succinate) and so we insure that we are measuring the amount (the concentration) of the enzymes available to consume O_2 and to maintain the proton-motive force in the mitochondria. In this manner we also insure that the measurement of ETS activity is insulated from variability in the substrate supply systems which can be modulated by starvation and other forms of physiological stress.

R in mitochondria can be impacted by the availability of ADP as a substrate for phosphorylation. Outside the mitochondria, when the speed of some cellular energy-requiring processes such as protein synthesis increases, there is an increased rate of ATP degradation to ADP. Transported back to the mitochondria, this increases the availability of ADP for oxidative phosphorylation which, by lowering the pH and emf (voltage) gradient across the mitochondrial inner membrane, can stimulate the ETS and hence R (Chance and Williams, 1955; Nelson and Cox, 2005; Lane, 2005). R increases with feeding known as "specific dynamic action" (SDA) has been noted by Kiorboe et al. (1985) and Thor (2003) in *Acartia tonsa*. They found that the respiration rate in copepods during food-saturated conditions was 4 times greater than during conditions of starvation, and postulated that this increase is mainly related to the biosynthesis and transport. They argue that gut activity, amino acid oxidation and urea excretion contributes less to the SDA. In any case, SDA

is another zooplankton process likely to disturb the ADP/ATP ratio via the demand for ATP throughout the organism. Most physiological mechanisms that disturb the ADP/ATP ratio will lead to considerable variability in R. Ingestion, for example, such as studied in *Euphausia superba* by Ikeda and Dixon (1984) will also fall in this group.

In this study, R in well fed mysids is three times higher than R in mysids starved for 46 h or more. In mysids that have an active metabolism, but a low nutrient reserve, the lack of substrates is rapidly reflected in R. The variation in the ratios R/ϕ is a direct consequence of all of the above processes. Hernández-León and Gómez (1996) studied the causes of the high variability of the R/ϕ ratios obtained in other zooplankton studies for different oceanic areas and oceanographic conditions and showed that the factors affecting this relationship are: chlorophyll, primary production, temperature and size of organisms. The variability of the R/ϕ in relation to chlorophyll and primary production suggests that these indices of the quantity or quality of food impact R, but not ETS (Hernández-León and Gómez, 1996). In this study, R and R/ϕ in fed mysids was three times higher than in mysids starved for 46 h or more.

In addition, in figure 5.1 (R vs starvation period) there are two peaks in R that coincide with the start of the dark period at 20:00 h. These are likely maxima in the circadian R rhythm. Many mysids in the hyperbenthos have endogenous rhythms of activity. Mysids of the genus, *Gastrossacus*, rest on the sediment during the day and ascend swimming at night, this behavior persists even under experimental conditions of darkness for several days (Mauchline, 1980). Mediterranean species of *Leptomysis* also have this type of feeding behavior (Dauby, 1995). Hecq et al. (1984) have conducted studies on the influence of experimental and environmental conditions in the consumption of O_2 in *L. lingvura*. They show that during the day R ranges between 20 and 24 mg O_2 h^{-1} mg $protein^{-1}$. It increases progressively during the night reaching a maximum value at the end of the night (48.2 mg O_2 h^{-1} mg $protein^{-1}$). Amylase activity increases in parallel.

It is not purpose of this paper to investigate how circadian rhythms affect respiration and respiration/ϕ ratio, but when we observe the behavior of respiratory metabolism during the dark period we have seen that the model

that includes two variables: starvation time and time of day have a better fit than the one that only includes the starvation time (Figures 5.1 and 5.3), but this model is rather descriptive and missing data in both periods (light and dark) in the last days of the experiment to assess how influence both variables in the respiration. Experiments are needed for this purpose. In organisms that have internal daily rhythms as mysids in applying models to estimate metabolic rates is necessary to consider this variable and the feeding conditions because that directly affect these processes.

Regarding the relationship between respiratory metabolism and biomass, other authors (Martínez, 2007; Gómez et al., 2008; Packard and Gómez, 2008; Herrera, 2009) have found similar correlations in different groups of zooplankton (table 5.2). In these studies the slope b is also ranges between 0.5 and 1. This variability has lead others to question Kleiber's law and the MTE to describe the oxygen consumption in a small range of sizes, short time scale or in different physiological states (Dodds et al., 2001; Lane, 2005; Packard and Gómez, 2008; Kolokotrones et al., 2010). The enzyme-kinetic-model (EKM) developed by (Packard et al., 1996, 2004) and Roy and Packard (2001) proposed that R is the product of ϕ and substrate availability that regulates it. These are the fundamental bases for regulating R. Biomass is indirectly related to R because it packages the mitochondria and the ETS enzymes (Martínez et al., 2010), but by itself biomass is an irrelevant factor. Here, the ETS-biomass relationship in the three treatments showed a better fit than the R-biomass ratio (Figures 5.4, 5.5, 5.6, table 5.2). ETS activity is determined by the concentration of Complex 1-NADH dehydrogenase in the mitochondria, and this concentration varies with the number of cells in the mysid and hence the biomass. ETS, being a constituent part of the mitochondria, the cells, and the mysid, should not change rapidly with environmental conditions or the amount of metabolizable substrate, as does respiration. However, with prolonged acclimation to different conditions (as with forced activity) the ETS activity could change.

If we analyze the data of the table 5.2, and assume that organisms in the regions of upwelling, coastal and eddies are well-fed (as in treatments A and B), the higher values of b ($\geq$0.75) become understandable. Likewise, assuming that zooplankton in oceanic regions are less-well fed (as in treatment C), the lower values of b (<0.75) become understandable.

5.5. Conclusions

1. *L. lingvura* respiratory activity shows a variability related to feeding conditions and circadian rhythms. Since the activity of enzymes of the ETS is not altered in the short term, this variability of respiratory rate forces parallel variability in the R/ϕ ratio, this ratio can be three times higher in feeding mysids than in starved ones. When performing *in vivo* experiments of respiratory metabolism in zooplankton it is necessary to take into account the physiological conditions and endogenous daily rhythms because they directly impact the respiratory activity.

2. In *L. lingvura* the Kleiber coefficient, b, of the regression equation: R=a W^b, varies with food conditions, it is lower than 0.75 when the organisms are exposed to minimum conditions of food for long periods of time. Further testing of this hypothesis will require studies of respiration and ETS activity in other zooplanktonic organisms exposed to different feeding conditions.

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Capítulo 6

Application of ETS analysis in the estimation of respiratory metabolism in deep-sea suprabenthic crustaceans

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Capítulo 6

Application of ETS analysis in the estimation of respiratory metabolism in deep-sea suprabenthic crustaceans

Abstract: Respiration is an index of physiological activity in zooplankton that can be used to estimate the energy production. The direct measurement of respiration rates in the marine environment is difficult, because incubations in laboratory conditions cannot exactly reproduce the natural conditions. ETS is an acronym for the activity of the respiratory electron transport system. The ETS assay is a biochemical method for estimating the "potential" respiration. The aim of this study was to apply this technique in suprabenthic species captured during the IDEADOS survey during summer 2010 in two different locations: Cabrera (Algerian sub-basin) and Sóller (Balearic sub-basin); and at three depths (250 m, 650 m and 850 m).

Decapods were the species with highest mean value of potential respiration. It averaged 17.4 μl O_2 h^{-1} ind^{-1} for *Plesionika heterocarpus* , 54.7 μl O_2 h^{-1} ind^{-1} for *Gennadas elegans* and 102 μl O_2 h^{-1} ind^{-1} for *Sergestes arcticus*. Euphausiids showed intermediate values averaging 16.2 μl O_2 h^{-1} ind^{-1} for *Thysanopoda aequalis* and 18.4 μl O_2 h^{-1} ind^{-1} for *Meganyctiphanes norvegica*. Mysids had lower respiration rates, 7.8 μl O_2 h^{-1} ind^{-1} for *Boreomysis arctica* and 8.8 μl O_2 h^{-1} ind^{-1} for *Eucopia unguiculata*.

Spatial differences in the mean specific ETS activity were found bet-

ween the two locations. It was higher in Cabrera and increased with depth; in Sóller it starting lower and then decreased with depth. The slope in the log ETS-log biomass plot in well-fed organisms, is close to or higher than 0.75 according to Gómez et al. (2008). In our measurements the slope was 0.93 for Cabrera and 0.60 in Sóller, the difference could indicate that the suprabenthos was well-fed for long periods of time in Cabrera.
Decapoda, Mysidacea and Euphausiacea represented 56 % of the suprabenthos in the area and we estimated average carbon demand for these taxons, due to respiration, in Cabrera to be 7.48, 15.27 and 3.87 mg C yr^{-1} m^{-2} at 250, 650 and 850 m, respectively. The equivalent values in Sóller were 43.69, 6.97 and 4.86 mg C yr^{-1} m^{-2} at 250, 650 and 850 m, respectively.

6.1. Introduction

Respiration rates are fundamental measures of biological activity and especially of its energy production process. In metazoans these rates are limited by their mitochondria and the biochemical mechanisms that control them. Historically, biomass has served as an easily measured proxy for respiration (Prosser and Brown, 1961; Ikeda, 1970) even though it was well know that the function of the biomass was to package the mitochondria (Fruton and Simmonds, 1958; Nelson and Cox, 2005; Packard and Gómez, 2008). The role of temperature in modulating respiration has been recognized, at least since the time of Arrhenius (1915), but has its own history in oceanography (Seiwell, 1937; Packard et al., 1975; Ikeda, 1985). From these studies it was clear that zooplankton respiration increased with the weight and size associated with biomass and increasing seawater temperature.

Respiratory rates are also related to swimming activity, when zooplankton are slowly maintaining their position in the water column their respiration is low, when they swim rapidly to escape predators or to capture prey their respiration speeds up. Cowles and Childress (1988) observed this respiratory shift in mysids. In addition, respiration is stimulated during feeding and mating (Thor, 2003; Kiorboe et al., 1985; Lampert, 1986; Bohrer and Lampert,

1988; Hernández-León and Gómez, 1996). However during these conditions ETS activity is likely to be relatively constant (Cammen et al., 1990; Herrera et al., 2011b).

The direct measurement of respiration rates in the oceanic environment is difficult because the rates are so low. Furthermore, they cannot be made by using incubations in the laboratory because the conditions cannot exactly reproduce the natural conditions. The ETS technique was developed by Packard (Packard, 1971; Packard et al., 1971, 1974) and then has been applied to estimate respiration in zooplankton (Packard et al., 1974; King and Packard, 1975; King et al., 1978; Owens and King, 1975; Bämsted, 1980; Schalk, 1988; Minutoli and Guglielmo, 2009; Hirch et al., 2009), phytoplankton (Packard, 1971; Kenner and Ahmed, 1975) and bacteria (Packard et al., 1983, 1996; Arístegui and Montero, 1995). Since its inception the ETS method has been investigated and improved to provide increasingly reliable estimates of respiration (Hernández-León and Gómez, 1996; Gómez et al., 1996; Packard and Gómez, 2008; Maldonado et al., 2012). The basis of this technique is that the ETS is the biochemical origin of respiration and controls energy production via oxidative phosphorylation. This technique uses the reduction of an artificial electron acceptor, a tetrazolium-salt (INT), to stoichiometrically measure the capacity of the mitochondria to consume O_2. This can be done because the reduction of 2 moles of INT by the ETS is equivalent of the ETS-driven reduction of 2 atoms of oxygen (or 1 molecule of O_2) (Packard, 1971). The respiratory enzimatic system is saturated with substrates (NADH, NADPH and succinate) to obtain the "potential.[a]ctivity or maximum activity of the electron transport chain, as demonstrated in a recent study by Maldonado et al. (2012).

Suprabenthos or hyperbenthos (Mees and Jones, 1997), characterized by their swimming capacity, occupy the 2 m water layer immediately adjacent to the seabed. They consist of peracaridan crustaceans such as amphipods, cumacean, isopods and mysids; and eucaridan crustaceans as euphausiids and decapods (Sainte-Marie and Brunel, 1985). Among other free swimming metazoans, the suprabenthos are an important community in coastal ecosystems exploiting a diversity of food resources: organic particles, detritus, phytoplankton and zooplankton (Cunha et al., 1999; Cartes et al., 2001) and have great importance in the transfer of organic matter and energy due their

particular populations dynamics related to their swarming behavior, their high activity level, and their tendency to make vertical and horizontal migrations (Mees and Jones, 1997). Their transfer supports many demersal fish and epibenthic crustaceans, such as *Merluccius merluccius* (Bozzano et al., 1997; Cartes et al., 2004), and the red shrimp *Aristeus antennatus* (Cartes, 1994).

Suprabenthonic assemblages and some aspects of their trophic relationships (Madurell et al., 2008; Polunin et al., 2001) have been defined previously in depth waters of Balearic Islands (Maynou and Cartes, 2000; Cartes et al., 2001, 2008). These waters are located between two sub-basins in the western Mediterranean Sea off the NE Spanish coast. These sub-basins (Algerian and Balearic) have different geomorphological and oceanographic characteristics and their boundaries are influenced by both seasonal and mesoscale processes in the adjacent areas (Pinot et al., 2002; López-Jurado et al., 2008).

Previous studies have demonstrated differences in trophic web structure between sub-basins (Maynou and Cartes, 2000; Cartes et al., 2001). However, suprabenthos assemblages and abundance seem be more a function of depth gradients, in both sub-basins. These variations are related to the nature of the sediment (e.g. grain size) and its trophic condition (e.g. total organic matter content (%OM), potential REDOX) (Cartes et al., 2008). One can also assume that the depth can be an important factors influence the body condition and metabolism of suprabenthonic species. However, the effect of nutritional conditions on the respiration rate in plankton animals is little known (Ikeda, 1971).

The aims of this study are to apply the ETS technique to estimate the respiration of three representative groups of suprabenthos (decapoda, mysidacea and euphausiacea), and to detect their spatial and depth variations. Then these estimates were used to calculate carbon demand in order to elucidate the importance of these three groups in the biogeochemical cycles in the waters around the Balearic Islands.

6.2. Material and Methods

6.2.1. Study area and sampling methods

The study was performed in two established fishing areas situated in the Northwest and Southern waters off Mallorca (Balearic Islands, western Mediterranean) within the framework of the multidisciplinary project IDEADOS (http://www.ba.ieo.es/ideados). The suprabenthos samples were collected during a summer survey in July 2010 at three depths (250 m, 650 m and 850 m) in two different locations (Cabrera and Sóller), separated by a distance of ca. 60 nm. The Northwest location was close to the harbour of Sóller, in the Balearic sub-basin. The Southern location was close to the Cabrera Archipelago, in the Algerian sub-basin (Figure 6.1).

The samples were collected with a rectangular net rigged in a beam-trawl used to catch megabenthic fauna within 0.6 m above the bottom. The dimensions of net were 1.25 m by 0.3 m. At each location and depth three samples of suprabenthos were colected. Between 3-5 individuals of different species of decapods (*Gennadas elegans* (Smith, 1884), *Plesionika heterocarpus* (A. costa, 1871) and *Sergestes arcticus* (Kröyer, 1855)), mysids (*Boreomysis arctica* (Kröyer, 1855) and *Eucopia unguiculata* (Willemoes-Suhm, 1875)) and euphausiids (*Meganyctiphanes norvegica* (M. Sars, 1857) and *Thysanopoda aequalis* (Hansen, 1905)) were caught with each sample, and immediately frozen on board at -196℃ in liquid nitrogen. The number of collected individuals depended of the availability of live specimens in the sample. In the laboratory samples were stored at -80℃ until measurements of ETS activity and protein biomass could be made.

The rest of the sample was fixed in buffered formaldehyde (4 %) for further sorting, identification at a high taxa level, and counting under a stereomicroscope. The abundance of selected taxa was estimated as individuals 100 m^{-2} in order to characterize the sampling stations and estimate carbon demand for each group. Temperature, salinity and depth was recorded by a CTD on transects with a SB39 profiler mounted on the beam-trawl. At each profile, the measurements were made at 1 m above the bottom. Data on oxygen and fluorometry were obtained from the IDEADOS database that were performed simultaneously in the same stations where collecting suprabenthos.

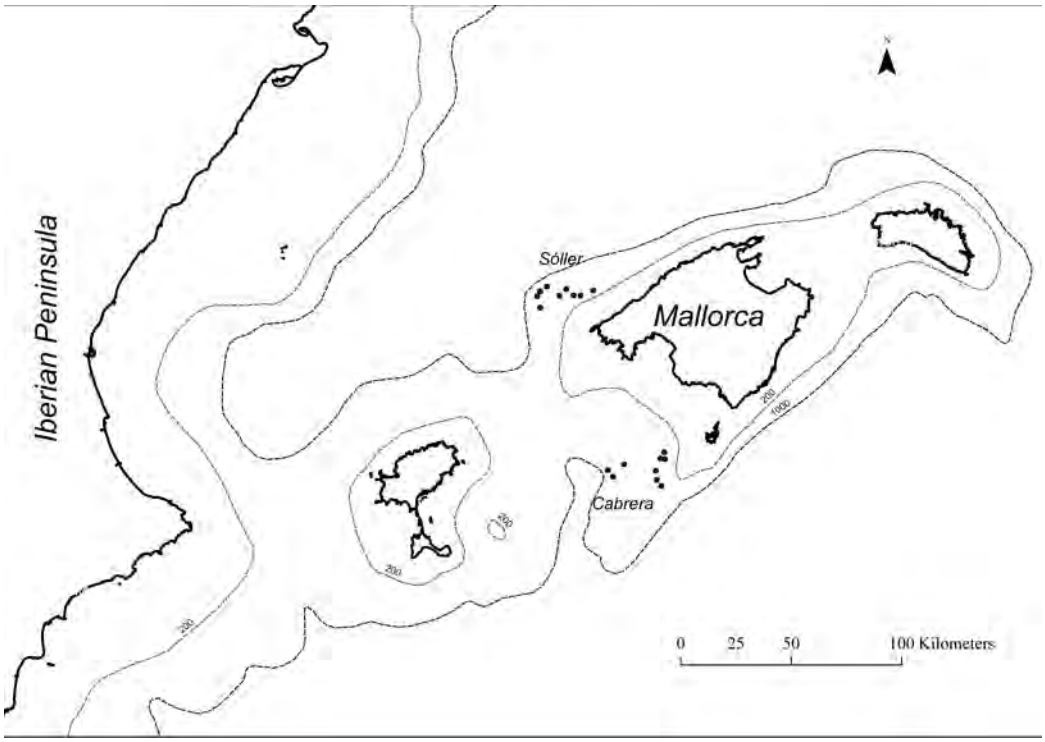

Figura 6.1: Map of study area, indicating the continental shelf location of the suprabenthos sampling area: Sóller (Balearic sub-basin) and Cabrera (Algerian sub-basin).

6.2.2. ETS analysis

Potential respiration was estimated according to ETS method (Packard, 1971) with modifications (Owens and King, 1975; Gómez et al., 1996; Packard and Christensen, 2004). Samples were homogenized with ultrasound for 45 seconds in 1.5 ml of Milli-Q double-distilled water, then centrifuged for 10 minutes at 4000 rpm at 0°C. A 0.5 ml aliquot of the supernatant was added 1.5 ml of solution containing the substrates (0.2 % (v / v) Triton X-100, 50 mM sodium phosphate buffer pH8, 0.133M disodium succinate, 0.835mM of 0.24mM NADH and NADPH) and 0.5 ml of 4 mM INT (Sigma Lab). Each sample was controlled by a blank without substrates. Samples were incubated at 18°C for 20 minutes after which the reaction was stopped with a quench solution consisting of 50 % phosphoric acid 0.1M and 50 % formaldehyde to 36 %. The absorbance reading was performed in a spectrophotometer (Beckman DU 650, USA) at 490 nm and 750 nm to correct for turbidity. ETS activity was calculated according to the following equation:

$$ETS = COD \cdot 60 \cdot H \cdot AS / (1{,}42 \cdot t \cdot L \cdot F) \tag{6.1}$$

Where COD is the absorbance of the sample at 490 nm corrected for blank and reagents, H is the homogenate volume in ml, AS is the volume of the reaction mixture in ml, the factor 60 converts min to h, 1.42 is the conversion factor of INT-formazan into O_2 as μl, L is the cubette length (1cm), F is the volume of the homogenate in the assay in ml and t is the incubation time in min. ETS activity was corrected for *in situ* temperature using Arrenhius equation and activation energy (Ea) of 15 kcal mol^{-1} (Packard et al., 1975).

$$ETS_{assay} = ETS_{insitu} \cdot e^{(Ea/R \cdot (1/T_{assay} - 1/T_{insitu}))} \tag{6.2}$$

Where R is the gas constant, T_{assay} is the temperature of the assay and T_{insitu} is the *in situ* temperature where the sample was taken.

The specific ETS activity indicates oxygen consumption per unit biomass and it is an indicator of the physiological activity of living biomass. Biomass was estimated in mg of protein by the method of Lowry et al. (1951), as amended by Rutter (1967).

The rate of potential oxygen consumption (ETS activity) is converted to carbon demand rate assuming an respiratory quotient (RQ) of 0.85 (King et al., 1978). The potential respiratory carbon demand by groups at different locations and depth levels was calculated as the product of the mean ETS activity and the mean abundance by group at the corresponding sampling location and depth, and converted to μg C d^{-1} m^{-2}.

6.2.3. Statistical analysis

Specific ETS activity per unit biomass was used in order to test interspecific and spatial changes in respiration rates. To confirm normality of residuals, specific ETS activity data were analyzed by the Shapiro Wilk test and the homoscedasticity of the data was confirmed with Levene's test. The variability of specific ETS activity among species was tested by one way ANOVA test. Comparison between locations and among depths was defined by ANOVA analysis. The test included two orthogonal factors: location (Cabrera/Soller) and depth (250 m, 650 m and 850 m), with 3 replicates for treatment. This statistical design was also applied in the comparison of environmental parameters (temperature, salinity, oxygen and fluorometry), abundance of suprabenthos and estimations of carbon demand. Relationships between ETS-protein biomass in different locations and depths were obtained by the regression equations, using confidence limits of 95 % and the Pearson correlation coefficient.

6.3. Results

6.3.1. Characterization of sampling area

Mean environmental parameters and suprabenthos abundance at sampling stations are shown in table 6.1. Despite being significant, the differences in percentage of oxygen (0.21 ml L^{-1}) and temperature (0.04°C) were very small, thus not affecting respiration rates. ANOVA analysis demonstrated differences between locations in the case of temperature ($p<0.05$) where it was slightly higher in Cabrera at three depth levels. At both locations it was statistically higher at 250 m ($p<0.001$). Oxygen also significantly differed

between locations and depths ($p<0.001$). The mean value was always higher in Sóller, largely because the value at 250 m (4.50 ml L^{-1}) was so high (Table 6.1).

Suprabenthos abundance was not statistical different between locations and depths. However, at both locations, decapods were lower at 250 m, euphausiids higher at 250 m and mysids higher at 650 m than at 850 m and, in both cases, higher than at 250 m ($p<0.001$).

Tabla 6.1: Summary of environmental characteristics and suprabenthos abundance in individuals 100 m^{-2} (mean±SE) of sampling stations in two locations at S (Cabrera) and NW (Sóller) Balearic Islands, at three depths levels (250 m, 650 m and 850 m).

	Cabrera				Sóller			
	Total	250	650	850	Total	250	650	850
Temperature (°C)	13.15±0.20	13.24±0.02	13.11±0.01	13.09±0.00	13.11±0.23	13.21±0.02	13.07±0.01	13.04±0.00
Salinity (PSU)	38.49±0.02	38.50±0.01	38.49±0.00	38.48±0.00	38.49±0.02	38.50±0.02	38.48±0.00	38.48±0.00
Oxygen ($ml.L^{-1}$)	4.19±0.01	4.22±0.02	4.15±0.01	4.21±0.01	4.40±0.02	4.50±0.02	4.25±0.02	4.34±0.02
Fluorimeter ($mg.m^{-3}$)	0.09±0.01	0.02±0.00	0.02±0.00	0.03±0.00	0.19±0.06	0.21±0.18	0.17±0.14	0.12±0.09
Decapods	1.98±1.25	0.02±0.02	4.85±3.51	1.06±0.19	0.61±0.27	0.05±0.03	0.69±0.47	1.10±0.61
Euphausiids	5.12±2.01	10.34±5.17	3.35±0.72	1.67±0.44	22.48±15.01	60.61±40.09	4.47±2.31	2.36±1.15
Mysids	3.21±1.43	0.05±0.05	8.25±2.23	1.34±0.13	3.45±1.23	0.44±0.44	7.26±1.92	2.65±1.45
Suprabenthos	19.04±3.10	18.37±6.72	26.87±2.41	11.87±2.90	35.45±14.53	69.13±39.14	24.76±9.34	12.45±5.19

6.3.2. Biomass, ETS activity and specific ETS activity

Decapods had the highest ETS activities. The mean values were 17.44 μl O_2 h^{-1} ind^{-1} for *P. heterocarpus*, 54.66 μl O_2 h^{-1} ind^{-1} for *G. elegans* and 101.97 μl O_2 h^{-1} ind^{-1} for *S. arcticus* (Table 6.2). These activities correlate with the higher range of protein biomass, between 5 and 14 mg ind^{-1} (Table 6.2). Euphausiids showed intermediate values of ETS, 18.44 μl O_2 h^{-1} ind^{-1} for *M. norvegica* and 16.23 μl O_2 h^{-1} ind^{-1} for *T. aequalis*, with the corresponded range of proteins biomass ranging from 1 to 4 mg ind^{-1}.
Mysids showed lower values of ETS: 7.8 μl O_2 h^{-1} ind^{-1} for *B. arctica* and 8.84 μl O_2 h^{-1} ind^{-1} for *E. unguiculata*. The mysids protein biomass range was: 1-3 mg ind^{-1}. A good correlation between ETS and biomass was found (Figure 6.2).

Specific ETS activity, expressed as potential respiration in unit of μl O_2 h^{-1} mg $prot^{-1}$, was not significantly different between species (ANOVA test $p>0.05$), with mean values ranged between 6.54 μl O_2 h^{-1} mg $prot^{-1}$ for *T. aequalis* and 9.76 μl O_2 h^{-1} mg $prot^{-1}$ for *M. norvegica* (Table 6.2).

Overall, the specific ETS activity or potential respiration per unit of biomass was significantly higher in Cabrera than in Sóller ($p<0.05$)(Figure 6.3). However, the activities were not significant differences between depths ($p>0.05$), but the trend with depth in each area was different, the mean specific ETS activity increased with depth in Cabrera while in Sóller it decreased ($p<0.05$) (Figure 6.3).

Tabla 6.2: Average biomass, ETS activity and specific ETS activity in representative species of suprabenthos at 250 m, 650 m and 850 m. All Sóller and Cabrera data were included in the averages.

Taxon	**Species**	**n**	**Biomass** (mg protein ind^{-1})	**ETS activity** ($\mu l\ O_2\ h^{-1}\ ind^{-1}$)	**specific ETS activity** ($\mu l\ O_2\ h^{-1}\ mg\ prot^{-1}$)
DEC	*G. elegans*	7	6.56±1.09	54.66±11.27	8.34±1.09
DEC	*P. heterocarpus*	3	5.07±3.36	17.44±8.06	8.43±4.58
DEC	*S. arcticus*	5	14.19±1.95	101.97±31.37	7.29±2.04
EUPH	*M. norvegica*	11	2.63±1.42	18.44±5.92	9.76±2.55
EUPH	*T. aequalis*	3	2.00±0.57	16.23±7.59	6.54±2.10
MYS	*B.arctica*	15	1.31±0.24	7.80±1.03	7.70±1.14
MYS	*E. unguiculata*	3	2.79±1.23	8.84±0.95	9.72±7.66

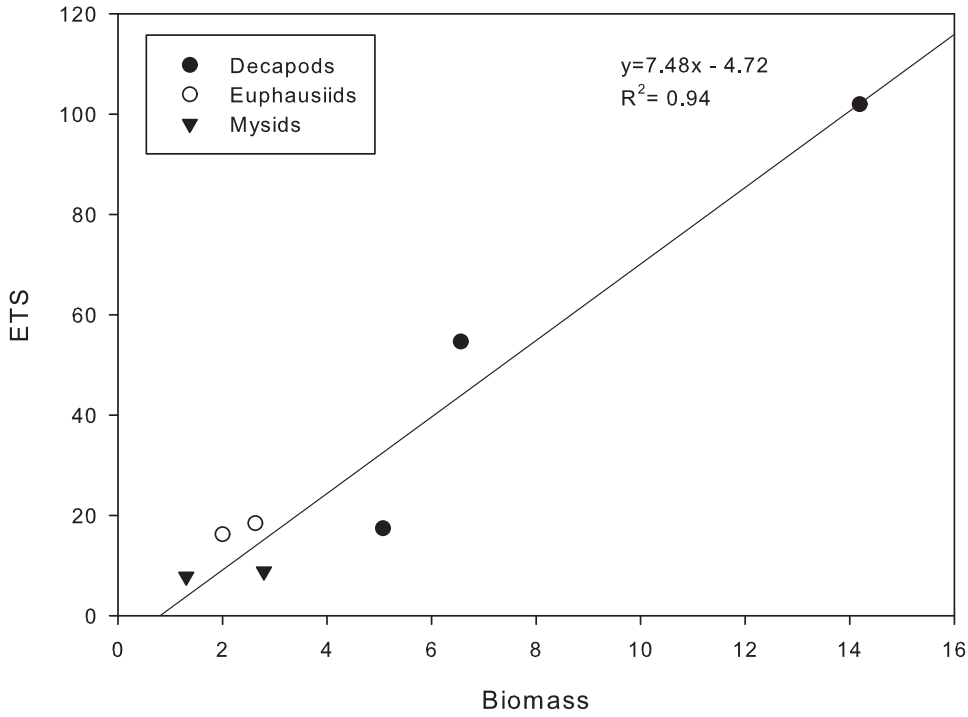

Figura 6.2: Correlation between ETS activity in μl O_2 h^{-1} ind^{-1} and biomass (mg protein) averaged by species.

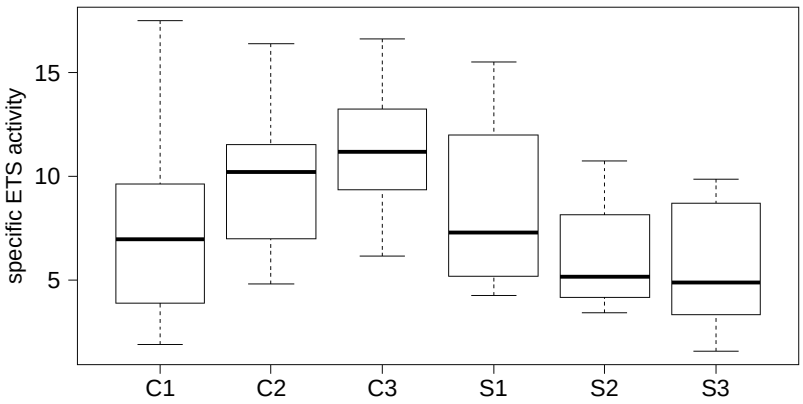

Figura 6.3: Specific ETS activity (μl O_2 h^{-1} mg $prot^{-1}$) by stations: Cabrera 250 m (C1), Cabrera 650 m (C2), Cabrera 850 m (C3), Sóller 250 m (S1), Sóller 650 m (S2) and Sóller 850 m (S3).

6.3.3. Relationship between ETS activity and protein biomass

The relationship between ETS activity and biomass (mg protein ind^{-1}) on an individual basis, expressed in logarithmic terms, is represented by the equation:

$$log\ ETS = b\ log\ W + log\ a \tag{6.3}$$

or

$$ETS = aW^b \tag{6.4}$$

Figure 6.4 represents the relationship between biomass and ETS activity at the two different locations, the coefficients of regression and correlation coefficients are shown in table 6.3. The ETS activity increased with increasing body mass, but the slope of the regression is higher in Cabrera.

Tabla 6.3: Coefficients of regression equations of log weight (mg protein ind^{-1}) and log ETS activity (μl O_2 h^{-1} ind^{-1}) and correlation coefficients (r^2) for different locations.

Location	a	b	r^2	n	p value
Cabrera	2.18	0.93	0.84	22	<0.01
Sóller	2.07	0.60	0.58	27	<0.01

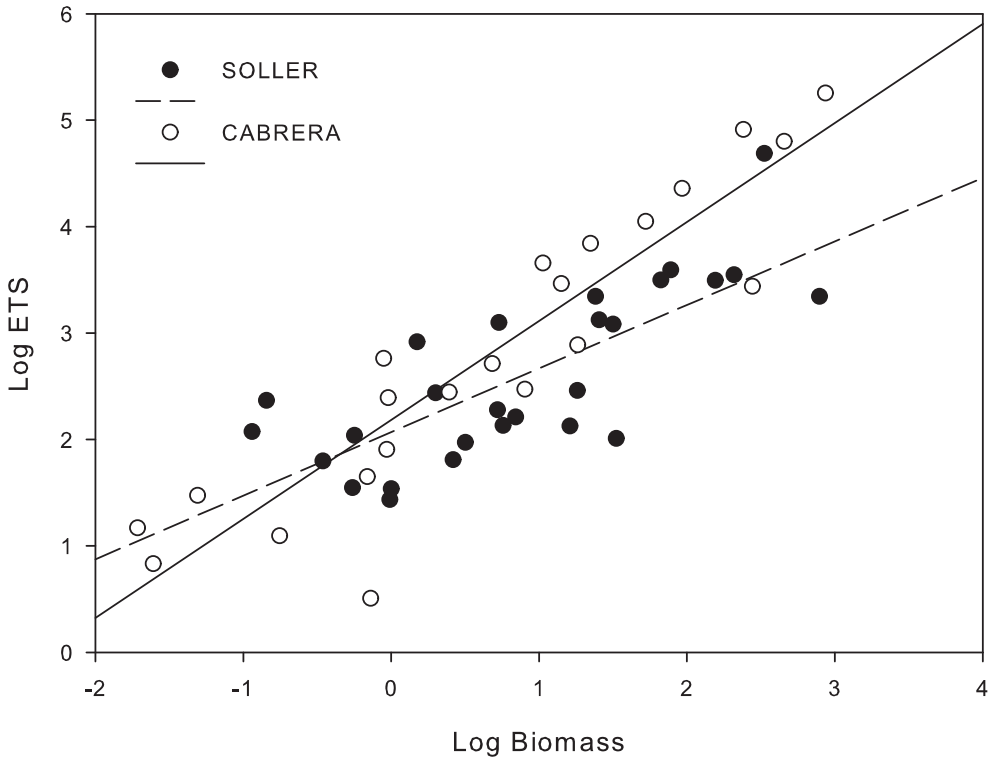

Figura 6.4: Relationship between potential respiration and biomass (mg protein) at two locations: Cabrera and Sóller, on a natural logarithmic scale.

6.3.4. Carbon Demand

The total carbon demand (in μg C d^{-1} m^{-2}) of three taxons was, in Cabrera, 20.48±10.33, 41.84±23.67 and 10.60±2.28 at 250, 650 and 850 m respectively, while in Sóller it was 119.70±79.25, 19.10±8.95 and 13.32±7.06 at 250, 650 and 850 m respectively (Table 6.4). The largest contribution at 250 m in both Sóller and Cabrera was due to the euphausiids, and at 650 in Cabrera was due to the decapods, because of their high abundances in these stations (Table 6.1).

Tabla 6.4: Carbon demand (μg C d^{-1} m^{-2}) ± SE estimate from abundance data in Sóller and Cabrera at 250 m, 650 m and 850 m in the summer of 2010 and mean ETS activity in decapods, mysids and euphausiids. Percentage of mean annual primary production (PP) was calculated from Bosc et al. (2004).

Locality	Depth	Decapods	Euphausiids	Mysids	Total	% of PP
Cabrera	250	0.14±0.14	20.30±10.15	0.04±0.04	20.48±10.33	0.012
	650	28.07±20.31	6.59±1.42	7.18±1.94	41.84±23.67	0.025
	850	6.16±1.10	3.27±0.87	1.17±0.11	10.60±2.28	0.006
Sóller	250	0.29 ±0.15	119.03±78.72	0.38±0.38	119.70±79.25	0.073
	650	3.99 ±2.74	8.78±4.54	6.33±1.68	19.10±8.95	0.009
	850	6.38 ±3.54	4.63±2.25	2.31±1.26	13.32±7.06	0.007

6.4. Discussion

This study provides comparative data on respiratory metabolism of three representative groups of suprabenthos crustaceans in intermediate waters off the Balearic Islands. Calculation of respiratory activity based in the ETS method, used here, has broadly been reported for natural zooplankton communities (King and Packard, 1975; King et al., 1978; Owens and King, 1975; Bämsted, 1980; Schalk, 1988; Minutoli and Guglielmo, 2009; Hirch et al., 2009), but rarely on suprabenthos assemblages. The results demonstrate the effectiveness of this approach in estimating oxygen consumption, in providing an index of physiological state of organisms, in detect spatial changes

due to different environmental conditions, and in demonstrating the possible implications in carbon flux research (Schalk, 1988; Hernández-León et al., 2001; Hirch et al., 2009; Minutoli and Guglielmo, 2009).

Respiration rates were related to body size (figure 6.2), so one can deduce differences in ETS activity per individual among species or groups depending on their average size (Ikeda, 1985; Ivleva, 1980). The widely studied decapod *Gennadas elegans* and *Sergestes arcticus* had an average adult body length of 40 mm and 45 mm, respectively (Zariquiey Alvarez, 1968). These shrimp showed the highest values of ETS, coherent with the high values of proteins biomass. *Pleisonika sp.* is a genus of decapods which can attain a size of 50-70 mm (Zariquiey Alvarez, 1968), but the individuals caught in the study were small, and had correspondingly lower amounts of protein. This can explain the relatively low ETS activity in this decapod. The species of euphausiids and mysids analyzed in this study had similar body lengths of 20-40 mm (Tattersall and Tattersall, 1951; Brinton et al., 2000) and their ETS activities were lower than *Gennadas elegans* and *Sergestes arcticus*, reflecting their lower biomass (Table 6.2). We found a good correlation between ETS and protein biomass (Figure 6.2) and can conclude that ETS is a good index of living biomass, the biomass that has enzymatic activity. This confirms the results found by other authors (Packard et al., 1983; Packard and Gómez, 2008; Martínez, 2007; Gómez et al., 2008).

The specific ETS activity (μl O_2 h^{-1} mg $prot^{-1}$) is really the parameter that enables one to compare potential respiration rates in organisms of different sizes. However, in this study inter-specific differences were not detected in specific ETS. Probably, the analyzed species have similar life habits and swimming activity, that are known to be important factors affecting variability in respiration rates (Torres and Childress, 1983; Ikeda, 1985; Cowles and Childress, 1988). The studied species have similar feeding habits, detritus feeding and omnivorous species, that actively filtrated particulate organic matter, but can also feed on plankton (phyto and zooplankton). For example, *Boreomysis arctica* feed higher quantities of copepods (Polunin et al., 2001). The diet of *G. elegans* included a high proportion of green detritus which probably originated from radiolaria, whereas *E. unguiculata* food consisted mainly of small copepods and celenterates in the North East Atlantic (Roe, 1984).

Comparing respiration rates with literature data is difficult, because of the differences in methodologies and environmental conditions. For example, Herrera et al. (2011b) measured potential respiration rates, normalized by protein biomass under laboratory condition at 20.5°C for the mysid *Leptomysis lingvura*, and found that they ranged from 30.1 to 42.6 μl O_2 h^{-1} mg $prot^{-1}$. ETS activities in epipelagic zooplankton collected around Mallorca averaged 17.45±1.64 μl O_2 h^{-1} mg $prot^{-1}$ (Herrera et al., present issue); and Minutoli and Guglielmo (2009) found that ETS activities in Western Mediterranean region averaged 0.026±0.001 μl O_2 h^{-1} mg wet $weight^{-1}$. For sure, because of the temperature difference the ETS here will be lower compared to the measurements made by Herrera et al. (2011b) but specific respiration in the mesopelagic oceans is always slower than in the epipelagic ocean (Torres et al., 1979; Childress, 1975; Cowles and Childress, 1988; Mahaut et al., 1995). Nevertheless, ETS activities here are in the range of values predicted from zooplankton studies in the region by Herrera et al. (submitted) and Minutoli and Guglielmo (2009).

Spatial differences were found in specific ETS activities between the two locations, Sóller and Cabrera. They are separated by only 60 kms but are located in different sub-basins with different oceanographic conditions (EUROMODEL Group, 1995). Sóller is more influenced by atmospheric forcing and by cold, saline Mediterranean waters, while Cabrera is more influenced by forcing due to the density gradients and receives warmer and less saline Atlantic waters (Pinot et al., 2002). Neither the difference in O_2 (0.21 $ml.L^{-1}$) or the difference in temperature (0.04°C) can explain the difference in specific ETS between the two areas.

No significant difference was found in specific ETS between depths, but a decreasing trend with depth in Cabrera and increasing one in Sóller was found. Other authors found, in studies of zooplankton, that specific ETS activity changes between areas or decreases with depth is related to the temperature and also to the limited availability of food for zooplankton in deep waters (Schalk, 1988; Hirch et al., 2009). In deeper suprabenthos food availability may increase at greater depths, depending on the to nature of the sediments and their trophic conditions (%OM, potential REDOX). This is in contrast to the zooplankton that depend almost exclusively on Chl from

the surface (Cartes et al., 2008). Studies in the area shows that the percent of OM increases with depth from the shelf-slope break (1.6-4.5 %) to bathyal stations (2.5-9 %), and that increment generally paralleled the increase in the proportion of mud in the sediments (Cartes et al., 2008). However no conclusive evidence at present has indicated increased nutrient availability in the Algerian or Balearic sub-basin.

Possible indicators of food availability for suprabenthos can be the percentage, content and quality of organic matter, and the availability of phyto and zooplankton in the water column. In this sense, the percentage of OM was similar in both areas (Cartes et al., 2011), but at Sóller, the more energetic environment may induce higher settling velocity of particles and thus lower degradation of OM (Pasqual et al., submitted). Therefore, higher nutritional value of OM associated with fresh marine organic matter inputs was found in this area, where primary production may be induced by the occurrence of stronger frontal systems linked to Northern and Balearic currents flowing along the slope (López-Jurado et al., 2008). However, the sediments in Cabrera showed higher contents of lipids and carbohydrates, whereas the proportion of protein was lower (indicative of higher degradation of organic matter) (Pasqual et al., present issue).

The Algerian basin is subject to more unpredictable events such as eddies generated by entry of Atlantic waters through the Straits of Gibraltar (López-Jurado et al., 2008). Punctual inputs of fresh marine organic matter during phytoplankton blooms during spring can explain the better condition of suprabenthos at summer in Cabrera. In the upper water column, differences in the mesozooplankton biomass and carbon and nitrogen content has been observed between the water masses in Sóller and in Cabrera affecting the trophic pattern in fish larvae (Laiz-Carrión et al., 2013). Herrera et al. (present issue) found higher zooplankton biomass and ETS activity in Cabrera than in Sóller in studies carried in summer 2010. Also, high productivity events (Estrada, 1996; Bosc et al., 2004) induced by oceanographic or current events may contribute to increased zooplankton biomass (Cartes et al., 2008). Cartes et al. (2011) concluded that secondary production for overall suprabenthos was similar in both areas, but on the upper slope at Sóller (350-450 m), the increase of natural disturbance in the area, increasing P/B and diversity of suprabenthic peracarids. Moreover, the production may depend, further than

the scale adopted, on the trophic levels under analysis, and the proximity of target taxa to the primary food sources that they exploit. However, diverse studies evidences better conditions of top predators inhabiting the Balearic sub-basin (Sóller), evidenced by higher food consumption, energy content of diets and fecundity (i.e. *Merluccius merluccius*: (Cartes et al., 2008; Hidalgo et al., 2008); *Aristeus antennatus*: (Cartes et al., 2009; Guijarro et al., 2008)).

Respiration rates are generally expressed as power functions of body size according to the equation: $R = a\ W^b$ (Prosser and Brown, 1961). This so called Kleiber's law (Kleiber, 1961) established a value of 0.75 for the b exponent in this equation. However, other authors found that this value of 0.75 is highly variable (Glazier, 2005, 2006), in fish larvae b values can range from 0.65 to 1.69 (Giguere et al., 1988). Herrera et al. (2011b) found that in mysids this is related to feeding conditions. Gómez et al. (2008) suggested that this exponent is less than 0.75 in oligotrophic regions and greater than or equal to 0.75 in regions with higher food availability such as coastal or upwelling regions. Others have found similar relationships related to food availability in cultures (Martínez et al., 2010; Herrera et al., 2011b). This sensitivity of the exponent in Kleiber's law suggests that the biomass-ETS could be used as an indicator of physiological state of individual organisms and ecological communities, or in other words, an index of what proportion of the biomass of organism is being used for the production of energy.

The ETS activity measures potential respiration (the maximum oxygen consumption under substrate saturating conditions). It is not directly related to food intake or associated processes that increase respiration rates like SDA (Specific Dynamic Action) (Kiorboe et al., 1985; Thor, 2003). ETS, being a constituent part of the mitochondria, should not change rapidly with environmental conditions or with the amount of metabolizable substrate, as does respiration (Herrera et al., 2011b). Changes in ETS has more to do with long-term processes such as the production of structural proteins and more respiratory complexes related to metabolic process.

In our results, the exponent b was 0.93 in Cabrera and 0.60 in Sóller. This difference in the relationship between biomass and ETS activity suggests a better physiological state of the organisms in Cabrera. Other authors suggested that this may be due to greater food availability (Gómez et al., 2008;

Martínez et al., 2010; Herrera et al., 2011b), unfortunately, as mentioned above, there are no data from the region to support this hypothesis.

The decapods, euphausiids and mysids comprised 56 % of the total in the suprabenthos samples. As heterotrophs, these groups require organic carbon for metabolism, growth and reproduction. From the abundances and ETS activities for each group one can calculate the potential carbon demand in these suprabenthic groups due the respiration. These estimates, although they have an error associated with a seasonally varying uncertainty in the biomass of organisms and the abundances enable us to quantify the carbon, consumed annually by these taxons, due the respiration, and to determine their importance in the carbon cycle. The resulting annual mean estimates of total carbon demand summarizing the value of suprabenthic mysids, decapods and euphausiids, was in Cabrera, 7.48±3.77, 15.27±8.64 and 3.87±0.76 mg C yr^{-1} m^{-2} at 250, 650 and 850 m respectively, while in Sóller it was 43.69±28.93, 6.97±3.27 and 4.86±2.58 mg C yr^{-1} m^{-2} at 250, 650 and 850 m respectively (Table 6.4). These values represent a mean of 0.03 % of the total primary production in Sóller and 0.015 % in Cabrera according to primary production data obtained by Bosc et al. (2004) from the 4-year SeaWiFS time series in the area between 1998-2001.

6.5. Conclusions

1. The use of the ETS technique facilitated estimations of the suprabenthos respiratory activity and the detection of the physiological changes in the organisms between the two areas.

2. ETS activity is a good index of living biomass.

3. Specific ETS activity in decapods, mysids and euphausiids show not differences.

4. The ETS-biomass ratio showed significant differences between Cabrera and Sóller which suggests that organisms are in a better physiological state in Cabrera than in Sóller.

5. The average annual carbon demand for suprabenthic mysids, decapods

and euphausiids represents 0.03 % of the total primary productivity in Sóller, and 0.015 % in Cabrera.

Acknowledgements

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Capítulo 7

Application of ETS analysis in the calculation of carbon demand and the role of zooplankton in the Balearic coastal ecosystem

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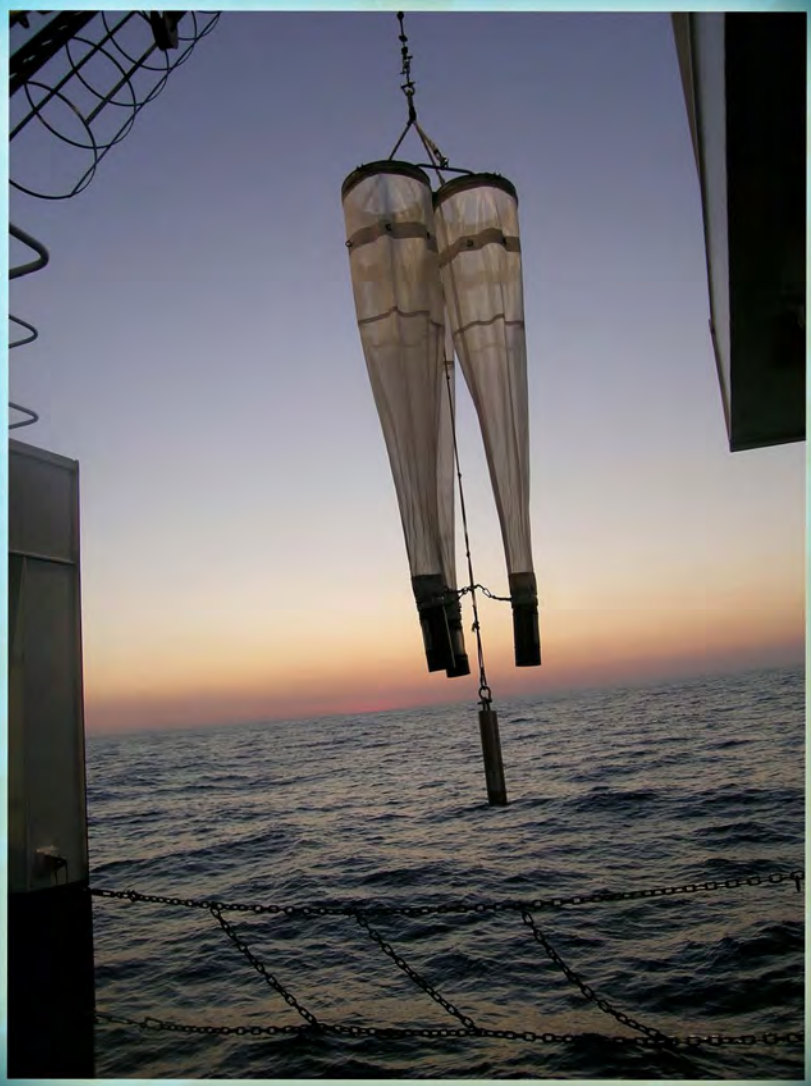

Capítulo 7

Application of ETS analysis in the calculation of carbon demand and the role of zooplankton in the Balearic coastal ecosystem

Abstract: Measuring electron transport system (ETS) activity in zooplankton provides an index of respiration, theoretically, the potential respiration rate. It could be also an index of the vitality (the health) of the plankton as well as it's living biomass. The measurement is largely based on the rate-limiting step (Complex 1) and thus should register the maximum level of electron flow through the respiratory electron transport system. This enzyme system is one of the most ancient and perhaps the most widepread of all enzyme systems on the planet (Lane, 2005). From its measurements we can calculate zooplankton carbon demand to elucidate the role of the zooplankton in the carbon cycle in the epipelagic zone.

In an oligotrophic Mediterranean region near the Balearic Islands we carried out a study of the zooplankton community in the upper 200 meters of the water column. We focused on two areas with different oceanographic conditions: the Balearic and Algerian sub-basins. We compared the biomass, ETS activity and specific ETS activity of different size fractions (53-200, 200-500, $>500\mu$m) in both areas. In both regions the largest contribution to respiration is found in the larger sizes. The specific respiration (per unit biomass) is greater in smaller fractions, indicating that they have a more active metabolism. Both

biomass and ETS activity increased in the region of Cabrera (Algerian sub-basin) and for both regions biomass and ETS activity is greater in shallow waters (200 m)
Using Kleiber's law as a tool to investigate these relationships, we found that the coefficient b was less than 0.75, indicating that the respiration was depressed (shifted down). In cultures and in eutrophic ocean waters (upwelling areas) b normally is greater than 0.75, consequently we intuit that the low value of b over the Balearic and Algerian sub-basins may indicate that the zooplankton are not well fed and that they are living under oligotrophic stress.

7.1. Introduction

Zooplankton play an important role in carbon transfer and vertical flux in the oceans. They are part of the "biological pump" because they transfer carbon fixed by phytoplankton through the food web to deeper levels of the ocean (Longhurst and Harrison, 1989). For this reason zooplankton respiration has been widely studied in recent decades by the scientific community (Conover, 1960; Childress, 1968; Ikeda, 1970; Packard, 1971; Packard et al., 1974; King and Packard, 1975; Bämsted, 1980; Hernández-León and Gómez, 1996; Gómez et al., 1996; Del Giorgio and Williams, 2005; Mayzaud et al., 2005; Hernández-León and Ikeda, 2005; Packard and Gómez, 2013). However, *in situ* measurements of zooplankton respiration remains difficult to carry out in practice. Capturing a natural zooplankton community and isolating it in good physiological condition in a simulated *in situ* environment long enough to make a physiological and ecologically meaningful respiration measurement, challenges the possible. Hence, the drive to develop biochemical indices of respiration. The activity of the respiratory electron transport system (ETS), theoretically measures potential respiration and since the early work of King and Packard (1975) and Finlay et al. (1983) its relationship with respiration has been shown to be better than the Kleiber relationship between respiration and biomass (Packard and Gómez, 2008). Accordingly, we can use ETS activity to calculate respiration thereby reducing the problems associated with incubation of zooplankton in a controlled environment (Gómez, 1991).

Oxygen is the final electron acceptor of the aerobic respiratory electron transport system. The ETS method (Packard et al., 1971, 1974) saturates the mitochondrial ETS with NADH and succinate, and ETS microsomal with NADPH. It then uses a tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloryde (INT), often called tetrazolium violet, as the electron acceptor. The reaction generates the reduced tetrazolium which is, in the parlance of organic chemistry, a formazan. In this process 1 mole of O_2 consumed is equivalent, stoichiometrically, to 2 mol of INT reduced and 2 mol of formazan produced. The formazan is measured spectrophotometrically at 490 nm. This is a measurement of the ability of mitochondria and microsomes to transfer, physiologically, electrons from substrates (NADH, NADPH and succinate) to a final electron acceptor. It is the respiratory capacity and in the parlance of enzymology, the maximum velocity of the ETS, its V_{max}.

Respiratory O_2 consumption is affected by several factors that do not affect the V_{max} of the respiratory ETS. Among these short-term factors are nutritional level, activity level, and behavioral shifts. They can change respiration at the physiological level, but they do not change the potential respiration at the biochemical level. The potential respiration is determined by the concentration of the enzyme complex NADH dehydrogenase-lipoprotein in the inner membrane of mitochondria. This enzyme complex is constitutive and therefore part of the mitochondrial machinery. This V_{max} does not change rapidly with changing external nutritional conditions, behavior, or the amount of substrate metabolized. Indeed, it is a permanent characteristic of the cell and as such varies along with variations in carbon, nitrogen, protein and total biomass of the cell. However because this V_{max} is a property of organic catalysts it will vary with the temperature, pressure, pH, and ionic strength of the surrounding chemical and physical fields. Furthermore, because this V_{max} is the respiratory potential, it and the respiration rate will move in parallel with these four factors.

In the long term, the relationship between biomass and ETS activity may be affected by conditions such as nutritional state. Other authors have found that the ETS-biomass exponential relation coefficient (b) is close to or above 0.75 in organisms that are well fed, while it is lower in organisms found in oligotrophic conditions (Martínez, 2007; Gómez et al., 2008; Packard and Gómez, 2008; Herrera et al., 2011b). Here, we investigate this ratio for zoo-

plankton in the Balearic Sea.

The Balearic archipelago is located in the boundary area between the northern, more saline and colder waters, originated in the nearby Gulf of Lion, and the southern, less saline and warmer oligotrophic waters of the Algerian basin, where the Balearic channels control the meridional mass transport and fluxes of the water masses (Pinot et al., 2002). Those authors indicate that through the Mallorca channel, severe winters are associated with an increased inflow of northern waters, whereas milder winters result in a higher northward flow of recent Atlantic water and can be related with the zooplankton biomass and its biodiversity (Fernández de Puelles et al., 2007, 2009). In relation to that, other biological studies in the same area showed, that whether seasonal or interannual variability was investigated, the distribution of the zooplankton communities were strongly influenced by the inflow/outflow of those waters (Fernández de Puelles et al., 2003, 2004).

Temporal studies during a decade (1994-2003) found out that hydrographic changes were reflected in the planktonic distribution and linked to large atmospheric factors (Fernández de Puelles et al., 2004). Accordingly, due to the proximity of the Atlantic, the Mallorca channel could be considered as a suitable place for a long-term studies to observe zooplankton changes in relation to large-scale climatic fluctuations (Fernández de Puelles and Molinero, 2007; Fernández de Puelles and Molinero, 2008). This study focuses on the zooplankton ETS activity in three size fractions in the upper 200 meters of the water column in these two Water Masses.

7.2. Material and Methods

7.2.1. Study area and sampling methods

Samples were collected during the oceanographic cruise IDEADOS 0710 (11 to 29 of July 2010) in the western Mediterranean Sea off the northwest and southwest of Mallorca, the largest of the Balearic Islands. The sample sites were on the slope of the Balearic sub-basin and the Algerian sub-basin respectively over a range of depths from 200 and 900m (Figure 7.1). The zooplankton collecting was performed by vertical net hauls being raised at

a speed of 1m s^{-1}. The microzooplankton (53-200μm) were sampled with a Calvet net of 53μm mesh net. The mesozooplankton were captured with WP2 200μm after which the haul was divided in fractions of 200-500μm and >500μm. The samples were taken at different times of day (morning, midday, afternoon and midnight) at each station. They were immediately frozen in liquid nitrogen at -196℃ and stored at -80℃ until ETS activity and protein analyses could be performed.

7.2.2. Hydrographic data

Hydrographic data was obtained with a SBE-911 CTD recorder operating at a sampling rate of 24Hz, deployed at an average speed of less than 1 m s^{-1}. The CTD transects were separated by 4.5 nmi, with stations placed 2.8 nmi apart at the Sóller ground, in the north, and 5nmi apart at the Cabrera ground, in order to reach the 1000 depth isobath in each case, hydrographic parameters (salinity, potential temperature, dissolved oxygen, turbidity, fluorescence and Photosythetically Available Radiation (PAR)) were processed using the Sea Bird Electronic Data Processing routines and according to the standard protocols developed for IEO data analysis (López-Jurado et al., 1995). Salinity and oxygen were calibrated on board using standard salinity bottles and winkler method, respectively. Temperature and salinity was considered accurate within 0.005°C and 0.003 units respectively.

7.2.3. ETS analysis

Potential respiration was estimated according to ETS method (Packard et al., 1971, 1974) with modifcations (Owens and King, 1975; Gómez et al., 1996; Packard and Christensen, 2004). Samples were sonified with an ultrasonic processor (Sonic Vibra-Cell, Model VCX130, USA) for 45 seconds in 1.5 ml of Milli-Q double-distilled water, then centrifuged for 10 minutes at 4000 rpm at 0℃. A 0.5 ml aliquot of the supernatant was added to a 1.5 ml of solution containing the substrates (0.2 % (v / v) Triton X-100. Samples were incubated at 18°C for 20 minutes after which the reaction was stopped with a quench solution consisting of 50 % phosphoric acid (0.1M) and 50 % of formaldehyde (36 %). The absorbance reading was performed in a spectrophotometer (Beckman DU 650, USA) at 490 nm and 750 nm to co-

rrect for turbidity. ETS activity was calculated for *in situ* temperature using Arrenhius equation and activation energy of 15 kcal mol^{-1} (Packard et al., 1975). The rate of potential oxygen consumption (ETS activity) became carbon demand rate assuming an RQ of 0.85 (King et al., 1978). Biomass was estimated in mg of protein by the method of Lowry et al. (1951), as amended by Rutter (1967).

7.2.4. Statistical analysis

The data were analyzed using the program R Development Core Team 2010 (R Foundation for Statistical Computing,Vienna, Austria). To confirm normality, ETS activity, biomass and specific ETS activity data were analyzed by the Shapiro Wilk test and the homoscedasticity of the residuals was assessed graphically. ETS, biomass and specific ETS activity data were not normal and statistical differences between stations, times of day and fractions were tested using Kruskal-Wallis test; and between regions of Cabrera and Sóller were assessed using Wilcoxon Mann-Whitney test. To study the correlation between ETS activity-biomass we obtain the regression equations, using a confidence limits of 95 % and the Pearson correlation coefficient.

7.3. Results

7.3.1. Hydrographic data

In both sampling sites the water column was stratified with a thermocline between 20 and 40 m in which the temperature dropped from 26°C to 14°C (Figure 7.2). Surface chlorophyll a values ranged from 0.06 to 0.15 mg m^{-3} in Sóller and from 0.03 to 0.10 mg m^{-3} in Cabrera (Figure 7.3), the deep chlorophyll maximum (DCM) was at 50-70 m depth with values between 0.20 to 0.50 mg m^{-3} in Sóller, and in Cabrera the DCM was between 60-80 m with values between 0.21 to 0.27 mg m^{-3}.

7.3.2. Zooplankton biomass and ETS activity

ETS activity, biomass and carbon demand were estimated for the different size fractions (53-200, 200-500,$>$500μm) at different times of day (morning,

midday, afternoon and midnight) at each station as shown in tables 7.1, 7.2 and 7.3.
No significant differences at different times of day were observed in ETS activity ($p>0.05$) and biomass ($p>0.05$) as shown in figure 7.4.

Significant differences between Cabrera and Sóller in ETS activity m^{-3} ($p<0.01$) for all size of zooplankton as shown in figure 7.5A and in total biomass (mg protein m^{-3}) ($p<0.01$) as shown in figure 7.5B being higher in the area of Cabrera. Both variables are higher in the Cabrera region. Furthermore in Cabrera, ETS activity is greater in the shallower stations (200 m) ($p<0.05$). The biomass is also greater in the 200m stations, but the difference is not significant ($p>0.05$). In the Sóller region, no significant differences were observed in ETS activity ($p>0.05$) and in the biomass ($p>0.05$) in the different depth zones shown in figure 7.6.

In the Sóller region the contribution of ETS activity m^{-3} appears greater in the larger sizes, but not significant different ($p>0.05$). However, if we analyze the ETS activity per unit of biomass (specific ETS) in smaller fractions it is significant greater ($p<0.01$) (Figures 7.7A and B). In Cabrera the greatest contribution to the potential respiration per m^{-3} is done by the fraction 200-500μm but not significantly different than the other fractions ($p>0.05$), while specific ETS activity was higher in fractions of smaller size ($p<0.01$)(Figures 7.8A and B).

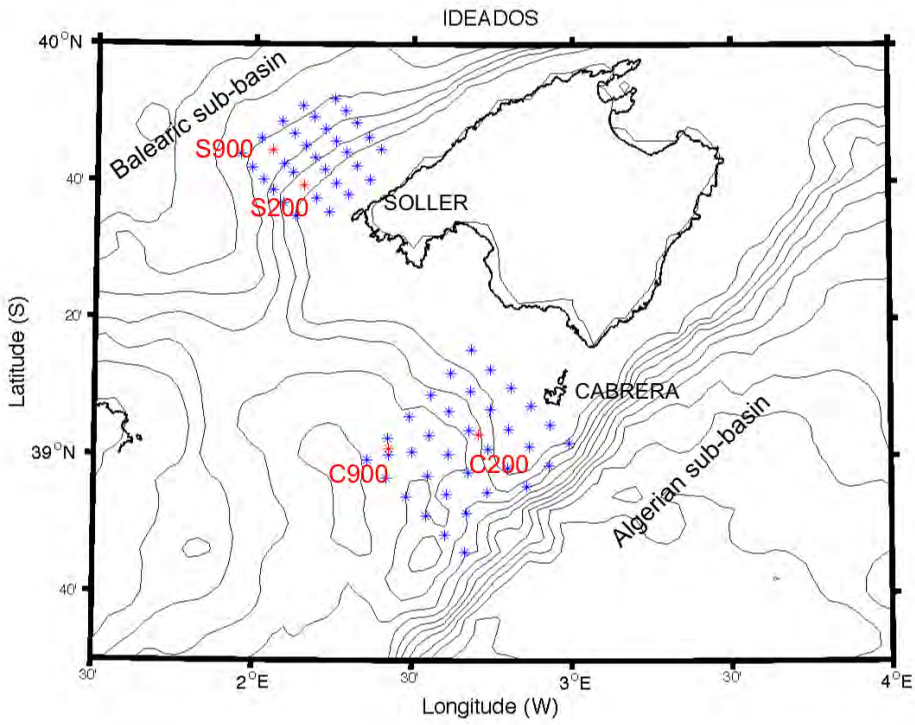

Figura 7.1: Map of study area, indicating hydrographic stations in blue and zooplankton sampling stations in red. S200 and S900 were located in Balearic sub-basin (Sóller) at 200 and 900m depth respectively and C200 and C900 in Algerian sub-basin (Cabrera) at 200 and 900m respectively.

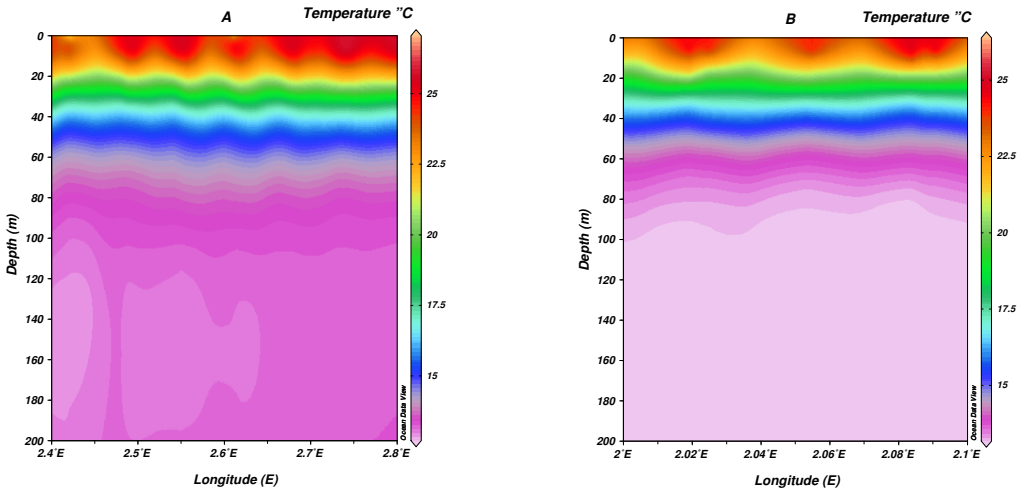

Figura 7.2: Temperature profiles showing the thermocline in A: Cabrera and B: Sóller.

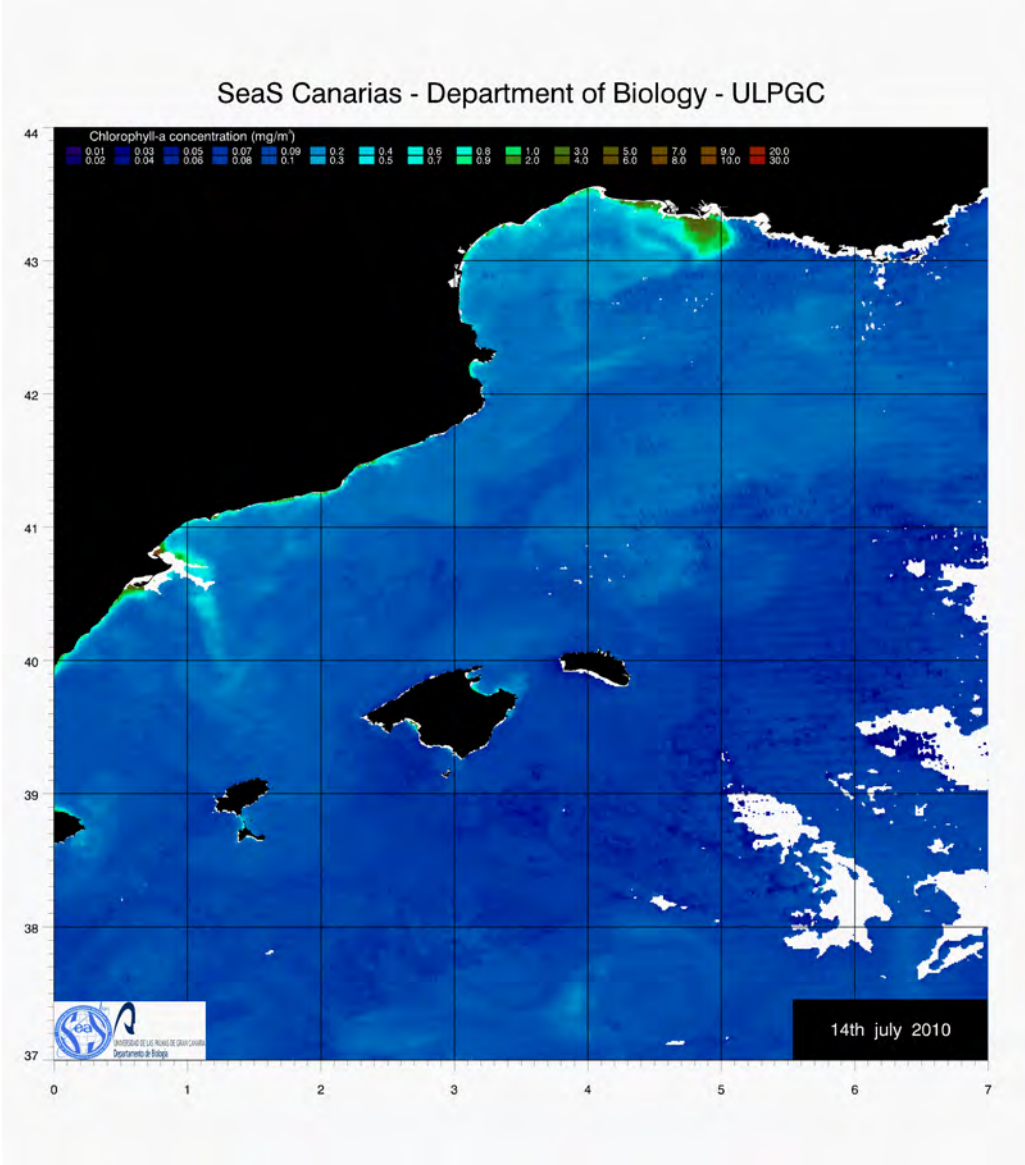

Figura 7.3: Satellite image of chlorophyll a surface concentration (mg m^{-3}) at the beginning of the sampling.

Tabla 7.1: Biomass, ETS activity and carbon demand for the zooplankton 53-100 μm size

Station	Sampling time	ETS activity (μmol O_2 h^{-1} m^{-3})	Biomass (mg prot m^{-3})	sp. ETS activity (μmol O_2 h^{-1} mg $prot^{-1}$)	Carbon demand (μmol C h^{-1} mg $prot^{-1}$)
S200	Midday	0.12	0.15	0.82	0.70
S200	Midnight	0.14	0.10	1.38	1.17
S200	Morning	0.11	0.09	1.14	0.97
S200	Midday	0.10	0.12	0.84	0.71
S200	Afternoon	0.11	0.04	2.78	2.37
S200	Midnight	0.07	0.08	0.97	0.82
S900	Morning	0.07	0.20	0.36	0.31
S900	Midday	0.09	0.09	1.01	0.86
S900	Midnight	0.14	0.17	0.84	0.71
S900	Afternoon	0.10	0.09	1.15	0.98
C200	Midday	0.28	0.16	1.77	1.51
C200	Midnight	0.20	0.13	1.52	1.29
C200	Midday	0.10	0.14	0.67	0.57
C200	Midnight	0.30	0.60	0.51	0.43
C200	Morning	0.11	0.04	2.92	2.48
C200	Midday	0.17	0.30	0.58	0.49
C900	Midnight	0.25	0.14	1.79	1.53
C900	Midday	0.10	0.07	1.48	1.25
C900	Afternoon	0.26	0.18	1.44	1.23
C900	Morning	0.25	0.54	0.46	0.39
C900	Midday	0.19	0.76	0.25	0.22

Tabla 7.2: Biomass, ETS activity and carbon demand for the zooplankton 200-500 μm size

Station	Sampling time	ETS activity (μmol O_2 h^{-1} m^{-3})	Biomass (mg prot m^{-3})	sp. ETS activity (μmol O_2 h^{-1} mg $prot^{-1}$)	Carbon demand (μmol C h^{-1} mg $prot^{-1}$)
S200	Midday	0.09	0.20	0.44	0.38
S200	Midnight	0.23	0.27	0.87	0.74
S200	Morning	0.15	0.19	0.78	0.66
S200	Midday	0.14	0.15	0.93	0.79
S200	Afternoon	0.22	0.28	0.80	0.68
S200	Midnight	0.09	0.14	0.65	0.56
S900	Morning	0.06	0.02	2.59	2.20
S900	Midday	0.08	0.19	0.45	0.39
S900	MIdnight	0.21	0.27	0.75	0.64
S900	Midday	0.17	0.22	0.79	0.67
S900	Afternoon	0.17	0.29	0.59	0.50
C200	Midday	0.32	0.39	0.81	0.69
C200	Midnight	0.47	0.58	0.81	0.69
C200	Midday	0.24	0.51	0.47	0.40
C200	Midnight	0.50	1.09	0.46	0.39
C200	Morning	0.24	0.50	0.49	0.42
C200	Midday	0.44	0.83	0.54	0.46
C900	Midnight	0.16	0.29	0.56	0.48
C900	Midday	0.11	0.28	0.41	0.34
C900	Afternoon	0.29	0.47	0.63	0.53
C900	Morning	0.17	0.37	0.46	0.39
C900	Midday	0.21	0.22	0.96	0.81

Tabla 7.3: Biomass, ETS activity and carbon demand for the zooplankton >500 μm size

Station	Sampling time	ETS activity (μmol O_2 h^{-1} m^{-3})	Biomass (mg prot m^{-3})	sp. ETS activity (μmol O_2 h^{-1} mg $prot^{-1}$)	Carbon demand (μmol C h^{-1} mg $prot^{-1}$)
S200	Midday	0.20	0.50	0.40	0.34
S200	Midnight	0.27	0.81	0.33	0.28
S200	Morning	0.30	0.59	0.51	0.43
S200	Midday	0.13	0.19	0.68	0.57
S200	Afternoon	0.10	0.52	0.20	0.17
S200	Midnight	0.05	0.21	0.26	0.22
S200	Morning	0.17	0.12	1.36	1.16
S900	Morning	0.09	0.16	0.55	0.47
S900	Midday	0.04	0.14	0.30	0.26
S900	Midday	0.17	0.48	0.36	0.30
S900	Afternoon	0.19	0.76	0.25	0.21
C200	Midday	0.28	0.65	0.43	0.37
C200	Midnight	0.31	0.86	0.36	0.30
C200	Midday	0.30	1.09	0.28	0.24
C200	Midnight	0.14	0.60	0.24	0.20
C200	Morning	0.26	0.79	0.32	0.27
C200	Midday	0.17	0.54	0.32	0.27
C900	Midnight	0.26	0.96	0.27	0.23
C900	Midday	0.10	0.35	0.29	0.25
C900	Afternoon	0.15	0.43	0.35	0.30
C900	Morning	0.10	0.11	0.91	0.78

The relationship between ETS activity per m^{-3} and biomass (mg protein m^{-3}) expressed in logarithmic terms is represented by the equation:

$$log\ ETS = b\ log\ W + loga \tag{7.1}$$

or

$$ETS = aW^b \tag{7.2}$$

The figure 7.9 show the regression lines for different size fractions (53-200, 200-500, >500μm), the ETS activity increase with increase body mass, but the slope of regression is different for each sizes, see table7.4.

Tabla 7.4: Coefficients of regression equations of log weight (mg protein m^{-3}) and log ETS activity (μmol O_2 h^{-1} m^{-3}) and correlation coefficients (r^2) for different size fractions

Size	a	b	r^2	n
53-200μm	-1.29	0.35	0.37	21
200-500μm	-0.88	0.63	0.73	22
>500μm	-1.32	0.59	0.58	21

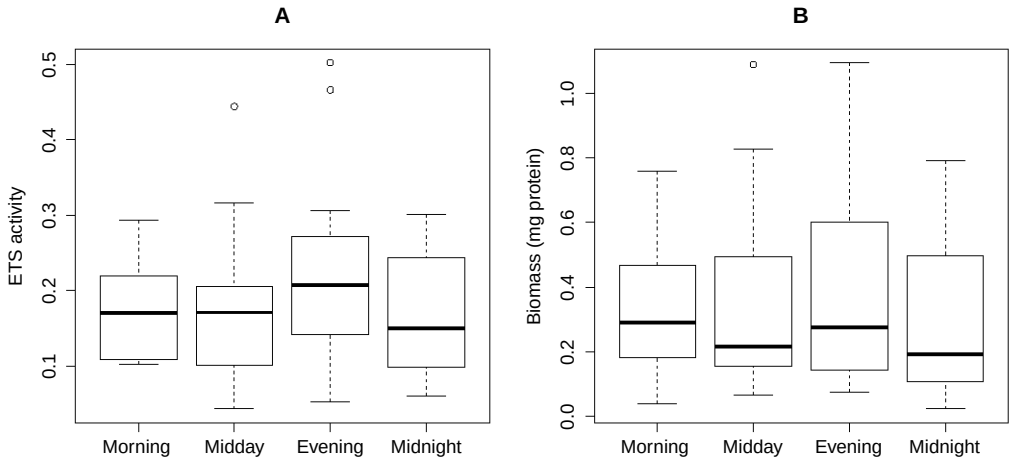

Figura 7.4: A: ETS activity (μmol O_2 h^{-1} m^{-3}) and B: biomass (mg protein m^{-3}); at different times of day.

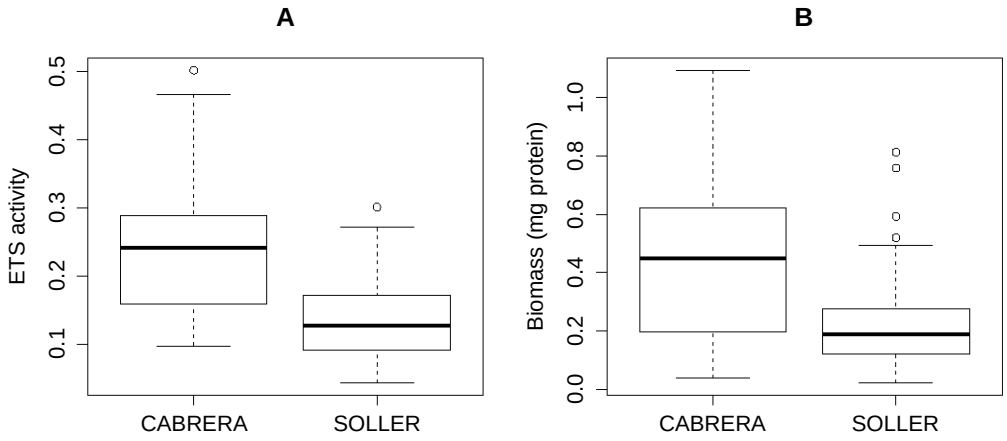

Figura 7.5: A: total zooplankton ETS activity (μmol O_2 h^{-1} m^{-3}) (average for all samples) and B: total zooplankton biomass (mg protein m^{-3}) (average for all samples); in Cabrera and Sóller.

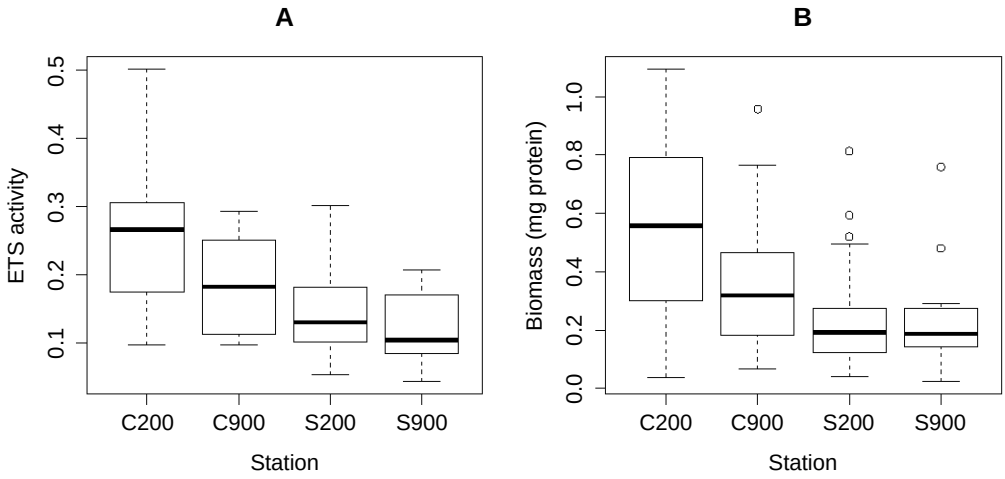

Figura 7.6: A: Total zooplankton ETS activity (μmol O_2 h^{-1} m^{-3}) and B: Biomass (mg protein m^{-3}); in each station at different depths.

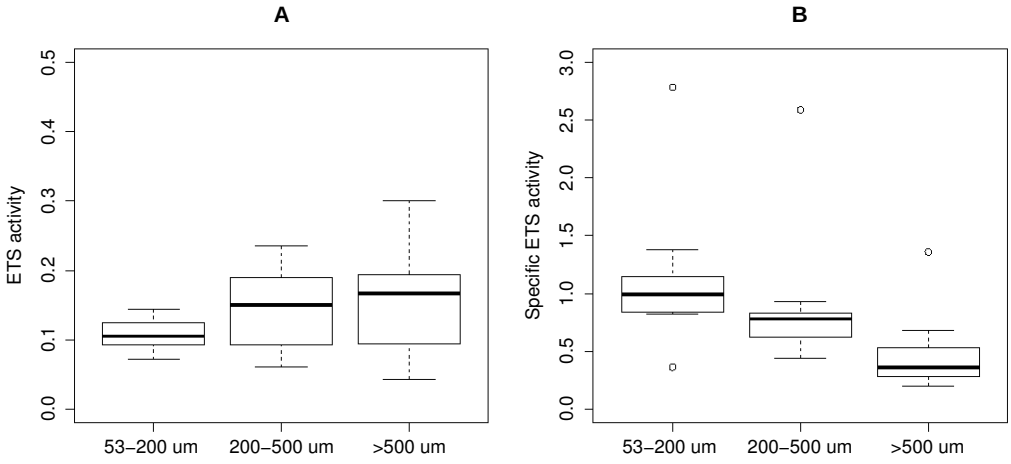

Figura 7.7: Sóller data. A: ETS activity (μmol O_2 h^{-1} m^{-3}) for the different size fractions (53-200, 200-500,>500μm); B: Specific ETS activity (μmol O_2 h^{-1} mg $prot^{-1}$) for the different size fractions (53-200, 200-500,>500μm).

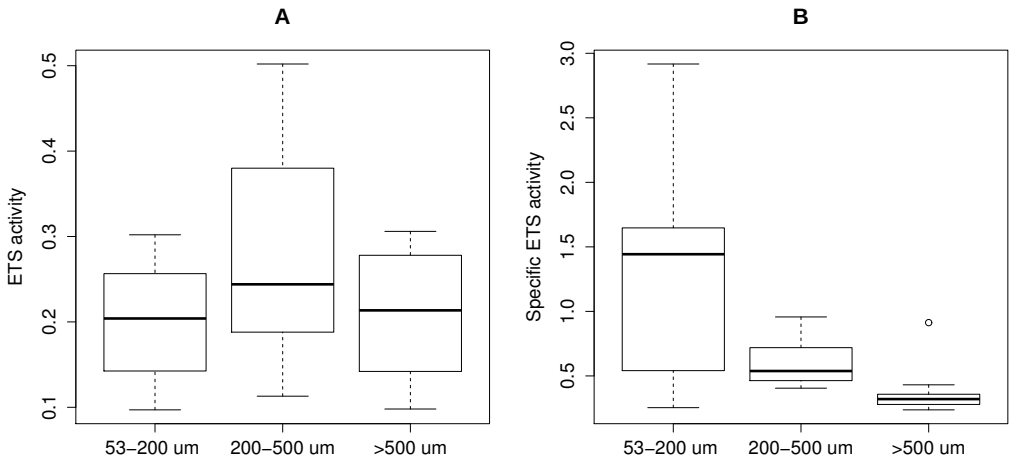

Figura 7.8: Cabrera data. A: ETS activity (mg protein m^{-3}) for the different size fractions (53-200, 200-500,>500μm); B: Specific ETS activity (μmol O_2 h^{-1} mg $prot^{-1}$) for the different size fractions (53-200,200-500,>500μ).

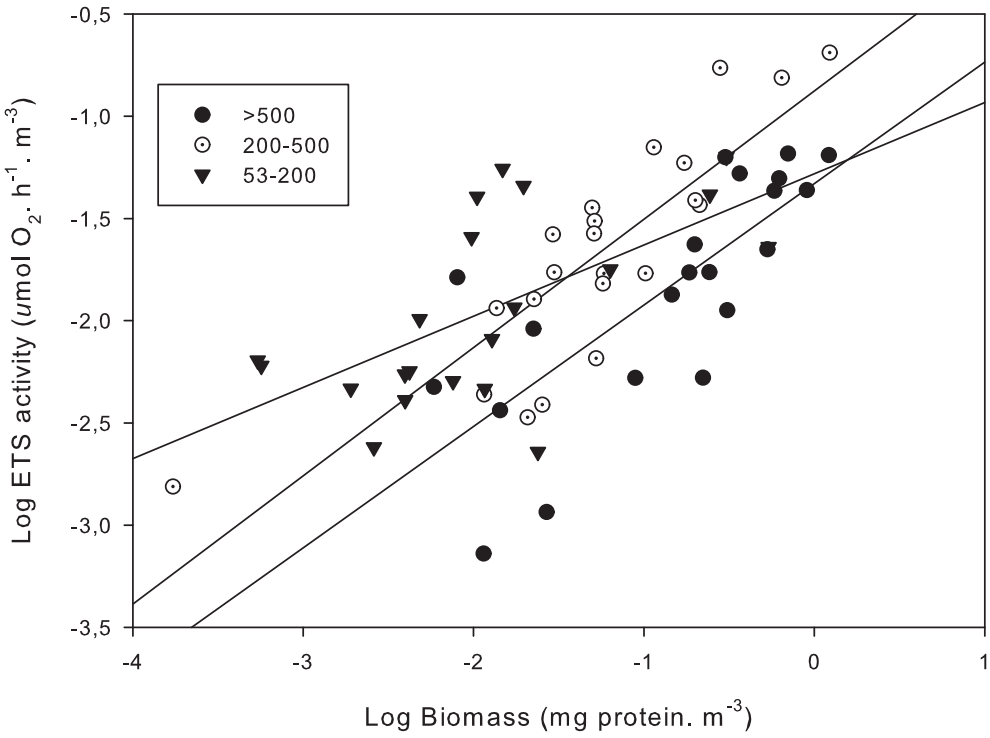

Figura 7.9: Relationship between ETS and biomass for different size fractions (53-200, 200-500,>500μm) around Balearic Island (western Mediterranean).

Tabla 7.5: Review of published data of biomass and ETS activity. ETS data were converted to μl O_2 h^{-1} m^{-3}, protein data were converted to dry weight using the dry weight-protein ratio of 5.2 (Postel et al., 2000). Carbon demand (μg C h^{-1} m^{-3})= ETS (μl O_2 h^{-1} m^{-3})* 0.85 *12/22.4 where 0.85 is the RQ (King et al., 1978) and 22.4 is the volume in μl of 1 μmol of O_2 and 12 is the weight of 1 mol of carbon.

Reference	Size (μm)	Region	st	Deep	Biomass (mg dry wt)	ETS activity (μl O_2 h^{-1} m^{-3})	sp. ETS activity (μl O_2 h^{-1} DW^{-1})	C demand (μg C h^{-1} m^{-3})
Present work	53-200	Western	S200	0-200	0.50	2.42	5.69	1.10
	53-200	Mediterranean	S900	0-200	0.72	2.30	3.62	1.05
	53-200		C200	0-200	1.19	4.38	5.72	1.99
	53-200		C900	0-200	1.76	4.71	4.68	2.15
	200-500		S200	0-200	1.08	3.48	3.21	1.59
	200-500		S900	0-200	1.03	3.11	4.46	1.41
	200-500		C200	0-200	3.37	8.25	2.57	3.76
	200-500		C900	0-200	1.68	4.23	2.59	1.93
	>500		S200	0-200	2.19	3.91	2.29	1.78
	>500		S900	0-200	2.00	2.76	1.58	1.26
	>500		C200	0-200	3.92	5.44	1.40	2.48
	>500		C900	0-200	2.40	3.42	1.97	1.56
King et al. (1978)	>212	Eastern tropical	2	0-83	2.86	15.54	5.44	7.08
	>212	North Pacific	6	0-79	4.97	37.34	7.51	17.00
	>212		10	0-44	4.75	27.73	5.84	12.63
	>212		14	0-78	4.18	26.15	6.26	11.91
	>212		18	0-79	8.34	64.05	7.68	29.17
	>212		20	0-68	30.74	257.35	8.37	117.19
	>212		25	0-70	14.43	126.71	8.78	57.70
	>212		29	0-65	31.08	238.46	7.67	108.59
	>212		33	0-65	20.46	152.92	7.47	69.63
	>212		34	0-65	27.38	108.00	3.94	49.18
	>212		37	0-65	22.15	139.08	6.28	63.33
	>212		40	0-78	10.10	34.74	3.44	15.82
	>212		44	0-78	7.95	44.10	5.55	20.08
	>212		46	0-81	9.25	60.62	6.56	27.60

Reference	Size (μm)	Region	st	Deep	Biomass (mg dry wt)	ETS activity (μl O_2 h^{-1} m^{-3})	sp. ETS activity (μl O_2 h^{-1} DW^{-1})	C demand (μg C h^{-1} m^{-3})
Packard (1979)	>102	Northwest Africa	30	0-200	26.00	80.08	3.08	36.47
	>102	upwelling	31	0-200	59.00	150.45	2.55	68.51
	>102		36	0-200	32.00	213.44	6.67	97.19
	>102		37	0-200	19.50	197.93	10.15	90.13
	>102		62	0-200	14.50	136.01	9.38	61.93
	>102		70	0-200	13.00	147.55	11.35	67.19
	>102		78	0-200	20.00	269.60	13.48	122.76
	>102		85	0-200	11.50	156.06	13.57	71.06
	>102		89	0-200	14.50	184.01	12.69	83.79
	>102		97	0-200	19.50	95.55	4.90	43.51
	>102		99	0-200	5.00	133.00	26.60	60.56
	>102		104	0-200	11.00	95.48	8.68	43.48
	>102		105	0-200	9.50	87.02	9.16	39.63
	>102		119	0-200	13.00	62.53	4.81	28.47
	>102		122	0-200	18.00	165.06	9.17	75.16
Minutoli and	>335	Western	VA	0-200	0.79	0.18	0.23	0.08
Gugliemo(2009)	>335	Mediterranean	V4B	0-200	1.22	0.42	0.34	0.19
	>335		V3B	0-200	0.64	0.23	0.35	0.10
	>335		V1A	0-200	0.90	0.20	0.23	0.09
	>335		V2	0-200	0.55	0.14	0.25	0.06
	>335	Eastern	V6	0-200	0.39	0.12	0.31	0.05
	>335	Mediterranean	V7	0-200	0.50	0.14	0.31	0.06
	>335		V8	0-200	0.26	0.08	0.32	0.04
	>335		VIERA	0-200	0.40	0.12	0.33	0.06
	>335		V10	0-200	1.01	0.35	0.36	0.16

7.4. Discussion

The average biomass is higher in Cabrera than Sóller and decrease in deeper areas. Other authors have studied the abundance of zooplankton in the area, although the diversity of methods and nets used make difficult the comparison.The study conducted by Fernández de Puelles et al. (2003) on seasonal and interannual variability at southern of Mallorca, during 1994-1999 in 3 stations at 75, 100 and 200 m depth using a Bongo net of 250μm, showed that the zooplankton decreases from the coast to the oceanic stations. During these 6 years the authors argue that the biomass of zooplankton were low, similar to other Western Mediterranean areas (Fernández de Puelles et al., 2003). There were also peaks in late spring in neritic and coastal areas, and peaks in winter in most oceanic areas. Other authors also show the importance of winter mixing that enable the entry of nutrients from deep zones to the surface, this fertilization of the euphotic zone increases primary and secondary production.

In previous studies in the area between 2003 and 2004, although it was difficult to identify regularities in the temporal dynamics of zooplankton, were observed biomass maximum peaks in late winter and summer (February and June), (Cartes et al., 2008). We have carried out the sampling in the summer when there is a marked stratification and nutrients have not upwelled from deeper waters.

ETS activity is higher in the Cabrera region. It represents potential respiration and is related to biomass, since it is controlled by constituent mitochondrial proteins and thus is a good living biomass predictor. However, it should be noted that although there is a good relationship between ETS activity and biomass, it is not a causal one, that is why higher zooplankton biomass in Cabrera is reflected in higher ETS activity. The biomass simply packages the enzyme complexes that constitute the ETS. It is the enzyme kinetics of these enzymes that is the causal basis of the ETS activity (Packard and Gómez, 2008). Note that here, as we speak of "ETS activity", we are referring to the in vitro ETS activity, the activity measured by our enzyme assay for ETS activity. This contrasts with the *in vivo* ETS activity which is the time-varying ETS activity that changes with food availability, hormonal levels, and swimming activity. It is the *in vivo* ETS activity of the whole

organism that is the respiration rate.

With regard to respiration and biomass, the relationship is also not causal and is even less coupled than the ETS-biomass relationship because of the influence of variables such as food availability, hormonal levels, and swimming activity. Herrera et al. (2011b) has shown food availability in mysids impacts the respiration rate but not the in vitro ETS activity. The relationship between biomass and ETS can be a good indicator of the physiological state of an organism. For many years the paradigm of "Kleiber's law" (Kleiber, 1961) has been accepted dogma in animal physiology. This "law" is based on an exponential relationship between biomass and respiration with a coefficient close to 0.75 or 3/4. Recently, the metabolic theory of ecology (MTE) was developed on the basis of Kleiber's law (Brown et al., 2004) and has been touted by Whitfield (2006) as biology's new unifying theory. However, more recently, many authors have found variations in Kleiber's coefficient (b) in different taxonomic groups (Glazier, 2005, 2006; Atanasov, 2010). These findings shed doubt on the reliability of this exponent and and argue that it is not a constant for all species and sizes of organisms as claimed.

Packard and Gómez (2008) argue that the MTE and Kleiber's law describe respiration over a large size range but not short periods of time or different physiological states; and propose the EKM based on potential respiration and availability of substrates, not in biomass. Aguiar-González et al. (2012) show this to be the case in experiments with marine bacteria. Field studies of zooplankton studies show different ratios close to or greater than 0.75 in optimal feeding conditions, and below 0.75 when the organisms are found in oligotrophic areas or poor feeding (Martínez, 2007; Gómez et al., 2008; Packard and Gómez, 2008; Herrera et al., 2011b). In this study, the coefficient b is lower than 0.75 in all sizes (CI 95 %) (see table 7.4) which suggests that the zooplankton are nutrient limited and that both areas (Cabrera and Sóller) are oligotrophic, this is consistent with the hydrographic data.

However, the slope of the regression equation in the smaller size (53-200μm) is significantly different from the other sizes, and has a lower correlation between ETS and biomass. One reason may be that this fraction has greater diversity of organisms including autotrophs, herbivores and omni-

vores. Conover (1960) showed that carnivorous zooplankton has higher respiratory rate than herbivorous; otherwise the nutritional conditions of the organisms also affects the slope of the log ETS-log biomass equation (Herrera et al., 2011b) which could indicate that this fraction is in worse feeding conditions. As expected total ETS activity generally increased, while specific ETS activity decreased with increasing body weight, this indicates that the small size fractions are metabolically more active, Ikeda (1970) found a similar correlations in studies in different groups of planktonic animals.

Comparing our biomass and ETS data with those published by other authors, the biomass values obtained are lower than in the tropical eastern North Pacific (King et al., 1978) and in areas of upwelling (Packard, 1979) which can be two orders of magnitude greater than those obtained in the Sóller stations. In contrast, compared with the values obtained by Minutoli and Guglielmo (2009) in different Mediterranean areas, the biomass is similar at some of our stations, but ETS values are lower, resulting in a lower specific ETS and lower carbon demand per cubic meter in these areas of the Mediterranean Sea (Table 7.6).

Considering carbon demands presented in table 7.6), it seems clear that Sóller and Cabrera are oligotrophic areas, however compared to the values presented by Minutoli and Guglielmo (2009) it seems that within the Mediterranean, the Balearic Sea is an area richer in nutrients and supports, via the food chain, a zooplankton energy demand that is up to 10 times higher in this area than outside. Frontal mesoscale events between Mediterranean waters and waters of Atlantic origin, and input of cold northern water into the channels (Pinot et al., 1995; Fernández de Puelles et al., 2004), could be act as fertilization mechanisms that increase productivity off the Balearic Islands.

Based on estimations of carbon demand, and assuming an error associated with a seasonally varying uncertainty, we can estimate the average annual carbon demand. Epipelagic zooplankton respiration, was 12.14 g C yr^{-1} m^{-2} in Cabrera, while in Sóller was 7.17 g C yr^{-1} m^{-2}. According to data of primary production (PP) obtained by Bosc et al. (2004) from the 4-year SeaWiFS time series over the 1998-2001 period, our values represent a 19.7 % of PP in Cabrera and 12 % in Sóller. Assuming that the study area

is a moderately productive (PP between 250-1000 mg C d^{-1} m^{-2}), carbon consumption by metazooplankton (>200 μm) is 22.2 % of the PP according to Calbet (2001). Of this carbon, an average of 25 % is loss by respiration, which means that 5.5 % of the PP is consumed due the respiration (Calbet, 2001). These estimates are consistent with the data obtained in this study using the ETS technique, if we consider that our carbon demand estimate is the "potential" carbon demand, and in this estimate is included the microzooplankton (53-200μm) demand.

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Conclusiones generales

Capítulo 2

1. A partir del estudio taxonómico y genético se han identificado seis especies de misidáceos asociados a *C. nodosa*: *Siriella armata* (Milne-Edwards, 1837), *Leptomysis* sp. aff *heterophila*, *Leptomysis lingvura* ssp., *Paramysis arenosa* (G.O. Sars, 1877), *Anchialina agilis* (G.O. Sars, 1877) y *Gastrosaccus roscoffensis* (Bacescu, 1970). Una de ellas, *L. lingvura lingvura* ssp., ha sido citada por primera vez en aguas de Gran Canaria.

2. *G. roscoffensis*, *Leptomysis lingvura* ssp. y *Leptomysis* sp. aff *heterophila* han sido secuenciados por primera vez y registrados en la base de datos de GenBank.

Capítulo 3

3. Se ha determinado que los misidáceos son el principal componente del suprabentos asociado a praderas de *C. nodosa* en la costa de Gran Canaria.

4. La comunidad de misidáceos mostró el mismo patrón de variabilidad estacional que *C. nodosa* y la ictiofauna asociada, siendo más abundantes en primavera que en otoño.

Capítulo 4

5. *Paramysis nouveli* y *Leptomysis lingvura* han demostrado su potencial como alimento vivo en acuicultura; los niveles de lípidos, ácidos grasos

y proteínas satisfacen los requerimientos para el cultivo de peces y cefalópodos según la FAO.

6. *Paramysis nouveli* y *Leptomysis lingvura* presentan porcentajes de DHA, EPA y ácido araquidónico (AA) superiores a los que presentan las presas vivas utilizadas tradicionalmente en acuicultura, por lo que podrían ser alimentos de mejor calidad para cultivos de peces ornamentales o para las primeras etapas del desarrollo de peces y cefalópodos.

Capítulo 5

7. En *Leptomysis lingvura* la actividad respiratoria se ve afectada por las horas de inanición, no así la actividad ETS, por lo cual la relación R/ϕ puede ser hasta 3 veces superior en organismos recién alimentados respecto a la de organismos en inanición.

8. La ley de Kleiber, que establece un coeficiente de 0.75 en la relación exponencial ETS-biomasa (ETS=a W^b) solo es aplicable en cultivos de *L. lingvura* que se encuentran en condiciones óptimas de alimentación. En misidáceos con déficit de alimento este coeficiente es inferior a 0.75.

Capítulo 6

9. El estudio de la relación ETS-biomasa en suprabentos colectado durante la campaña oceanográfica IDEADOS sugiere que los organismos se encuentran en mejores condiciones fisiológicas en Cabrera (subcuenca Argelina) que en Sóller (subcuenca Balear).

10. En base a los datos de abundancia y actividad ETS se estimó la demanda de carbono de los principales componentes del suprabentos, que representó un 0.03 % de la producción primaria total en Sóller y un 0.015 % en Cabrera.

Capítulo 7

11. El coeficiente b de la relación ETS-biomasa fue menor a 0.75 en todas las tallas de zooplancton, tanto en Sóller como en Cabrera, lo que sugiere que estos se encuentran en condiciones mínimas de alimento, esto

coincide con los datos hidrográficos que muestran que ambas zonas son oligotróficas.

12. En base a los datos de biomasa y de la actividad ETS se estimó una demanda anual de carbono por parte del zooplancton epipelágico de 12.14 g C en Sóller y de 7.17 g C en Cabrera. Estos valores representan un 19.7 % de la producción primaria en Cabrera y un 12 % en Sóller.

Bibliografía

Aguiar-González, B., Packard, T., Berdalet, E., Roy, S., Gómez, M., 2012. Respiration predicted from an enzyme kinetic model and the metabolic theory of ecology in two species of marine bacteria. Journal of Experimental Marine Biology and Ecology 412, 1–12.

Anderson, G., 2010a. Mysida classification. january 20, 2010. Available from http://peracarida.usm.edu/MysidaTaxa.pdf (accessed 10 may 2012) .

Anderson, G., 2010b. Peracarida taxa and literature (Cumacea, Lophogastrida, Mysida, Stygiomysida and Tanaidacea). Available from http://peracarida.usm.edu (accessed 10 may 2012) .

Ansell, A., Gibson, R., Barnes, M., Press, U., 1997. The hyperbenthos. Oceanography and Marine Biology: an Annual Review 35, 221–255.

AOAC, 1995. Official Methods of Analysis. AOAC. USA. Washington D.C., USA.

Arístegui, J., Montero, M., 1995. The relationship between community respiration and ETS activity in the ocean. Journal of plankton research 17, 1563–1571.

Atanasov, A., 2010. The change of power coefficent in metabolism-mass relationship across life's taxons during evolution: Prediction for mass-dependent metabolic model. Trakia Journal of Sciences 8, 12–24.

Bämsted, U., 1980. ETS activity as an estimator of respiratory rate of zooplankton populations. The significance of variations in an environmental factor. Journal of Experimental Marine Biology and Ecology 42, 267–283.

Barberá, C., Tuya, F., Boyra, A., Sánchez-Jerez, P., Blanch, I., Haroun, R., 2005. Spatial variation in the structural parameters of *Cymodocea nodosa* seagrass meadows in the Canary Islands: a multiscaled approach. Botanica Marina 48, 122–126.

Barberá-Cebrián, C., Ribeiro da Cunha, M., Sánchez Jerez, P., Ramos Esplá, A.A., 2001. Mysidacea associated with seagrass meadows off the southeast iberian coast. Boletin Instituto Español de Oceanografía 17, 97–106.

Barberá-Cebrián, C., Sánchez-Jerez, P., Ramos-Esplá, A.A., 2002. Fragmented seagrass habitats on the mediterranean coast, and distribution and abundance of mysid assemblages. Marine Biology 141, 405–413.

Bohrer, R., Lampert, W., 1988. Simultaneous measurements of the effects of food concentration on assimilation and respiration in *Daphnia magna* Straus. Functional Ecology 2, 463–471.

Bosc, E., Bricaud, A., Antoine, D., 2004. Seasonal and interannual variability in algal biomass and primary production in the Mediterranean Sea, as derived from 4 years of SeaWiFS observations. Global Biogeochemical Cycles 18, GB1005.

Bozzano, A., Recasens, L., Sartor, P., 1997. Diet of the European hake *Merluccius merluccius* (Pisces: Merlucciidae) in the western Mediterranean (Gulf of Lions). Scientia Marina 61 (1), 1–8.

Brinton, E., Ohman, M., Townsend, A., Knight, M., Bridgeman, A., 2000. Euphausiids of the world ocean (world biodiversity database cd-rom series). Expert Center for Taxonomic Identification, University of Amsterdam, Amsterdam, Netherlands .

Brown, J., Allen, A., Gillooly, J., 2007. Body Size: The Structure and Function of Aquatic Ecosystems.. Cambridge University Press. chapter 1. The metabolic theory of ecology and the role of body size in marine and freshwater ecosystems.

Brown, J., Gillooly, J., Allen, A., Savage, V., West, G., 2004. Toward a metabolic theory of ecology. Ecology 85 (7), 1771–1789.

Calbet, A., 2001. Mesozooplankton grazing effect on primary production: a global comparative analysis in marine ecosystems. Limnology and Oceanography 46, 1824–1830.

Calder III, W., 1983. Ecological scaling: Mammals and birds. Annual Review of Ecology Systems 14, 213–230.

Cammen, L., Corwin, S., Christensen, J., 1990. Electron transport system (ETS) activity as a measure of benthic macrofaunal metabolism. Marine Ecology Progress Series 65, 171–182.

Cartes, J., 1994. Influence of depth and season on the diet of the deep-water aristeid *Aristeus antennatus* along the continental slope (400 to 2300 m) in the Catalan Sea (western Mediterranean). Marine Biology 120, 639–648.

Cartes, J., Madurell, T., Fanelli, E., López-Jurado, J., 2008. Dynamics of suprabenthos-zooplankton communities around the Balearic Islands (western Mediterranean: Influence of environmental variables and effects on the biological cycle of *Aristeus antennatus*. Journal of Marine Systems 71, 316–335.

Cartes, J., Mamouridis, V., Fanelli, E., 2011. Deep-sea suprabenthos assemblages (Crustacea) off the Balearic Islands (western Mediterranean): Mesoscale variability in diversity and production. Journal of Sea Research 65, 340 – 354.

Cartes, J., Maynou, F., Fanelli, E., Papiol, V., Lloris, D., 2009. Long-term changes in the composition and diversity of deep-slope megabenthos and trophic webs off Catalonia (western mediterranean): Are trends related to climatic oscillations? Progress in Oceanography 82, 32–46.

Cartes, J., Maynou, F., Morales-Nin, B., Massutí, E., Moranta, J., 2001. Trophic structure of a bathyal benthopelagic boundary layer community south of the Balearic Islands (southwestern Mediterranean). Marine Ecology Progress Series 215, 23–35.

Cartes, J., Rey, J., Lloris, D., De Sola, L., 2004. Influence of environmental variables on the feeding and diet of European hake (*Merluccius merluccius*) on the Mediterranean Iberian coasts. Journal of the Marine Biological Association of the UK 84, 831–835.

Castro, J., 1995. Mysids and euphausiids in the diet of *Scomber japonicus* Houttuyn,1782 off the Canary Islands. Boletín del Instituto Español de Oceanografía. II, 77–86.

Chance, B., Williams, G., 1955. Respiratory enzymes in oxidative phosphorylation. Journal of Biological Chemistry 217, 409–427.

Childress, J., 1975. The respiratory rates of midwater crustaceans as a function of depth of occurrence and relation to the oxygen minimum layer off southern california. Comparative Biochemistry and Physiology Part A: Physiology 50, 787–799.

Childress, J, J., 1968. Oxygen minimum layer: Vertical distribution and respiration of the mysid *Gnathophausia ingens*. Science 160, 1242–1243.

Christie, W., 1982. Lipid Analyses. Pergamon. Oxford, UK. 2 edition.

Clutter, R.I., 1969. The microdistribution and social behavior of some pelagic mysid shrimps. Journal of Experimental Marine Biology and Ecology 3, 125–155.

Cockcroft, A., Webb, P., Wooldridge, T., 1988. Nitrogen regeneration by two surf-zone mysids, *Mesopodopsis slabberi* and *Gastrosaccus psammodytes*. Marine Biology 99, 75–82.

Conover, R., 1960. The feeding behavior and respiration of some marine planktonic Crustacea. The Biological Bulletin 119, 399–415.

Cowles, D., Childress, J., 1988. Swimming speed and oxygen consumption in the bathypelagic mysid *Gnathophausia ingens*. Biological Bulletin 175, 111–121.

Cunha, M., Sorbe, J., Moreira, M., 1999. Spatial and seasonal changes of brackish peracaridan assemblages and their relation to some environmental variables in two tidal channels of the Ria de Aveiro (NW Portugal). Marine Ecology Progress Series 190, 69–87.

Dauby, P., 1995. A study of the feeding habits in four Mediterranean *Leptomysis* species (Crustacea: Myisidacea). Marine Ecology 16, 93–102.

Del Giorgio, P., Williams, P., 2005. Respiration in aquatic ecosystems. Oxford University Press, New York.

Del Giorgio, P.A., Duarte, C.M., 2002. Respiration in the open ocean. Nature 420, 379–384.

Dodds, P.S., Rothman, D.H., Weitz, J., 2001. Re-examination of the 3/4 - law of metabolism. Journal of Theoretical Biology 209, 9–27.

Domingues, P., Fores, R., P.G., L.T.P., Andrade, J.P., 2000. Mysid culture: lowering costs with alternative diets. Aquaculture Research 31, 719–728.

Domingues, P., Turk, P., Andrade, J., Lee, P., 1999a. Culture of the mysid, *Mysidopsis almyra* (Bowman),(Crustacea: Mysidacea) in a static water system: effects of density and temperature on production, survival and growth. Aquaculture Research 30, 135–143.

Domingues, P., Turk, P., Andrade, J., Lee, P., 2001a. Effects of enriched *a*rtemia nauplii on production, survival and growth of the mysid shrimp *Mysidopsis almyra* Bowman 1964 (Crustacea: Mysidacea). Aquaculture Research 32, 599–603.

Domingues, P., Turk, P.E., Andrade, J.P., Lee, P.G., 1999b. Culture of the mysid, *Mysidopsis almyra* (Bowman), (Crustacea: Mysidacea) in a static water system: effects of density and temperature on production, survival and growth. Aquaculture Research 30, 135–143.

Domingues, P.M., Sykes, A., Andrade, J.P., 2001b. The use of *Artemia* sp. or mysids as food source for hatchlings of the cuttlefish *Sepia officinalis*; effects on growth and survival throughout the life cycle. Aquaculture International 9, 319–331.

Duarte, C., Cebrian, J., 1996. The fate of marine autotrophic production. Limnology and Oceanography , 1758–1766.

Duarte, C., Chiscano, C., 1999. Seagrass biomass and production: a reassessment. Aquatic Botany 65, 159–174.

Duarte, C., et al., 2002. The future of seagrass meadows. Environmental conservation 29, 192–206.

Duarte, C.M., Regaudie-de Gioux, A., Arrieta, J.M., Delgado-Huertas, A., Agustí, S., 2013. The oligotrophic ocean is heterotrophic. Annual Review of Marine Science 5, 551–569.

Espino, F., Tuya, F., Brito, A., Haroun, R., 2011a. Ichthyofauna associated with *Cymodocea nodosa* meadows in the Canarian Archipelago (central eastern Atlantic): Community structure and nursery role. Ciencias Marinas 37, 157–174.

Espino, F., Tuya, F., Brito, A., Haroun, R., 2011b. Variabilidad espacial en la estructura de la ictiofauna asociada a praderas de *Cymodocea nodosa* en las Islas Canarias, Atlántico nororiental subtropical. Revista de Biología Marina y Oceanografía 46, 391–403.

Estrada, M., 1996. Primary production in the northwestern Mediterranean. Scientia Marina 60 (2), 55–64.

EUROMODEL Group, 1995. Progress from 1989 to 1992 in understanding the circulation of the Western Mediterranean Sea. Oceanologica Acta 18 (2), 255–271.

Falkowski, S., Wilson, C., 1992. Phytoplankton productivity in the North Pacific Ocean since 1900 and implications for absorption of anthropogenic CO2. Nature 358, 741–743.

Fernández de Puelles, M., Alemany, F., Jansá, J., 2007. Zooplankton time-series in the Balearic Sea (Western Mediterranean): Variability during the decade 1994-2003. Progress in Oceanography 74, 329–354.

Fernández de Puelles, M., Lopéz-Urrutia, A., Morillas, A., Molinero, J., 2009. Seasonal variability of copepod abundance in the Balearic region (western Mediterranean) as an indicator of basin scale hydrological changes. Hydrobiologia 617, 3–16.

Fernández de Puelles, M., Molinero, J., 2007. North Atlantic climate control on plankton variability in the Balearic Sea, western Mediterranean. Geophysical research letters 34, L04608.

Fernández de Puelles, M., Molinero, J., 2008. Decadal changes in hydrographic and ecological time-series in the Balearic Sea (western Mediterranean), identifying links between climate and zooplankton. ICES Journal of Marine Science: Journal du Conseil 65, 311–317.

Fernández de Puelles, M., Pinot, J., Valencia, J., 2003. Seasonal and interannual variability of zooplankton community in waters off Mallorca island

(Balearic Sea, Western Mediterranean): 1994-1999. Oceanologica Acta 26, 673–686.

Fernández de Puelles, M., Valencia, J., Jansá, J., Morillas, A., 2004. Hydrographical characteristics and zooplankton distribution in the Mallorca channel (Western Mediterranean): spring 2001. ICES Journal of Marine Science: Journal du Conseil 61, 654.

Fernández-Urruzola, I., Packard, T., Gómez, M., 2011. GDH activity and ammonium excretion in the marine mysid, *Leptomysis lingvura*: Effects of age and starvation. Journal of Experimental Marine Biology and Ecology 409, 21–29.

Finlay, B., Span, A., Ochsenbein-Gattlen, C., 1983. Influence of phisiological state on indices of respiration rate in protozoa. Comparative Biochemistry and Physiology 74A, 211–219.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipid from animal tissues. J. Biol. Chem. 226 (1), 497–509.

Fruton, J., Simmonds, S., 1958. General Biochemistry. John Wiley, New York, NY. pp 1077 2nd edition.

Giguere, L., Cote, B., St-Pierre, J.F., 1988. Metabolic rates scale isometrically in larval fishes. Marine Ecology Progress Series. 50, 13–19.

Gillooly, J., Allen, A., Savage, V., Charnov, E., West, G., Brown, J., 2006. Response to Clarke and Fraser: Effects of temperature on metabolic rate. Functional Ecology 20, 400–404.

Gillooly, J., Brown, J., West, G., Savage, V., Charnov, E., 2001. Effects of size and temperature on metabolic rate. Science 293, 2248–2251.

Glazier, D., 2005. Beyond the '3/4-power law': Variation in the intra- and interespecific scaling of metabolic rate in animals. Biological Reviews 80, 611–661.

Glazier, D., 2006. The 3/4-power law is not universal: evolution of isometric, ontogenetic metabolic scaling in pelagic animals. BioScience 56, 325–332.

Gómez, M., 1991. Biomasa y actividad metabólica del zooplancton en relación con un efecto de masa de isla en aguas de Gran Canaria. Ph.D. thesis. Universidad de las Palmas de Gran Canaria.

Gómez, M., Martínez, I., Hernández-León, S., Packard, T., 2008. Does ETS activity follow Kleiber's law?, International Symposium on Eastern Boundary Upwelling Ecosystems (2-6 Junio 2008). Las Palmas de Gran Canaria, Canary Islands, Spain.

Gómez, M., Torres, S., Hernández-León, S., 1996. Modification of the electron transport system (ETS) method for routine measurements of respiratory rates of zooplankton. South African Journal of Marine Science 16, 15–20.

Guijarro, B., Massutí, E., Moranta, J., Díaz, P., 2008. Population dynamics of the red shrimp *Aristeus antennatus* in the Balearic Islands (western Mediterranean): Short spatio-temporal differences and influence of environmental factors. Journal of Marine Systems 71, 385–402.

Hecq, J., Sainvitu, E., Dauby, P., 1984. Influence des conditions experimentales et environmentales sur la consommation d' oxygène et l' activité nutritionelle de *Leptomysis lingvura* (Sars) (crustacé: Mysidacé). Annals of the Royal Zoological Society of Belgium 114 (1), 200–201.

Hemmingsen, A.M., 1960. Energy metabolism as related to body size and respiratory surfaces, and its evolution. volume 9 (2), 7-110. Reports of the Steno Memorial Hospital and the Nordisk Insulin Laboratorium (Copenhagen).

Hernández-León, S., Gómez, M., 1996. Factors affecting the respiration-ETS ratio in marine zooplankton. Journal of Plankton Research 18, 239–255.

Hernández-León, S., Gómez, M., Pagazaurtundua, M., Portillo-Hahnefeld, A., Montero, I., Almeida, C., 2001. Vertical distribution of zooplankton in Canary Island waters: implications for export flux. Deep Sea Research Part I: Oceanographic Research Papers 48, 1071–1092.

Hernández-León, S., Ikeda, T., 2005. A global assessment of mesozooplankton respiration in the ocean. Journal of plankton research 27, 153–158.

Herrera, A., 2009. Desarrollo de las técnicas de cultivo y estudio del metabolismo respiratorio en misidáceos. Master's thesis. Universidad de las Palmas de Gran Canaria. ISBN 978-84-693-0479-2.

Herrera, A., Gómez, M., Molina, L., Otero, F., Packard, T., 2011a. Rearing techniques and nutritional quality of two mysids from Gran Canaria (Spain). Aquaculture Research. 42, 677–683.

Herrera, A., Packard, T., Santana, A., Gómez, M., 2011b. Effect of starvation and feeding on respiratory metabolism in *Leptomysis lingvura* (G.O. Sars, 1866). Journal of Experimental Marine Biology and Ecology 409, 154–159.

Hidalgo, M., Massutí, E., Moranta, J., Cartes, J., Lloret, J., Oliver, P., Morales-Nin, B., 2008. Seasonal and short spatial patterns in European hake *Merluccius merluccius L.* recruitment process at the Balearic Islands (western Mediterranean): The role of environment on distribution and condition. Journal of Marine Systems 71, 367–384.

Hiller-Adams, P., Childress, J.J., 1983. Effects of prolonged starvation on O_2 consumption, NH_4^+ excretion, and chemical composition of the bathypelagic mysid *Gnathophausia ingens*. Marine Biology 77, 119–127.

Hirch, S., Martin, B., Christiansen, B., 2009. Zooplankton metabolism and carbon demand at two seamounts in the NE Atlantic. Deep Sea Research Part II: Topical Studies in Oceanography 56, 2656–2670.

Iglesias, J., Sánchez, F., Bersano, J., Carrasco, J., Dhont, J., Fuentes, L., Linares, F., Muñoz, J., Okumura, S., Roo, J., et al., 2007. Rearing of *Octopus vulgaris* paralarvae: Present status, bottlenecks and trends. Aquaculture 266, 1–15.

Ikeda, T., 1970. Relationship between respiration rate and body size in marine plankton animals as a function of the temperature of habitat. Bulletin of the Faculty of Fisheries Hokkaido University 21, 91–112.

Ikeda, T., 1971. Changes in respiration rate and in composition of organic matter in *Calanus cristatus* (Crustacea Copepoda) under starvation. Bulletin of the Faculty of Fisheries Hokkaido University 21, 280–298.

Ikeda, T., 1985. Metabolic rates of epipelagic marine zooplankton as a function of body mass and temperature. Marine Biology 85, 1–11.

Ikeda, T., Dixon, P., 1984. The influence of feeding on the metabolic activity of antarctic krill (*Euphausia superba* Dana). Polar Biology 3, 1–9.

Ivleva, I., 1980. The dependence of crustacean respiration rate on body mass and habitat temperature. Internationale Revue der gesamten Hydrobiologie und Hydrographie 65, 1–47.

Izquierdo, M., 1996. Essential fatty acid requirements of cultured marine fish larvae. Aquaculture Nutrition 2, 183–191.

Izquierdo, M., Watanabe, T., Takeuchi, T., Arakawa, T., Kitajima, C., 1990. In: The Current Status of Fish Nutrition in Aquaculture. Tokyo University of Fisheries, Tokyo. chapter Optimum EFA levels in *Artemi*a to meet the EFA requirements of red sea bream (*Pagrus major*). pp. 221–232.

Jumars, P., 2007. Habitat coupling by mid-latitude, subtidal, marine mysids: import-subsidised omnivores. Oceanography and Marine Biology: An Annual review. 45, 89–188.

Kenner, R., Ahmed, S., 1975. Measurement of electron transport activities in marine phytoplankton. Mar.Biol. 33, 119–127.

King, F., Packard, T., 1975. Respiration and the activity of respiratory electron transport system in marine zooplankton. Limnology and Oceanography 20, 849–859.

King, F.D., Devol, A.H., Packard, T.T., 1978. Plankton metabolic activity in the eastern tropical North Pacific. Deep Sea Research 25, 689 – 704.

Kiorboe, T., Mohlenberg, F., Hamburguer, K., 1985. Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. Marine Ecology Progress Series 26, 85–97.

Kleiber, M., 1961. The fire of life: An introduction to animal energetics. John Wiley and sons Inc, New York, USA. 454 pp.

Kolokotrones, T., Savage, V., Deeds, E.J., Fontana, W., 2010. Curvature in metabolic scaling. Nature 464, 753–756.

Kuhn, A., Bengtson, D., Simpson, K., 1991. Increased reproduction by mysids (*Mysidopsis bahia*) fed with enriched *Artemia* spp. nauplii. Mysids in fisheries: Hard lessons from headlong introductions 9, 192–199.

Labat, R., 1953. *Paramysis nouveli n.* sp. et *Paramysis bacescoin* sp. deux espèces de mysidacés confondues, jusqu'à présent, avec *Paramysis helleri* G.O. Sars, 1877. Bulletin Institut Océanographique 1034, 1–24.

Laiz-Carrión, R., Quintanilla, J.M., Torres, A.P., Alemany, F., García, A., 2013. Hydrographic patterns conditioning variable trophic pathways and early life dynamics of bullet tuna *Auxis rochei* larvae in the Balearic Sea. Marine Ecology Progress Series 475, 203–212.

Lampert, W., 1986. Response of the respiratory rate of *Daphnia magna* to changing food conditions. Oecologia 70, 495–501.

Lane, N., 2005. Power, sex, suicide: Mitochondria and the meaning of life. Oxford University Press, New York.

Lee-Montero, I., Navarro, A., Borrell, Y., García-Celdrán, M., Martín, N., Negrín-Báez, D., Blanco, G., Armero, E., Berbel, C., Zamorano, M., et al., 2013. Development of the first standardised panel of two new microsatellite multiplex pcrs for gilthead seabream (*Sparus aurata L.*). Animal Genetics 44 (5), 533–546.

Longhurst, A.R., Harrison, W.G., 1989. The biological pump: Profiles of plankton production and consumption in the upper ocean. Progress In Oceanography 22, 47 – 123.

López-Jurado, J., Garcia Lafuente, J., Cano Lucaya, N., 1995. Hydrographic conditions of the Ibiza channel during november 1990, march 1991 and july 1992. Oceanologica acta 18, 235–243.

López-Jurado, J., Marcos, M., Monserrat, S., 2008. Hydrographic conditions affecting two fishing grounds of Mallorca island (Western Mediterranean): during the IDEA project (2003-2004). Journal of Marine Systems 71, 303–315.

Lowry, O., Rosebrough, N., Farr, A., Randall, R., 1951. Protein measurements with a folin phenol reagent. Journal of Biological Chemistry 193, 265–275.

Lussier, S., Kuhn, A., Chammas, M., Sewall, J., 1988. Techniques for the laboratory culture of *Mysidopsis* species (Crustacea: Mysidacea). Environmental Toxicology and Chemistry 7, 969–977.

Madurell, T., Fanelli, E., Cartes, J., 2008. Isotopic composition of carbon and nitrogen of suprabenthic fauna in the NW Balearic Islands (western Mediterranean). Journal of Marine Systems 71, 336–345.

Mahaut, M., Sibuet, M., Shirayama, Y., 1995. Weight-dependent respiration rates in deep-sea organisms. Deep Sea Research Part I: Oceanographic Research Papers 42, 1575–1582.

Maldonado, F., Packard, T., Gómez, M., 2012. Understanding tetrazolium reduction and the importance of substrates in measuring respiratory electron transport activity. Journal of Experimental Marine Biology and Ecology 434-435, 110–118.

Martin, J.W., Davis, G.E., 2001. An updated Classification of the Recent Crustacea. Natural History Museum of Los Angeles County. Science Series 39, 124 pp.

Martínez, I., 2007. Relaciones entre biomasa, tasa de crecimiento, respiración y respiración potencial en el crustáceo *Artemia salina*. Master's thesis. Universidad de las Palmas de Gran Canaria.

Martínez, I., Gómez, M., Packard, T., 2010. Potential respiration is a better respiratory predictor than biomass in young *Artemia salina*. Journal of Experimental Marine Biology and Ecology 390, 78–83.

Mauchline, J., 1980. The biology of Mysids and Euphausiids. Advances in Marine Biology 18, 1–681.

Mauchline, J., Murano, M., 1977. World list of the Mysidacea, Crustacea. Journal of the Tokio University of fisheries 4, 39–88.

Maynou, F., Cartes, J., 2000. Community structure of bathyal decapod crustaceans off south-west Balearic Islands (Western Mediterranean): seasonality and regional patterns in zonation. Journal of the Marine Biological Association of the UK 80, 789–798.

Mayzaud, P., Boutoute, M., Gasparini, S., Mousseau, L., Lefevre, D., 2005. Respiration in marine zooplankton: The other side of the coin: CO2 production. Limnology and Oceanography 50, 291–298.

Mees, J., Dewicke, A., Hamerlynck, O., 1993. Seasonal composition and spatial distribution of hyperbenthic communities along estuarine gradients in the Westerschelde. Netherland Journal of Aquatic Ecology 27, 359–376.

Mees, J., Fockedey, N., Hamerlynkck, O., 1995. Comparative study of the hyperbenthos of three European estuaries. Hydrobiologia 311, 153–174.

Mees, J., Jones, M., 1997. The hyperbenthos. Oceanography and marine biology 35, 221–256.

Meland, K., Willassen, E., 2007. The disunity of mysidacea (crustacea). Molecular Phylogenetics and Evolution 44, 1083–1104.

Minutoli, R., Guglielmo, L., 2009. Zooplankton respiratory electron transport system (ETS) activity in the Mediterranean Sea: spatial and diel variability. Marine Ecology Progress Series 381, 199–211.

Murano, M., 1999. South Atlantic Zooplankton. Backhuy Publishers, Leiden, Holland. volume 2. chapter Mysidacea. pp. 1099–1140.

Navarro, A., Badilla, R., Zamorano, M., Pasamontes, V., Hildebrandt, S., Sánchez, J., Afonso, J., 2008. Development of two new microsatellite multiplex PCRs for three sparid species: Gilthead seabream *Sparus auratus* l., red porgy *Pagrus pagrus* and redbanded seabream *P. auriga*, (Valenciennes, 1843) and their application to paternity studies. Aquaculture 285, 30–37.

Nelson, D., Cox, M., 2005. Lehninger Principles of Biochemistry. Freeman and Company, New York. 1216 pp.

Norton, B., Lewis, M., Mayer, F., 1999. Storage duration and temperature and the acute toxicities of estuarine sediments to *Mysidopsis bahia* and *Leptocheirus plumulosus*. Bulletin of environmental contamination and toxicology 63, 157–166.

Otero-Ferrer, F., 2012. Seahorses in Gran Canaria Island (Spain): ecology and aquaculture - combined tools for marine conservation issues. Ph.D. thesis. Universidad de Las Palmas de Gran Canaria.

Otero-Ferrer, F., Molina, L., Socorro, J., Herrera, R., Fernández-Palacios, H., Izquierdo, M., 2007. Efecto de la primera alimentación en la supervivencia y el crecimiento de criías de caballito de mar, *Hippocampus hippocampus* (Linnaeus, 1758)., XI Congreso Nacional de Acuicultura (24-28 de Septiembre). Vigo, España.

Otero-Ferrer, F., Molina, L., Socorro, J., Herrera, R., Fernández-Palacios, H., Izquierdo, M.S., 2010. Live prey first feeding regimes for short-snouted seahorse *Hippocampus hippocampus* (Linnaeus, 1758) juveniles. Aquaculture research 41, e8–e19.

Owens, T., King, F., 1975. The measurement of respiratory electron transport system activity in marine zooplankton. Marine Biology 30, 27–36.

Packard, T., 1971. The measurement of respiratory electron-transport activity in marine phytoplankton. Journal of Marine Research 29, 235–244.

Packard, T., 1979. Respiration and respiratory electron transport activity in plankton from the Northwest African upwelling area. Journal of Marine Research 37, 711–742.

Packard, T.., Devol, A., King, F., 1975. The effect of temperature on the respiratory electron transport system in marine plankton. Deep Sea Research 22, 237–249.

Packard, T., Garfield, P., Martinez, R., 1983. Respiration and respiratory enzyme activity in aerobic and anaerobic cultures of the marine denitrifying bacterium *Pseudomonas perfectomarinus*. Deep Sea Research 2, 227–243.

Packard, T., Gómez, M., 2008. Exploring a first-principles-based model for zooplankton respiration. ICES Journal of Marine Science 65, 371–378.

Packard, T., Harmon, D., Boucher, J., 1974. Respiratory electron transport activity in plankton from upwelled waters. Tethys 6, 213–222.

Packard, T., Healy, M., Richards, F., 1971. Vertical distribution of the activity of the respiratory electron transport system in marine plankton. Limnology and Oceanography 16, 60–70.

Packard, T.T., 1985. Oxygen Consumption in the Ocean: Measuring and Mapping with Enzyme Analysis. ACS Publications. volume 209. chapter 11. pp. 177–209. http://pubs.acs.org/doi/pdf/10.1021/ba-1985-0209.ch010.

Packard, T.T., Berdalet, E., Blasco, D., Roy, S., St-Amand, L., Lagacé, B., Lee, K., Gagnó, J., 1996. Oxygen consumption in the marine bacterium *Pseudomonas nautica* predicted from ETS activity and bisubstrate enzyme kinetics. Journal of Plankton Research 18, 1819–1835.

Packard, T.T., Blasco, D., Estrada, M., 2004. Modeling physiological processes in plankton on enzyme kinetic principles. Scientia Marina 68, 49–56.

Packard, T.T., Christensen, J., 2004. Respiration and vertical carbon flux in the Gulf of Maine water column. Journal of Marine Research 62, 93–115.

Packard, T.T., Gómez, M., 2013. Modelling vertical carbon flux from zooplankton respiration. Progress in Oceanography 110, 59–68.

Pavón-Salas, N., Herrera, R., Hernández-Guerra, A., Haroun, R., 2000. Distributional pattern of seagrasses in the Canary islands (central-east Atlantic ocean). Journal of Coastal Research 16, 329–335.

Peters, R.H., 1983. The ecological implications of body size. volume 2. Cambridge University Press. pp. 329.

Pinot, J., López-Jurado, J., Riera, M., 2002. The CANALES experiment (1996-1998). Interannual, seasonal, and mesoscale variability of the circulation in the Balearic Channels. Progress in Oceanography 55, 335–370.

Pinot, J., Tintoré, J., Gomis, D., 1995. Multivariate analysis of the surface circulation in the Balearic Sea. Progress in Oceanography 36, 343–376.

Polunin, N.V., Morales-Nin, B., Pawsey, W.E., Cartes, E., Pinnegar, K., Moranta, D., 2001. Feeding relationships in Mediterranean bathyal assemblages elucidated by stable nitrogen and carbon isotope data. Marine Ecology Progress Series 220, 13–23.

Postel, L., Fock, H., Hagen., W., 2000. ICES Zooplankton Methodology Manual. Academic Press. chapter Biomass and Abundance. pp. 83–192.

Prosser, C., Brown, F., 1961. Comparative Animal Physiology. W B. Saunders Company, Philadelphia. pp. 888.

Reitsema, L., 1980. A recirculating artificial seawater system for the laboratory culture of *Mysidopsis almyra* (Crustacea; Pericaridea). Estuaries and Coasts 3, 321–323.

Reyes, J., Sansón, M., Afonso-Carrillo, J., 1995. Distribution and reproductive phenology of the seagrass *Cymodocea nodosa* (Ucria) Ascherson in the Canary Islands. Aquatic Botany 50, 171–180.

Roe, H., 1984. The diel migrations and distributions within a mesopelagic community in the north east atlantic. 2. vertical migrations and feeding of mysids and decapod crustacea. Progress in Oceanography 13, 269–318.

Roo, F., Hernández-Cruz, C., Socorro, J., Fernández-Palacios, H., Montero, D., Izquierdo, M., 2009. Effect of DHA content in rotifers on the occurrence of skeletal deformities in red porgy *Pagrus pagrus* (linnaeus, 1758). Aquaculture 287, 84–93.

Roy, S., Packard, T., 2001. CO_2 production rate predicted from isocitrate dehydrogenase activity, intracellular substrate concentrations and kinetic constants in the marine bacterium *Pseudomonas nautica*. Marine Biology 138, 1251–1258.

Rutter, W., 1967. Methods in developmental biology. Academic Press, New York. chapter Protein determinations in embryos. pp. 671–684.

Sainte-Marie, B., Brunel, P., 1985. Suprabenthic gradients of swimming activity by cold-water gammaridean amphipod Crustacea over a muddy shelf in the Gulf of Saint Lawrence. Marine Ecology Progress Series. 23, 57–69.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual. volume 2. Cold Spring Harbor Laboratory Press, New York.

San Vicente, C., Sorbe, J.C., 1999. Spatio-temporal structure of the suprabenthic community from Creixell beach (western Mediterranean). Acta Oecologica 20, 377–389.

San Vicente, C., Sorbe, J.C., 2001. Temporal changes in the structure of the suprabenthic community from Hendaya beach (southern Bay of Biscay): A comparison with a northwestern Mediterranean beach community. Boletín del Instituto Español de Oceanografía. 17, 107–120.

Sánchez-Jerez, P., Esplá, A.R., 1996. Detection of environmental impacts by bottom trawling on *Posidonia oceanica* (L.) Delile meadows: sensitivity of fish and macroinvertebrate communities. Journal of Aquatic Ecosystem Health 5, 239–253.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177, 191–199.

Sargent, J., McEvoy, L., Bell, J., 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquaculture 155, 117–127.

Schalk, P., 1988. Respiratory electron transport system (ETS) activities in zooplankton and micronekton of the Indo-Pacific region. Marine Ecology Progress Series. 44, 25–35.

Schmidt-Nielsen, K., 1984. Scaling: why is animal size so important? Cambridge University Press. pp 241.

Seiwell, H., 1937. Consumption of oxygen in sea water under controlled laboratory conditions. Nature 140, 506–507.

Soldevilla, F.L., Landeira, J.M., Espinosa, J.M., Izquierdo, F., 2006. Catálogo del metazooplancton de las aguas de las islas canarias. Capitán 8 (1), 41–73.

Spears, T., DeBry, R.W., Abele, L.G., Chodyla, K., Boyko, C.B., 2005. Peracarid monophyly and interordinal phylogeny inferred from nuclear small-subunit ribosomal DNA sequences (crustacea: Malacostraca: Peracarida). Proceedings of the Biological Society of Washington 118, 117–157.

Tacon, A., 1989. Manual de Capacitación. Nutrición y alimentación de peces y camarones cultivados. FAO. Project reports No. 4.

Tattersall, W.M., Tattersall, O., 1951. The British Mysidacea. Ray Society, London. pp 460.

Thor, P., 2003. Elevated respiration rates of the neritic copepod *Acartia tonsa* during recovery from starvation. Journal of Experimental Marine Biology and Ecology 283, 133–143.

Torres, J., Belman, B., Childress, J., 1979. Oxygen consumption rates of midwater fishes as a function of depth of occurence. Deep Sea Research Part A. Oceanographic Research Papers 26, 185–197.

Torres, J., Childress, J., 1983. Relationship of oxygen consumption to swimming speed in *Euphausia pacifica*. Marine Biology 74, 79–86.

Tuya, F., Hernandez-Zerpa, H., Espino, F., Haroun, R.J., 2012. Drastic decadal decline of the seagrass *Cymodocea nodosa* at Gran Canaria (eastern Atlantic): interactions with the green algae *Caulerpa prolifera*. Aquatic Botany 105, 1–6.

Tuya, F., Martín, J., Luque, A., 2006. Seasonal cycle of a *Cymodocea nodosa* seagrass meadow and of the associated ichthyofauna at Playa Dorada (Lanzarote, Canary Islands, eastern Atlantic). Ciencias Marinas 32, 695–704.

Tuya, F., Perez, J., Medina, L., Luque, A., 2001. Seasonal variation of the macrofauna from three seagrass meadows of *Cymodocea nodosa* off Gran Canaria (central-eastern Atlantic ocean). Ciencias marinas 27, 223–234.

Verslycke, T., Fockedey, N., McKenney Jr, C., Roast, S., Jones, M., Mees, J., Janssen, C., 2004. Mysid crustaceans as potential test organisms for the evaluation of environmental endocrine disruption: a review. Environmental Toxicology and Chemistry 23, 1219–1234.

Wang, Z., Dauvin, J.C., 1994. The suprabenthic crustacean fauna of the infralittoral fine sand community from the Bay of Seine (Eastern English Channel): composition, swimming activity and diurnal variation. Cahiers de Biologie Marine 35, 135–155.

Ward, S., 1984. A system for laboratory rearing of the mysid, *Mysidopsis bahia* Molenock. The Progressive Fish-Culturist 46, 170–175.

Whitfield, J., 2006. In the beat of a heart: Life, Energy, and the unity of Nature. The National Academies Press. pp. 278.

Wittmann, K., 1981. Comparative biology and morphology of marsupial development in *Leptomysis* and other Mediterranean Myisidacea (Crustacea). Journal of experimental marine biology and ecology 52, 243–270.

Wittmann, K., 1986. Saisonale und morphogeographische differenzierung bei *Leptomysis lingvura* und zwei verwandten spezies (Crustacea, Mysidacea). Annalen des Naturhistorischen Museums in Wien. 87, 265–294.

Wittmann, K., Moro, L., Riera, R., 2010. Sobre la distribución de *Gastrosaccus roscoffensis* (Crustacea: Mysida) en el Atlántico nororiental y primer registro para las Islas Canarias. Revista de la Academia Canaria de Ciencias 22, 91–101.

Wittmann, K., Wirtz, P., 1998. A first inventory of the mysid fauna (Crustacea: Mysidacea) in coastal waters of the Madeira and Canary archipelagos. Bol. Mus. Mun. Funchal 5, 511–533.

Wittmann, K.J., Hernández, F., Durr, J., Tejera, E., González, J.A., Jimenez, S., 2003. The epi-to bathypelagic Mysidacea (Peracarida) off the Selvagens, Canary, and Cape Verde Islands (NE atlantic), with first description of the male of *Longithorax alicei* H. Nouvel, 1942. Crustaceana 76, 1257–1280.

Wittmann, K.J., et al., 1977. Modification of association and swarming in North Adriatic Mysidacea in relation to habitat and interacting species. Biology of Benthic Organisms 493, 502.

Woods, C., Valentino, F., 2003. Frozen mysids as an alternative to live *Artemia* in culturing seahorses *Hippocampus abdominalis*. Aquaculture Research 34, 757–763.

Yamada, K., Hori, M., Tanaka, Y., Hasegawa, N., Nakaoka, M., 2007. Temporal and spatial macrofaunal community changes along a salinity gradient in seagrass meadows of Akkeshi-ko estuary and Akkeshi Bay, northern Japan. Hydrobiologia 592, 345–358.

Yamada, K., Hori, M., Tanaka, Y., Hasegawa, N., Nakaoka, M., 2010. Contribution of different functional groups to the diet of major predatory fishes

at a seagrass meadow in northeastern Japan. Estuarine, Coastal and Shelf Science 86, 71–82.

Yamada, K., Kumagai, N.H., 2012. Importance of seagrass vegetation for habitat partitioning between closely related species, mobile macrofauna *Neomysis* (Misidacea). Hydrobiologia 680, 125–133.

Zariquiey Alvarez, R., 1968. Crustáceos decápodos ibéricos. Investigación Pesquera 32, 510.

Zhang, Z.Q. (Ed.), 2011. Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. volume 3148, 1-237. Zootaxa.

Mysids are peracarida crustaceans with a highly relevant ecological role, especially in coastal areas where they are abundant. Lately mysids have become topical because of their importance in the food chain; they are the main food for many coastal fish, especially in their juvenile stages. In addition, mysids are one of the principal components of suprabenthos, and have recently been shown to remineralize a large portion of detritus and to regenerate inorganic nitrogen salts.

Despite their importance, until now there have been few studies on the abundance of mysids in the waters off Gran Canaria. This thesis advances our knowledge of Canary Island mysid species especially those that inhabit the coastal *Cymodocea nodosa* seagrass meadows of Gran Canaria. It highlights the important role of mysids in these coastal waters, as well as the need to preserve their habitat.

The cultivation of two species of mysids and a study of their nutritional quality shows that they are an excellent food to be used as live prey for fish and cephalopods. This part of the thesis is the first step in the research of new zooplankton species for aquaculture.

Studies of the respiratory metabolism of *L. lingvura*, one of the species of mysids that has been grown successfully in our laboratory, have led to significant discoveries in zooplankton ecophysiology. They have shown that factors such as starvation affect the respiration, but not the electron transport system (ETS) activity. Consequently, the relationship, R/ETS, is affected in parallel with the effect on respiration. It was also found that the long-term availability of food affects the ETS-biomass relationship.

These results have facilitated the interpretation of the ETS measurements and have been applied in zooplankton and suprabenthos oceanographic research corroborating results obtained on cultured mysids.