Distribution and population biology of pelagic decapod crustaceans of the western Mediterranean

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No hay diferencias entre sueño y realidad, basta vivirlo.

Daniela Silveira Simão

Eterno Aprendiz

Gonzaguinha

Eu fico
Com a pureza
Da resposta das crianças
É a vida, é bonita
E é bonita...

Viver!
E não ter a vergonha
De ser feliz
Cantar e cantar e cantar
A beleza de ser
Um eterno aprendiz...

Ah meu Deus!
Eu sei, eu sei
Que a vida devia ser
Bem melhor e será
Mas isso não impede
Que eu repita
É bonita, é bonita
E é bonita...

E a vida!
E a vida o que é?
Diga lá, meu irmão
Ela é a batida
De um coração
Ela é uma doce ilusão
Hê! Hô!...

Mas e a vida
Ela é maravida
Ou é sofrimento?
Ela é alegria
Ou lamento?
O que é? O que é?
Meu irmão...

Há quem fale
Que a vida da gente
É um nada no mundo
É uma gota é um tempo
Que nem dá um segundo...

Há quem fale
Que é um divino
Mistério profundo
É o sopro do criador
Numa atitude repleta de amor...

Você diz que é luta e prazer
Ele diz que a vida e viver
Ela diz que melhor é morrer
Pois amada não é
E o verbo é sofrer...

Eu só sei que confio na moça
E na moça eu ponho a força da fé
Somos nós que fazemos a vida
Como der ou puder ou quiser...

Sempre desejada
Por mais que esteja errada
Ninguém quer a morte
Só saúde e sorte...

E a pergunta roda
E a cabeça agita
Fico com a pureza
Da resposta das crianças
É a vida, é bonita
E é bonita...

Viver!
E não ter a vergonha
De ser feliz
Cantar e cantar e cantar
A beleza de ser
Um eterno aprendiz...

Ah meu Deus!
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Chapter 4:

Summary

The present thesis provides new information about pelagic decapod crustaceans, specially so concerning the Mediterranean pasiphaeids. Remarkable differences in the pattern of distributions of the pasiphaeids were observed according to the influence of Atlantic waters. Overall both abundance and biomass of the pasiphaeids in Alboran Sea was higher than in the rest of the Iberian Mediterranean populations. The depth on which the pasiphaeids started to occur in appreciable density was also shallower in the Alboran Sea where the *P. sivado* presented the widest depth range of distribution as well as the biggest size of first maturity of females. This size was progressively smaller with increasing distance from the Atlantic water source, i.e the Gibraltar Strait area. Overall the two species presented a size structure that changed with depth, with younger individuals occupying shallower depths and biggest adults occupying deeper bathymetries. *P. multidentata* juveniles were only observed in the Catalan Sea while they were absent in the Alboran Sea.

Size and morphological dimorphism of *P. sivado* were reported for the first time in the present thesis. All the five pleopods of females were thinner and more elongated than male pleopods, which in turn were rounded anteriorly and had robust shapes. A critical size from which the population sex ratio biases changed from females to males dominance was found in all *P. sivado* populations of the Mediterranean Iberian coast. This critical size was different and progressively larger again from populations nearest the Gibraltar Strait versus those placed in the Catalan Sea, in concordance with the female first maturity size gradient.

The present thesis also provides a first attempt to investigate the influence of the Gibraltar Strait on the genetic population structure of the species by using congener benthopelagic shrimps that dwell in different depth strata. The Gibraltar Strait was shown to be a strong and unique geographical barrier to the genetic flux of both *P. sivado* and *P. multidentata*, due the presence of a marked genetic structure characterized by two main haplotypes: one Atlantic and another one Mediterranean. Other new information related to the phylogeny of the genus *Pasiphaea* was that *P. sivado* a much higher genetic divergence when compared to the rest of pasiphaeid species analysed, indicating that several genetic subgroups do occur within the genus *Pasiphaea*.

The study of the pelagic decapod community in the waters around the island of Mallorca, placed between the Algerian and the Catalano-Balearic basins, showed the occurrence of two main assemblages: one above the shelf break and another one above the middle slope. The shelf community was characterized by the dominance of almost transparent species, such as *Sergestes arcticus,* the species that reached the greatest abundances, and *P. sivado* that was omnipresent in all pelagic strata. The novel registration of *Chlorotocus crassicornis* and *Plesionika heterocarpus* in the pelagic realm were restricted to the shelf-break at the Deep Scattering Layer. The red body species *Gennadas elegans*, *Pasiphaea multidentata*, and *Sergia robusta*, were restricted to the middle slope. No significant differences concerning the sampling geographic area or seasonality were found. Actually, the decapod pelagic community of the Balearic Sea was mainly structured by both the geomorphology (and associated hydrographic characteristics of the shelf/slope transition) and the influence of light in the water column. No decapod crustacean species occurred in epipelagic daytime samples. Size analysis showed the occurrence of species-specific patterns concerning the size/age movements into the water column throughout the day-night cycle. Moreover, the present work clearly confirms the presence of adult *P. multidentata* in the water column, evidencing that the adult fraction of the population has also the ability to perform vertical migrations.

In the present days many knowledge gaps still exist concerning the autoecology of pasiphaeids and several pelagic decapods. Many questions about population structure, distribution and phylogeny could be better endorsed by knowing the early life and dispersal histories of these species. The high sampling effort, and their associated high cost, still remains one of the principal reasons for the scarce information available nowadays.

Keywords: Macrozooplankton, Micronekton, benthopelagic decapods, Necktobentonic crutaceans, Diel vertical migrations, Size structure, Sexual dimorphism, Population genetics, Phylogenetics, Pasiphaeidae, Sergestidae.

Resum

Aquesta tesi presenta nova informació sobre crustacis decàpodes pelàgics, principalment sobre els pasifèids presents a la Mediterrània: *Pasiphaea sivado* i *Pasiphaea multidentata*. S'han trobat diferències importants en les pautes de distribució dels pasifèids en relació a la influència de les aigües atlàntiques a la Mediterrània. Globalment, tant l'abundància com la biomassa dels pasifèids del Mar d'Alboran van ser superiors que a la resta de poblacions mediterrànies mostrejades. La profunditat a la qual es comencen a trobar les diferents espècies de pasifèids de la Mediterrània és també menor a la Mar d'Alboran, on *P. sivado* va presentar el major rang de distribució en fondària, així com també la més gran talla de maduresa en femelles. Aquesta talla es va fent progressivament menor a mesura que augmenta la distància a la font d'aigües atlàntiques, és a dir, de la zona de l'Estret de Gibraltar. Amb dues espècies van presentar una estructura de talles variable en funció de la profunditat, amb individus juvenils ocupant profunditats més somes que els adults. Addicionalment, juvenils de *P. multidentata* només es van observar al Mar Català, mentre que van ser absents del Mar d'Alboran, fet que suggereix l'existència de diferències important en l'estacionalitat de la reproducció i/o de la dinàmica de les poblacions.

L'existència de dimorfisme sexual entre mascles i femelles de *P. sivado* es reporta per primera vegada en aquesta tesi, així com de diferències significatives en l'estructura de talles entre sexes. Els cinc pleopodis de les femelles adultes són clarament més fins i allargats que els pleopodis dels mascles adults, els quals són arrodonits anteriorment i tenen una forma més robusta. S'ha detectat una talla crítica a partir de la qual la proporció de sexes de les poblacions canvien cap a una dominància significativa dels mascles. Aquesta talla disminueix progressiva i significativament des de la població més propera a l'Estret de Gibraltar, la del Mar d'Alboran, passant per la població d'Alacant, fins a la població del Mar Català, en concordança amb els canvis observat a la talla de maduresa de les femelles.

Aquesta tesi també presenta una primera aproximació a investigar el paper de l'Estret de Gibraltar sobre l'estructura genètica de les poblacions de pasifèids, utilitzant les dues espècies utilitzades en els capítols precedents, les quals representen espècies congenèriques, simpàtriques geogràficament, però amb una marcada distribució batimètrica diferencial. Les anàlisis efectuades han mostrat que l'Estret de Gibraltar constitueix una forta i única barrera geogràfica per a la connectivitat genètica entre les poblacions d'ambdues espècies situades a una banda i altra de l'Estret. En ambdues esppècies s'ha detectat l'existència d'una marcada estructuració genètica caracteritzada per dos haplotips principals, un que es troba només en poblacions atlàntiques, i un altre únicament en poblacions mediterrànies. Basant-se també en característiques genètiques, s'ha obtingut nova informació referent a la filogènia del gènera *Pasiphaea*, que mostra l'existència d'una important divergència genètica entre *P. sivado* i la resta d'espècies de la família *Pasiphaeidae* de les quals en l'actualitat es té informació, fet que indica que dins del gènere *Pasiphaea* es troben diferents subgrups genètics ben delimitats.

L'estudi de la comunitat de crustacis decàpodes pelàgics en aigües al voltant de l'illa de Mallorca, localitzada entre les conques Algeriana i Catalano-Balear ha mostrat la presència de dues comunitats faunístiques diferenciades, no geogràficament sinó en funció de la batimetria: una en aigües localitzades sobre el límit plataforma-talús i una altra al damunt del talús mig. La comunitat més soma, la del límit plataforma-talús, es va caracteritzar per la dominància d'espècies pràcticament transparents, com Sergestes arcticus, l'espècie que va assolir les abundàncies més elevades, i *Pasiphaea sivado*, espècie omnipresent a tots els nivells pelàgics mostrejats. Els registres a l'ambient pelàgic de *Chlorotocus crassicornis* i *Plesionika heterocarpus*, no citades anteriorment en aquest ambient, es van restringir a les capes de difusió profunda del límit plataforma-talús. Les espècies de coloració més vermellosa (*Gennadas elegans, Pasiphaea multidentata* i *Sergia robusta*) es van localitzar únicament sobre fons del talús mig. No es van trobar diferències significatives en funció de la localització geogràfica o de les dues estacions de l'any mostrejades. Es considera que la comunitat de crustacis decàpodes pelàgics del Mar Balear està fonamentalment estructurada tant per la geomorfologia i característiques hidrogràfiques associades a la transició entre la plataforma i el talús continentals, com per la influència de la llum a la columna d'aigua. No es van trobar espècies de crustacis decàpodes en mostres epipelàgiques preses durant el dia. L'anàlisi de les talles dels individus capturats ha mostrat que els moviments de les diferents classes de talla (edat) a través de la columna d'aigua varien al llarg del cicle dia-nit d'una manera diferencial en funció de cada espècie. Adicionalment, aquest treball confirma clarament que la fracció adulta de la població de Pasiphaea multidenta presenta també la capacitat de dur a terme migracions verticals.

S'és conscient que existeixen avui dia encara molts forats en el coneixement de l'autoecologia i cicle vital tant dels pasifèids com de la resta de decàpodes pelàgics. Moltes qüestions romanents per a comprendre millor l'estructura i dinàmica de poblacions. L' distribució i filogènia podrien ser millor enteses i discutides si hagués més coneixença sobre el cicle vitat, els estadis larvaris i post-larvaris, així com sobre la dispersió i connectivitat de les poblacions. L'elevat esforç de mostreig, i el seu alt cost associat, són encara avui dia una de les raons principals de l'escassetat d'informació disponible.

Paraules clau: Macrozooplàncton, Micronècton, decàpodes bentopelàgics, crustacis nectobèntics, migracions diàries verticals, estructura de talles, dimorfisme sexual, genètica de poblacions, filogènia, Pasiphaeidae, Sergestidae.

Resumo

A presente tese aporta nova informação sobre crustáceos decápodas pelágicos, especialmente sobre os pasifeídeos mediterrâneos: *Pasiphaea sivado* e *Pasiphaea multidentata*. Relevantes diferenças em relação ao padrão de distribuição foram observadas devido a influência das águas atlânticas no mar Mediterrâneo.

Globalmente, tanto a abundância como a biomassa dos pasifeídeos do Mar de Alboran foram superiores ao restante das populações mediterrâneas amostradas. A profundidade na qual se inicia a distribuição das diferentes espécies de pasifeídeos do Mediterrânao é também menor no Mar de Alboran. E foi em Alborán que *P. sivado* apresentou o maior intervalo de distribução sobre o fundo, assim como também o maior tamanho de madurez dentre as fêmeas. O tamanho de madurez foi progressivamente menor a medida que aumenta-se a distância da fonte de águas atlânticas, ou seja, da zona do Estreito de Gibraltar. As duas espécies presentaram uma estrutura de tamanho variável em função da profundidade, com indivíduos juvenis ocupando menores profundidades que os adultos. Adicionalmente, juvenis de *P. multidentata* só foram observados no Mar Catalão, sendo ausentes do Mar de Alboran, fato que sugere a existência de diferênças relevantes na sazonalidade da reprodução e/ou da dinâmica destas populações.

A existência de dimorfismo sexual entre machos e fêmeas de *P. sivado* foi reportada pela primera vez nesta tese, assim como a existência de diferênças significativas na estrutura de tamanhos entre os sexos. Os cinco pleópodos das fêmeas adultas são claramente mais finos e alongados que os pleópodos dos machos adultos, os quais são arredondados anteriormente e têm uma forma mais robusta. Foi detectado um tamanho crítico a partir do qual a proporção de sexos das populações se alterna à uma dominância significativa de machos. Este tamanho diminui progressivamente e significativamente desda população mais próxima ao Estreito de Gibraltar, em sentido à do Mar d'Alboran, passando pela população de Alacante, até a populaçao do Mar Catalao; em concordância com as mudanças no tamanho de madurez observadas dentre as fêmeas.

Esta tese também apresenta uma primeira aproximação ao estudo do papel do Estreito de Gibraltar sobre a estructura genética das populaçoes de pasifeídeos, utilizando as mesmas espécies utilitzadas nos dois primeiros capítulos, as quais representam espécies congêneres, simpátricas geograficamente, porém com uma distribuição batimétrica marcadamente distinta. As análises efetuadas mostraram que o Estreito de Gibraltar constitui uma forte e única barreira geográfica à conectividade genética entre as populações das duas espécies situadas nos difrerentes lados do Estreito. Em ambas espécies foi detectada uma marcada estruturação genética caracterizada por dois haplótipos principais: um que se encontra apenas nas populações atlânticas, e um outro

encontrado somente nas populações mediterrânias. Baseando-se também em características genéticas, nova informação foi obtida referente a filogenia do gênero *Pasiphaea.* Foi observada uma alta divergência genética entre *P. sivado* e as demais espécies da família *Pasiphaeidae,* esta informação já foi encontrada por outros pesquisadores, fato que indica que dentro do gênero *Pasiphaea* existem diferentes subgrupos genéticos bem delineados.

O estudo da comunidade de crustáceos decápodas pelágicos em águas ao redor da ilha de Maiorca, localizada entre as concas Argeriana e Catalano-Balear revelou a presença de duas comunitades faunísticas diferenciadas, não geograficamente mas sim em função da batimetria: uma em águas localizadas sobre a quebra da plataforma continental e outra sobre o talude médio. A comunidade das águas mais rasas, i.e. a da quebra da plataforma, foi caracterizada pela dominância de espécies praticamente transparentes, como *Sergestes arcticus*, a espècie que apresentou as abundâncias mais elevadas, e *Pasiphaea sivado*, espécie onipresente em todos os níveis pelágicos amostrados. Os registros de *Chlorotocus crassicornis* e *Plesionika heterocarpus* no ambiente pelágico, não citados anteriormente neste ambiente, foi restrito à camada de difusão profunda da quebra da plataforma. As espécies de coloração mais avermelhada (*Gennadas elegans*, *Pasiphaea multidentata* e *Sergia robusta*) ocorreram apenas sobre o fundo do talude médio. Não foram encontradas diferênças significativas em função da localização geográfica ou das duas estações do ano amostradas. Sabe-se que a comunidade de crustáceos decápodas pelágicos do Mar Balear esta fundamentalmente estruturada tanto pela geomorfologia e características hidrográficas associadas à transição entre a plataforma e o talude continental, como pela influência da luz na coluna d'água. Não foram encontradas espécies de crustáceos decápodas nas amostras epipelágicas realizadas durante o dia. A análise de tamanho corporal dos indivíduos capturados evidenciou que o deslocamento vertical das diferentes classes de tamanho (idade) através da coluna d'água variou ao longo do ciclo dia-noite de forma diferente em cada espécie. Adicionalmente, este estudo confirma claramente que a fração adulta da população de *Pasiphaea multidenta* apresenta também a capacidade realizar migrações verticais.

Hoje em dia ainda existe muitas lacunas no conhecimento da autoecologia e ciclo de vida de pasifeídeos bem como sobre decápodas pelágicos em geral. Muitas questões remanescem para melhor compreender a estrutura e dinâmica das populações estudadas. A distribuição e filogenia, poderiam ser melhor entendidas e discutidas se houvesse mais conhecimento sobre o ciclo de vida, os estados larvários e post-larvários, assim como sobre a disperção e conectividade das populações. O elevado esforço amostral e o seu alto custo associado, são ainda hoje uma das principais razões da escassez de informação disponível.

Palavras-chave: Macrozooplâncton, Micronécton, decápodas bentopelágicos, crustaceos nectobentônicos, migracões verticais diárias, estrutura de tamanhos, dimorfismo sexual, genética de populações, filogenia, Pasiphaeidae, Sergestidae.

Resumen

Esta tesis presenta nueva información sobre crustáceos decápodos pelágicos, principalmente sobre los pasiféidos presentes en el Mediterráneo: *Pasiphaea sivado* y *Pasiphaea multidentata*. Se han encontrado diferencias importantes en las pautas de distribución de los pasiféidos en relación a la influencia de las aguas atlánticas en el Mediterráneo. Generalmente, tanto la abundancia como la biomasa de los pasiféidos en el Mar de Alborán fueron mayores que en el resto de las poblaciones muestreadas en el Mediterráneo de la Península Ibérica. La profundidad en la cual se comienzan a encontrar las diferentes especies de pasiféidos del Mediterráneo es también menor en el Mar de Alborán, donde *P. sivado* presenta el mayor rango de distribución de profundidad, así como la mayor talla de madurez en las hembras. Esta talla disminuye a medida que aumenta la distancia a la entrada de las aguas atlánticas, es decir, al Estrecho de Gibraltar. Ambas especies presentan una estructura de tallas variable en función de la profundidad, ocupando los individuos juveniles profundidades más someras que los adultos. Adicionalmente, solamente se han encontrado juveniles de *P. multidentata* en el Mar Catalán, mientras que no se observaron en el Mar de Alborán, lo que sugiere la existencia de importantes diferencias en la estacionalidad de la reproducción y/o de la dinámica de las poblaciones.

La existencia de dimorfismo sexual entre hembras y machos de *P. sivado* se describe por primera vez en esta tesis, así como las diferencias significativas en la estructura de tallas entre sexos. Los cinco pleópodos de las hembras adultas son claramente más finos y alargados que los de los machos adultos, los cuales son redondeados anteriormente y tienen una forma más robusta. Se ha detectado una talla crítica a partir de la cual la proporción de sexos de las poblaciones cambia hacia una dominancia significativa de los machos. Esta talla disminuye progresiva y significativamente desde la población más cercana al Estrecho de Gibraltar, la del Mar de Alborán, pasando por la población de Alicante, hasta la población del Mar Catalán, en concordancia con los cambios observados en la talla de madurez de las hembras.

Esta tesis también presenta una primera aproximación a la determinación del papel del Estrecho de Gibraltar sobre la estructura genética de las poblaciones de los pasiféidos, utilizando las dos especies empleadas en los capítulos precedentes, las cuales son especies congenéricas, simpátricas geográficamente, pero con una distribución batimétrica marcadamente diferente. Los análisis efectuados han mostrado que el Estrecho de Gibraltar constituye una fuerte y única barrera geográfica para la conectividad genética entre las poblaciones de las dos especies situadas a ambos lados del estrecho. En ambas especies se ha detectado la existencia de una marcada estructuración genética caracterizada por dos haplotipos principales, uno que se encuentra únicamente en

poblaciones atlánticas, y el otro únicamente en poblaciones mediterráneas. Basándose también en características genéticas, se ha obtenido nueva información referente a la filogenia del género *Pasiphaea*, la cual muestra la existencia de una importante divergencia genética entre *P. sivado* y el resto de especies de la familia Pasiphaeidae de las que se tiene información en la actualidad, hecho que indica que dentro del género *Pasiphaea* se encuentran diferentes subgrupos genéticos bien delimitados.

El estudio de la comunidad de crustáceos decápodos pelágicos en aguas circundantes a la isla de Mallorca, situada entre las cuencas Argelina y Catalano-Balear ha demostrado la presencia de dos comunidades faunísticas diferenciadas, no geográficamente sino en función de la batimetría: una en aguas localizadas sobre el límite plataforma-talud y otra sobre el talud medio. La comunidad más somera, la del límite plataforma-talud, se caracteriza por la dominancia de especies prácticamente transparentes, como *Sergestes arcticus*, la especie que mostró abundancias más elevadas, y *Pasiphaea sivado*, especie omnipresente en todos los niveles pelágicos muestreados. Los registros, *Chlorotocus crassicornis* y *Plesionika heterocarpus,* citados por primera vez en el ambiente pelágico, se restringieron a las capas de difusión profunda del límite plataforma-talud. Las especies de coloración rojiza (*Gennadas elegans*, *Pasiphaea multidentata* y *Sergia robusta*) se localizaron únicamente sobre el fondo del talud medio. No se encontraron diferencias significativas en función de la situación geográfica o de las dos estaciones del año muestreadas. Se considera que la comunidad de crustáceos decápodos pelágicos del Mar Balear está fundamentalmente estructurada tanto por la geomorfología y características hidrográficas asociadas a la transición entre la plataforma y el talud continental, como por la influencia de la luz en la columna de agua. No se observaron especies de crustáceos decápodos en muestras epipelágicas tomadas durante el día. El análisis de las tallas de los individuos capturados ha demostrado que los movimientos de las diferentes clases de talla (edad) a través de la columna de agua varían a lo largo del ciclo día-noche de manera diferencial en función de cada especie. Adicionalmente, este trabajo confirma claramente que la fracción adulta de la población de *Pasiphaea multidentata* presenta también la capacidad de llevar a cabo migraciones verticales.

Se es consciente que hoy en día aún existen carencias en el conocimiento de la autoecología y del ciclo vital tanto de los pasiféidos como del resto decápodos pelágicos. Muchas cuestiones remanentes sobre la estructura y dinámica de poblaciones, distribución y filogenia, podrían ser mejor comprendidas y discutidas si hubiera un mayor conocimiento sobre el ciclo vital, los estadios larvarios y post-larvarios, así como sobre la dispersión y conectividad de las poblaciones. El elevado esfuerzo de muestreo, y su alto coste asociado, son todavía hoy en día una de las principales razones de la escasez de información disponible.

Palabras clave:

Macrozooplancton, Micronecton, decápodos bentopelágicos, crustáceos nectobentónicos, migraciones diarias verticales, estructura de tallas, dimorfismo sexual, genética de poblaciones, filogénia, Pasiphaeidae, Sergestidae.

Introduction

Introduction

i.i. Pelagic decapod crustaceans

Pelagic decapod shrimps have a widespread geographic and bathymetric distribution from intertropical waters to high latitudes in both hemispheres (Casanova and Judkins 1977, Fasham and Foxton 1979, Clarke and Holmes 1987, Burghart *et al.* 2007). They comprise an important biomass fraction of the faunistic communities of the shelf break and slope (Omori 1974, Long *et al.* 1995, Sumida and Pires-Vanin 1997, Abelló *et al.* 2002, Company *et al.* 2004, Serejo *et al.* 2007, Callaway *et al.* 2007, Escobar-Briones *et al.* 2008, Delgado *et al.* 2013), which are continental margin areas of intense and increasing exploitation by the trawl fishery around the world (Orensanz *et al.* 1998, Callaway *et al.* 2007). These shrimps are relatively small and usually have no commercial interest. However, benthopelagic shrimps are very frequent in fishery hauls (Abelló *et al.* 2002), but, with some exceptions (e.g. *Pasiphaea japonica*) where they have become the targets of a specific fishery (Nanjo and Ohtomi 2009) or when they are sold as an accompanying by-catch in non-industrial fisheries (Ramirez-Llodra *et al.* 2007), virtually all their captures are discarded and consequently they are not landed nor sold. Notwithstanding they are a substantial, when not fundamental, part of epibenthic food chains on the continental slope (Rosecchi *et al.* 1988, Hassani, *et al.* 1997, Polunin *et al.* 2001, Serrano *et al.* 2003, Layman *et al.* 2005, Fanelli and Cartes 2008, Battaglia *et al.* 2013).

An inherent characteristic of the benthopelagic decapods inhabiting the slope is their marked behavioural rhythms (Mauchline 1972, Omori 1974, Macquart-Moulin and Patriti 1993). During the day hours these shrimps dwell on or close to the sea floor, in the so called *Benthic Boundary Layer* (BBL), whereas during the night they ascend in the water column to feed in surface or sub-surface layers of the ocean (Aguzzi *et al.* 2007). From the energetic point of view these shrimps act as exporters of primary production from the superficial photic zones to deep sea aphotic water masses (Maynou and Cartes 1998).

In oligotrophic zones, the primary production of the organisms that dwell near bottom areas of the slope and bathyal zones depends, almost exclusively, on the epipelagic planktonic production of the photic zone (Mauchline 1972, Eppley and Peterson 1979, Pauly and Christensen 1995, Cartes and Maynou 1998, Cartes *et al.* 2001, Cartes *et al.* 2002, Cusson and Bourget 2005, Tecchio *et al.* 2013). In this light, the role of the vertical migrators such as pelagic shrimps is the key for the functioning of the slope and bathyal ecosystems (Cartes and Maynou 1998). There are some families of pelagic decapods, with cosmopolitan distribution, such as the Pasiphaeidae or Sergestidae that are well adapted to inhabit the deep sea as well as to perform vertical migrations (Cartes *et al.* 1993, Cartes 1993b, Aguzzi *et al.* 2007).

Trophic relationship studies based on the stomach content analysis in fishes (Rosecchi *et al.* 1988, Hassani *et al.* 1997, Polunin *et al.* 2001, Serrano *et al.* 2003, Layman *et al.* 2005) showed that pasiphaeid shrimps are preyed upon by a variety of nektonic organisms with both commercial and ecological interest (Rosecchi *et al.* 1988, Orsi-Relini and Relini 1990, Cartes 1994, Hassani *et al.* 1997, Garison and Link 2000, Polunin *et al.* 2001, Serrano *et al.* 2003, Layman *et al.* 2005, Fanelli and Cartes 2008,Tecchio *et al.* 2013, Battaglia *et al.* 2013). The high frequency of occurrence and abundance associated to the vertical migratory behaviour in this family (Aguzzi *et al.* 2007) has demonstrated their key role in the trophic ecology of slope and demersal environments (Skjoldal and Bamstedt 1977, Maynou and Cartes 1998, Polunin *et al.* 2001, Cartes *et al.* 2007a, Tecchio *et al.* 2013).

In the Mediterranean Sea the Pasiphaeidae and Sergestidae are the most common families of pelagic decapods. In special, the congeneric pasiphaeid species *Pasiphaea sivado* and *Pasiphaea multidentata*, together with the sergestid *Sergestes arcticus*, are the most frequent and abundant benthopelagic decapod shrimps along the western Mediterranean coasts (Abelló *et al.* 1988, 2002, García-Rodríguez *et al.* 2011).

i.ii. General aim

The main aim of the present thesis is to provide new knowledge on pelagic decapod crustaceans of the western Mediterranean continental slope ecosystem, focusing on distribution characteristics and their association with geomorphology and water masses, population biology, genetics and benthopelagic behaviour. Specific goals are detailed below and in the presentation of each chapter. The main target species of the study are those belonging to the caridean shrimp family Pasiphaeidae, particularly *Pasiphaea sivado* and *Pasiphaea multidentata* since they are the most common and abundant benthopelagic species in the study area when sampled with epibenthic and demersal gears.

Since scientific knowledge constitutes the basis for fisheries and conservation management, it is expected that the information provided will be useful both as an increase in basic knowledge and as a contribution for the sustainable management of the exploited ecosystems of the continental slope. The cosmopolitism of the crustacean families focus of this study implies that a specific approach in a particular pelagic/benthopelagic ecosystem can be widely extrapolated to similar ecosystems functioning in geographically distant areas. In this light, the description of the ecology of pelagic decapods provides also a basis for the knowledge of the dynamics, functioning and interrelationships between the pelagic and deep sea systems.

i.iii. Study area

The overall study area encompassed the western Mediterranean Sea along the coasts of the Iberian Peninsula from the Gibraltar Strait in the SW to Cape Creus in the NE, including the Balearic Islands. For the genetic studies, the study area was enlarged as to comprise the Gulf of Cadiz, west of Gibraltar, in the NE Atlantic. The semi-arid climatology of the Mediterranean Sea area is characterised by an excess of evaporation in relation to pluviometry and river input. This disequilibrium, together with the seasonal increase of sea surface temperatures in summer, confers to these semi-closed

Mediterranean Sea high values of both salinity and density. High evaporation rates due to strong, extremely dry, cold, northerly winds from the European subcontinent also contribute to the salinity increase, especially so in winter.

Figure 1: Map of the Mediterranean iberic Sea showing the morphology of the hidrographic basins and the conection with the Atlantic ocean.

The Gibraltar Strait is the most important entrance of the Mediterranean Sea with receives large amounts of Atlantic surface water (in an order of 106 m^3s^{-1}) to compensate the water loss due to evaporation. The western Mediterranean Sea is composed by two geomorphologic basins: The Algerian and the Balearic (or Catalano-Balearic) basins. The Algerian basin is located east of the Gibraltar Strait, becoming the first Mediterranean basin that receives Atlantic water. Density differences imply that Atlantic water enters the Mediterranean Sea through the surface water layer towards the east, while denser Mediterranean water leaves this sea through deep layers in smaller volumes. Incoming Atlantic water is slowly altered during their trajectory by mixing with resident Mediterranean water masses and small river input, and also by evaporation (Tizperman and Malanotte-Rizzoli 1991, Cacho *et al.* 1999, Millot and Taupier-Letage 2005) (Figure 1). Details about the oceanography of the area will be presented in the next chapters as specific information will be required.

i.iv. Specific Goals and chapters

The present thesis is structured in four main chapters, in agreement with four main specific goals.

i.iv.i. Chapter 1:

Pasiphaeid shrimp populations in the western Mediterranean: geographical variability in distribution and population patterns

The aim of this first chapter is to analyse the geographic and bathymetric distribution of the congeneric species *P. sivado* and *P. multidentata* along the Mediterranean coasts of the Iberian Peninsula in terms of both density and biomass, to determine some population characteristics, namely size structure and size at maturity along 10 geographic subareas, as well as to analyse the relationships between abundance and hydrological characteristics, namely bottom temperature and salinity. The study is based on an extensive data set of 15 annual fishery research cruises (1994-2008) along the study area. The specific objectives that guided this chapter were:

- To determine the depth range and preferential depth of occurrence and abundance (density and biomass) of both species along the Iberian Mediterranean coast (10 geographic/geomorphologic subareas) and along the sampled depth gradient (40-800 m).

- To analyse the population size structure of the species along the Iberian Mediterranean coast and the sampled depth gradient.

- To assess the variability in size of maturity of *P. sivado* along the Iberian Mediterranean coast.

- To analyse the relationships between abundance and hydrological characteristics, namely bottom temperature and salinity, taking into account the ontogeny of the species (juveniles vs. adults).

i.iv.ii. Chapter 2:

Sexual dimorphism in the benthopelagic shrimp *Pasiphaea sivado* (Crustacea: Caridea: Pasiphaeidae)

Indications of the existence of sexual dimorphism in pleopod shape were observed in *P. sivado* during field biological data acquisition. Accordingly, pleopod shape was tested using morphological and geometrical morphometrics techniques. Gender differences in sex size structure were also tested to verify the occurrence of additional sexual dimorphism characteristics. The main objectives of this chapter were:

- To verify whether the pleopod protopod overall shape, as well as its length-width ratio, varies between genders in adult individuals.
- To assess whether size structure varies between genders, globally, geographically and bathymetrically.
- To verify whether the sex ratio varies as a function of size, depth and geographic sector.

i.iv.iii. Chapter 3:

Population genetics and phylogeny of pasiphaeid shrimps from the western Mediterranean Sea

This chapter is a first attempt to investigate the influence of the Gibraltar Strait on the genetic population structure of both *P. sivado* and *P. multidentata*. Additionally, the chapter also aims at assessing the internal phylogeny of the Pasiphaeidae using own data and all the available sequences of pasiphaeid species in Genbank database. The goals intended were:

- To characterize the genetic structure of pasiphaeid species populations (*P. sivado* and *P. multidentata*) across the influence of the Atlantic-Mediterranean transition.

- To verify whether there is a genetic gradient within the Mediterranean in the target species as a function of the Atlantic water influence in this sea.

- To quantify the genetic distance within the Pasiphaeidae.

- To provide new insights on the phylogenetic relationships within the Pasiphaeidae.

i.iv.iv. Chapter 4:

Vertical and seasonal distribution of pelagic decapod crustaceans over the shelf-break and middle slope in two contrasting zones around Mallorca (western Mediterranean Sea)

The aim of this chapter was to characterize the pelagic/benthopelagic decapod community, as well as to describe their diel vertical movements and determine their size distribution across the pelagic environment in two extreme photoperiod seasons in two areas around Mallorca characterized by different oligotrophic regimes as well as water mass characteristics and dynamics. The main objectives that conducted this chapter were:

- To characterize the faunistic composition of the pelagic decapod species according to the different sampling cells (SCs): season (late autumn / summer), geographic sector (Cabrera / Sóller), bathymetry (shelf edge / slope) and day time (day / night).

- To quantify the abundance of pelagic decapod species in the different SCs, and therefore estimate their role in the benthopelagic environment.

- To determine the depth range of each pelagic decapod species.
- To verify whether and how the depth distribution of each species varies along the day time.
- To assess the occurrence of differential species assemblages as a function of the SCs.
- To estimate the diversity indexes of the pelagic decapod assemblages identified.
- To describe the size structure of the pelagic decapods according to depth and the significant assemblages identified.

i.v. Presentation of the main benthopelagic species target of this thesis

i.v.i. Infraorder *Caridea*

i.v.i.i. Family Pasiphaeidae

The Pasiphaeidae family (Dana 1852) (Pleocyemata, Caridea) is distributed along a wide latitudinal range, from tropical to arctic and subantarctic waters, mainly on and above continental slopes and in the open sea, including deep-sea habitats (Clarke and Holmes 1987, Kaartvedt *et al.* 1988, Gibbons *et al.* 1994, d'Udekem & d'Acoz 1999, Tavares and Cardoso 2006). This family is represented by seven genera and 98 species: *Alainopasiphaea* (Hayashi 1999) (2), *Eupasiphae* (Wood-Mason 1893) (4), *Glyphus* (Filhol 1884) (1), *Leptochela* (Stimpson 1860) (15), *Parapasiphae* (Smith 1884)(4), *Pasiphaea* (Savigny 1816) (67) and *Psathyrocaris* (Wood-Mason 1893)(5) (De Grave and Frasen 2011, Komai *et al.* 2012).

Morphological features in this family, such as lateral compression of the body and pleopods as well as reduced rostrum, are considered to be the result of selective pressures on adaptation to the pelagic environment (Omori 1974, Johnsen 2005, Sardà *et al.* 2005, Aguzzi *et al.* 2007). These body characteristics confer to these shrimps the ability to maintain their position in the pelagic environment as well as to migrate promoting fast and wide jumps horizontally above the substrate or even vertically in relation to the sea bed.

Notwithstanding the large amount of taxonomic and faunistics work on this family (e.g. Stephensen 1923, Zariquiey Álvarez 1957, Iwasaki 1990, Hayashi 1999, Tavares and Cardoso 2006) there are still few detailed studies on biological aspects of species of the family, and most of them refer mainly to the European species *P. sivado* and *P. multidentata* (Cartes 1993a, Company and Sardá 2000, Frank 2000, Company *et al.* 2001, Ramirez-Llodra *et al.* 2007), but also to Pacific Ocean species such as *Pasiphaea japonica* and *Leptochela gracilis* (Nanjo 2007, Oh *et al.* 2006). In the literature, the majority of the studies on Pasiphaeidae shrimps concern information on the distribution and abundance in relatively restrict geographic and bathymetric scales, almost always in the context of deep benthic communities analysis (Cartes *et al.* 1994, Maynou *et al.* 1996, Koukouras *et al.* 2000, Abelló *et al.* 2002, Serejo *et al.* 2007). Overall, information on the biology of the Mediterranean pasiphaeid species is not scarce (Casanova 1976, Cartes 1993b, Maynou & Cartes 1998, Frank *et al.* 2000, Company & Sardá 2000, Company *et al.* 2001, Ramirez-Llodra *et al.* 2007, Aguzzi *et al.* 2007). A specific work on the biologies of *Pasiphaea sivado* and *P. multidentata* in the northwestern Mediterranean was published by Company *et al.* (2001) (Table 1).

Table 1: Summary of aspects related to the life history of *Pasiphaea sivado* and *P. multidentata*. The minimum body size (Min.), maximum (Max.) and asymptotic length (L∞) are referred to cephalothorax length in millimetres. Adapted from Company and Sardá (2000) and Company *et al.* (2001).

Population biology

	Sex ratio fits 1:1	Sex	Min	Max	L∞	K	ϕ	age (L∞)	smallest ovigerous female	Reproductive period	Recruitment	No. eggs by Females (±SD)
P. sivado	no	female	10	23.2	26.0	0.55	0.057	2	14.7	February and October	late spring	49
		Male	10	24.3	27.5	0.62	0.126	2				(±21.5)
		All	7	24.3	29.5	0.55	0.162					
P. multidentata	no	female	21	46.7	48.5	0.85	0.833	3 to 4	29.9	February and November	late spring	12
		Male	19	42.7	44.4	0.77	0.807	3 to 4				(±4.5)
		All	6	46.7	50.0	0.80	0.687					

Among all the species studied in the present thesis the pasiphaeid species show the largest mean body length ($CL_{min\text{-}max}$= 7 – 24.3 mm for *P. sivado* and 6.5 - 46.7 mm for *P. multidentata*) which in addition to their high abundance provide a very high biomass. In the Japan Sea, *Pasiphaea japonica* is a key commercial species in Toyama Bay. Recently the annual catches of *P. japonica* in this bay have increased from 400-500 t annually up to an average of 600-700 t which consequently called for implementation of appropriate stock management strategies in order to ensure the sustainability of the fishery (Nanjo and Ohtomi 2009). Nevertheless, in the western Mediterranean both *P. sivado* and *P. multidentata* are very common and abundant species, particularly *P. sivado,* but hardly no economic interest nor policy rules exist concerning these species.

Pasiphaea sivado (Risso, 1816)

Also known as glass shrimp, this species has an overall transparent body with small red spots or aggregation of little spots restricted to a few edge areas of the extremities of the somites and pereiopods. The species performs diel vertical migrations, inhabiting near the bottom during the day and moving upwards at night to near surface layers (Stephensen 1923, Franqueville 1971) (Figure 2). *P. sivado* is commonly caught by demersal trawling during the day on the upper slope along the Mediterranean Sea and northeast Atlantic from Morocco to Norway (Sund 1913, Stephensen 1923, Maurin 1961, González-Gurriarán and Olaso 1987; Abelló *et al.* 2002). It is also a very common species when sampled by pelagic/micronekton trawl surveys (Franqueville 1971, Koukouras *et al.* 2000).

P. sivado is a typical temperate species (Udekem d'Acoz 1999). In the Mediterranean the species inhabits both the eastern and western basins (Stephensen 1923). Environmental variations due to seasonality appear to have no large effect in the distribution structure and abundance of the species in Atlantic and Mediterranean populations at around 40°N of latitude (Fariña *et al.* 1997, Maynou *et al.* 1996). *P. sivado* is often the dominant species of bottom trawl surveys rather than other species target of the fisheries, including fishes, molluscs, and other crustaceans. In south Portugal the species occurred in so high densities that was classified as the typical species of the slope zone (Fariña *et al.* 1997), being also the dominant species in the Gulf of Cadiz (Delgado *et al.* 2013).

In the Mediterranean it was the dominant species in the Spanish coast between 300-400 m strata (524 ind.h^{-1}) (Abelló *et al.* 1988, 2002) and in the central-western coast of Italy at 300-500 m (Fanelli *et al.* 2007).

The widespread geographic and bathymetric distributions, the daily vertical migration pattern together with their high abundance provides to *P. sivado* a very important role in the flux of matter and energy between the epipelagic productive zone and the demersal aphotic zone. Stomach content studies on Atlantic specimens have shown that *P. sivado* preys on euphausiids and calanoid copepods (Lagardère 1972). In the Catalan Sea the feeding activity of *P. sivado* is more intense during the night when suprabenthic crustaceans such as mysids and amphipods are the main food items. During the day mysids remain one of the most important food items together with mesopelagic fishes (Cartes 1993b). Pasiphaeid shrimps are preyed upon by several crustaceans and pelagic species including fishes, cephalopods, dolphins and even fin whales (Rosecchi *et al.* 1988, Orsi-Relini and Relini 1990, Cartes 1994, Hassani *et al.* 1997, Lorentsen *et al.* 1998, Serrano *et al.* 2003, Fanelli and Cartes 2008, Battaglia *et al.* 2013).

Figure 2: A fresh sampled *Pasiphaea sivado* female bearing eggs in the abdomen. Photography by Pere Abelló.

In the Japan Sea the sister species *P. japonica* feeds mainly on mysids and euphausiids, but also on other crustaceans, fishes and cephalopods. Euphausiids were preyed in higher amounts during the summer, following their seasonal abundance bloom in Toyama Bay. These euphausiids were the exclusive prey item of the youngest size class of *P. japonica* (15 mm CL). Cephalopods occurred in foregut contents only in July and November, being preyed manly by small individuals (16 mm CL) (Nanjo 2007).

Overall, the majority of the published data about *P. sivado* species reports their presence in the pelagic and epibenthic communities. However, due to its relevant ecological importance, *P.*

sivado biology and life history has been often studied in the western Mediterranean. In the Catalan Sea *P. sivado* sampled near the bottom has been reported to range from 7 to 24 mm of cephalothorax length (CL), its longevity was estimated to be around 2 years, the smallest ovigerous female sampled had 14.7 mm CL, and their reproduction is continuous throughout the year, although peaking in autumn-winter (Company and Sardà 2000, Company *et al.* 2001, see table 1). *P. sivado* females of the Ligurian Sea also reproduce continuously, however females parasitized by *Amallocystis* (Protozoa: Ellobiopsidae) were never seen reproducing (Orsi-Relini and Pinca 1990).

In the Irish Sea the species shows two reproductive peaks. According to Williamson (1960) final stage embryos occurred from June to December with two peaks, in July and November. In the same work the author reported for the first time the morphology of embryos, four zoeal stages and megalopa or post-larvae. The distribution of larvae and early juveniles remains unknown, except for the finding of about 20 post-larvae close to the bottom at around 100 m in the Irish Sea (Williamson 1960).

Within the genus *Pasiphaea*, *P. sivado* is the main representative of a group of nine pasiphaeid species that is distinguished from the rest of pasiphaeids by having a reduced branchial formulae (Hayashi 1999, 2006). The *P. sivado* group is composed by those species having five pleurobranchiae (*P. sivado* (Risso,1816), *P. propinqua* De Man, 1916, and *P. japonica* Omori, 1976) while the remaining group is composed by species that present four pleurobranchiae (*P. debitusae* Hayashi, 1999, *P. fragilis* Hayashi, 1999, *P. gracilis* Hayashi, 1999, *P. laevis* Hayashi, 1999, *P. marisrubri* Iwasaki, 1989, *P. philippinensis* Hayashi, 1999, and *P. mclaughlinae* Hayashi, 2006).

The report of morphological differences between Atlantic and Mediterranean Sea populations of pasiphaeids was firstly reported by Sivertsen and Holthuis (1956) and Zariquiey Álvarez (1957). In 1976, Omori related that Mediterranean specimens of *P. sivado* presented a higher number of spinules on the merus of the first and second pereiopods when compared to Atlantic specimens.

Pasiphaea multidentata Esmark, 1866

Pasiphaea multidentata is a cold temperate species (Udekem d'Acoz 1999) that inhabits benthic boundary layers in the middle and lower slope down to 2000 m depth (Cartes 1993a; Abelló *et al.* 2002; Tecchio *et al.* 2011) (Figure 3). The species also performs vertical migrations into upper water layers up to 50-200 m of the water column at night (Franqueville 1971), although vertical displacements have been apparently restricted to juvenile individuals (Aguzzi *et al.* 2007, Cartes 1993c) (see also Chapter 4). The species has a trans-oceanic distribution comprising northern latitudes of both the eastern and western north Atlantic from the Gulf of Maine (69° W) to the west coast of Europe (18° E) and latitudinally from Tromsö (69° N), Norwegian Sea, down to Mauritania (20° N), including the Mediterranean Sea (Udekem d'Acoz 1999). Concerning the bathymetric range of the species in the benthic environment, the upper limit of distribution overlaps with that of *P. sivado*. Younger individuals of *P. multidentata* with about 10-20 mm CL inhabit shallower waters (together with *P. sivado*) than adults, which can reach about 47 mm CL (Company *et al.* 2001).

In the Mediterranean Catalan Sea the reproductive cycle of *P. multidentata* is markedly seasonal (Company *et al.* 2001). However differences concerning the reproductive period and seasonality of the species have being reported along their geographical distribution. In the gulf of Maine the spawning period takes place in two seasons: spring and autumn (Apollonio 1969), while in the western coast of Norway ovigerous females occur all year round (Matthews and Pinnoi 1973).In the Catalan Sea the size structure of *P. multidentata* ranges from juveniles with about 6 mm CL to adults with about 47 mm CL (Company *et al.* 2001). Population dynamics estimations showed that *P. multidentata* can live as long as 3.5 years (Table 1) (Company *et al.* 2001; Ramirez-Llodra *et al.* 2007). The species present a very marked seasonal reproduction with ovigerous females being only present from September to February. A well defined recruitment period takes place in early spring, showing a clearly different pattern to that of the shallower congeneric species *P. sivado* that reproduces continuously in the same area. Different reproduction periodicity has been reported for both species depending on the geographic study area (Stephensen 1923, Williamson 1960, Apollonio 1969, Mattews and Pinnoi 1973).

Feeding habits studies show that the in the Gulf of Maine, northwest Atlantic, the main food items of *P. multidentata* are based on pelagic copepods and euphausiids (Apollonio 1969). In the Gulf of Gascogne, northeastern Atlantic, the diet of *P. multidentata* is based on mesopelagic fishes and juvenile cephalopods, decapods such as *S. arcticus* and euphausiids; no predatory activity was found during the day (Lagardère 1977). In the Catalan Sea *P. multidentata* specimens sampled near the bottom, preyed mainly on mesopelagic fishes, euphausiids and isopods (Cartes 1993b). Nocturnal feeding activity was just observed in large individuals (>28 mm CL) that stayed near the bottom during the night time, which fed on suprabenthic organisms such as *Gennadas elegans*, *P. sivado*, *P. multidentata*, sergestid shrimps and little mesopelagic fish as myctophids and *Cyclothone* spp., while individuals sampled during the day evidenced a foraging on pelagic hyperiids, fishes, euphausiids and chaetognaths ingested the night before (Cartes 1993b). Different dietary composition was also found by the same author depending on the sampled depth. Thus, on the middle slope, *P. multidentata, P. sivado* and *S. arcticus* were the main decapod prey items of *P. multidentata*, while in the lower slope *Gennadas elegans* and *Sergia robusta* were the most common decapod prey of *P. multidentata*. *P. sivado* was the second most important food item of *P. multidentata* individuals captured between 380-540 m (Cartes 1993b), with correspond to the preferential occurrence depth of this prey (Abelló *et al.* 2002). Overall, low dietary overlap was observed among *P. sivado* and *P. multidentata* by Cartes (1993b), who supposed that it could be due the different preferential size spectra of the food items preyed by the these pasiphaeids. In this sense,

Figure 3: (a) *Pasiphaea multidentata* individuals sampled by a micronekton trawl. One adult individual is placed vertically while juvenile individuals are placed horizontally. (b) a typical adult captured by a demersal trawl.

Lagardère (1977) stated that the different depth range among the same pasiphaeids and other sergestid species prevents any trophic competition between them.

Morphological divergences between western Mediterranean and Atlantic (North Sea) populations of *Pasiphaea multidentata* were described by Zariquiey Alvarez (1957), mainly concerning the postrostral spine shape (with Mediterranean specimens showing a longer and more pointed rostrum).The number of spinules on the merus of the first pereiopod, and on the basis and merus of the second pereiopod, showed also differences, as in *P. sivado*. The Mediterranean form of *P. multidentata* was even described as a different species, under the name of *Pasiphaea* (Phye) *sicula* Riggio, 1896, later on considered as a subspecies of *P. multidentata* (Zariquiey Álvarez 1946). Sivertsen and Holthuis (1956) did not accept the species status of *P. sicula*. After that Zariquiey Alvarez in 1957 reported that his father R. Zariquiey Cenarro had a paper in press detailing the differences between Mediterranean and Atlantic *P. multidentata* populations that was unfortunately lost during an air raid that destroyed the press during the Spanish civil war (1936-1939). He concluded that Mediterranean specimens could be assigned to *P. multidentata*, but clearly remarked the occurrence of these morphological differences that could in the future assign the Mediterranean specimens to a different "race", as then reported by him, or significantly different populations.

i.v.i.ii. Family *Pandalidae*

Chlorotocus crassicornis (A. Costa, 1871)

Chlorotocus crassicornis is a nektobenthic species with an almost cosmopolitan distribution ranging from the North Atlantic through the Mediterranean, south-east Africa and Indo-Pacific region with a reported depth distribution ranging from 3-597 m (d'Udekem d'Acoz 1999, Li 2006). In the Mediterranean the species occurs from the mid continental shelf to the upper slope (D'onghia *et al.* 1998, Abelló *et al.* 2002), information about their biology and distribution is scarce (Carbonell and Abelló 1998, Vafidis *et al.* 2004, 2008). It shows a marked rhythmic night pattern of catchability on continental shelf waters that shifts towards a dusk and dawn pattern on the upper slope (Aguzzi *et al.* 2007).

Plesionika heterocarpus (A. Costa, 1871)

Plesionika heterocarpus is a nektobenthic species distributed along the eastern Atlantic and Mediterranean Sea, being a very common species of epibenthic trawl surveys. In the western Mediterranean, its depth distribution is comprised berween the deep continental shelf to the middle slope (Abelló *et al.* 1988, 2002). In the Catalan Sea the species reproduces continuously around the year (Company and Sardà 2000, Company *et al.* 2003). Overall most knowledge on the species concerns its main life history characteristics, distribution, population genetics, role as a fishery bycatch, etc. (e.g. Maynou *et al.* 1996, Carbonell *et al.* 2003, Vafidis *et al.* 2005, Fanelli and Cartes 2008, Vafidis *et al.* 2008, Muñoz *et al.* 2012, Guijarro *et al.* 2012, García-Merchán *et al.* 2012).

i.v.ii. Suborder *Dendrobranchiata*

Species of the suborder Dendobranchiata comprise a group of phylogenetically basal decapods that, among other characteristics, differ from the Caridea and Stenopodidea shrimps by the branching form of the gills and by the fact that they release their eggs directly to the water instead of brooding them under the abdomen. Among the Dendobranchiata, shrimps of the family Sergestidae and Benthesicymidae are among the commonest pelagic decapod species (Figure 5).

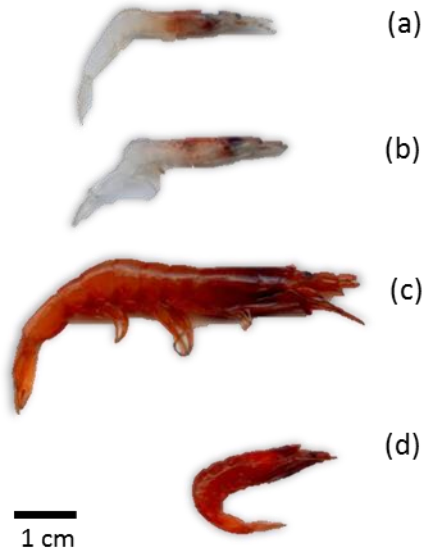


Figure 4: Scanner image of (a) *Sergestes arcticus*, (b) *Sergestes arachnipodus*, (c) *Sergia robusta* and (d) *Gennadas elegans*.

i.v.ii.i. Family *Sergestidae*

Judkins and Kensley (2008) made a thorough systematic study of this family and assigned some species to new genera. In the present thesis the proposed nomenclature of those authors was not followed since there is still some controversy about it and it is not completely accepted by the crustacean taxonomists. New changes are envisaged in the near future due to a wider used of genetic analyses, therefore the "classical" nomenclature was followed throughout this thesis.

Sergestes arcticus Krøyer, 1855

Sergestes arcticus is an almost transparent species with red pigmentation restricted to some areas of its cephalothorax (Figure 4) (Vestheim and Kaartvedt 2009). The species performs vertical migrations where adults are distributed from 80 to 2100 m (Koukouras *et al.* 2000, Gartner *et al.* 2008) and preferentially from 500 m below the surface during the day and below around 150 m during the night (Franqueville 1971, Koukuras 2000). In the northwestern Mediterranean *S. arcticus* is one of the most abundant pelagic decapod species captured by pelagic IKMT trawls representing 65.4 % of the total capture (Franquevile 1971) and reaching high densities when sampled in their preferential depth. However, the species is not very abundant when sampled by demersal commercial gears in the Mediterranean Sea, due the big mesh size of the gear in relation to the species body size. This small pelagic crustacean feeds manly on copepods, ostracods and euphausiids (Lagardere 1977), having a very important role within the continental slope and deep sea trophic nets (Cartes *et al.* 1998, 2002a, 2009). However, much information about their biology is still lacking (Company and Sardá 2000).

The species has a cosmopolitan distribution occurring in the Indo- Pacific south hemisphere (Australia and New Zealand), western and eastern Atlantic Ocean (from Canada, to Magellan Strait, and from Norway to South Africa) including also the whole Mediterranean Sea. Casanova (1977) identified morphological differences among south and north Atlantic and Mediterranean populations, concerning the shape of the petasma, and also among Atlantic and Mediterranean populations, concerning the presence/absence of a protuberance or spine in the first two pereiopods.

Sergestes vigilax Stimpson, 1860

S. vigilax is an almost all transparent body similar to *S. arcticus* in terms of coloration, and size, but not on its morphology which is characteristic due to its highly developed third maxillipeds. Information on its biology is scarce while some on its depth distribution is available (Casanova and Judkins 1977, Hopkins at al 1994). The geographical distribution of *S. vigilax* comprises the Indo-Pacific, western Atlantic, north-eastern Atlantic and Mediterranean, being mainly sampled near the surface (Udeken d' Acoz 1999). In the Mediterranean Sea the species is rarely collected by demersal gears, while it is more common in samples taken by pelagic trawls (Koukouras 2000). Its densities, however never reach as high values as those of *S. arcticus* (Fasham 1979).

Sergestes arachnipodus (Coco, 1832)

S. arachnipodus is a morphologically similar species to *S. arcticus*, which can be identified by the presence of little red spots on its dorsal area, both on its cephalothorax and abdomen, and by their slightly larger size (Figure 4).Its global distribution comprises the Mediterranean and the western and eastern North Atlantic from temperate to tropical waters. Its depth distribution ranges from the surface to 2300 m depth (d'Udekem d'Acoz 1999, Koukouras 2000, Abelló *et al.* 2002, Company *et al.* 2004, Cartes *et al.* 2009), but general information about their biology is lacking.

Sergia robusta (S.I. Smith, 1882)

S. robusta is a species with an overall red colouration that is more linked the deep sea regions compared to the before mentioned Sergestidae species (Figure 4 and 5). It is present across the temperate north-west and north-east Atlantic from the Faroe Islands (61 ° N) to Cabo Verde (14° N) and Mediterranean (d'Udekem d'Acoz 1999). Overall its depth distribution comprises the pelagic environment between around 200 m and almost 5000 m (Koukouras *et al.* 2000). However, its preferential depth during the night is located around 200-500 m while during the day it is found in deeper waters down to 2000 m (Crosnier and Forest 1973, Froglia and Giannini 1982, Vereshchaka

1994). There are no gaps in the distribution of the species across the Atlantic-Mediterranean transition, even so slight morphological differences between Atlantic and Mediterranean males were reported by Casanova and Judkins (1976).

In the Mediterranean, the species is often found in demersal trawl hauls targetting the red shrimp *Aristeus antenatus*, as also are *P. multidentata* and *Gennadas elegans* (Fanelli *et al.* 2007, Guijarro *et al.* 2011, García-Rodríguez *et al.* 2011) (Figure 4). It shows a trend of increasing size with depth (Morales-Nin *et al.* 2003), and it is one of the largest species within the Sergestidae (Company and Sardá 2000). *S. robusta* has also an important role in the throphic dynamics of the demersal system being preyed by both nektonic and benthic species and feeding on copepods, ostracods, chaetognathes and euphausiids (Lagardere 1977, Cartes *et al.* 2007a, 2009).

i.v.ii.ii. Family Benthesicymidae

Gennadas elegans (S.I. Smith, 1882)

Gennadas elegans is an all red pigmented shrimp that has the smallest body length compared to the before presented species (Figure 4 and 5). The species has also been shown to possess a differential high lipid storage which has been attributed to contribute to buoyancy and therefore maintain position in the water column (Childress and Nygaard 1974). The distribution of the species comprises the north-west and east Atlantic from Greenland to east Sahara and Mediterranean, dwelling in the pelagic environment from 30 to 2000 m below the surface (Franqueville 1971, Casanova and Judkins 1976, d'Udekem d'Acoz 1999). It mainly occurs on the bottom deeper than around 500 m but it can occur in much shallower waters in the pelagic environment during the night (Andersen *et al.*

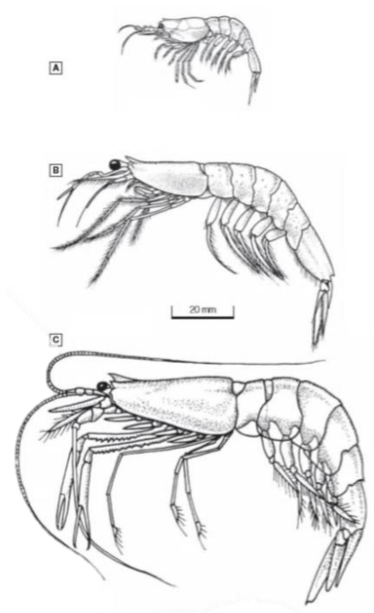


Figure 5: Drawings of: (A) *Gennadas elegans*, (B) *Sergia robusta*, (C) *Pasiphaea multidentata*. Source: Würtz (2010).

1998, Koukouras 2000).

Chapter

I

Pasiphaeid shrimp populations in the western Mediterranean: geographical variability in distribution and population patterns

Pasiphaeid shrimp populations in the western Mediterranean: geographical variability in distribution and population patterns

1.1. Introduction

Nektobenthic decapod crustacean shrimps have a widespread geographic and bathymetric distribution from high latitudes in both hemispheres to intertropical waters (Crosnier and Forest 1973, Casanova and Judkins 1976, Fasham and Foxton 1979, Clarke and Holmes 1987, Hayashi 1999, Burghart *et al.* 2007, Serejo *et al.* 2007). Many continental slope shrimps show cyclic movements associated to the photoperiod as shown in some pasiphaeid and sergestid shrimps (Macquart-Moulin and Patriti 1993, Omori 1974, Froglia and Giannini 1982, Cartes *et al.* 1993, Aguzzi *et al.* 2007). This vertical daily migration ability present in the behaviour of these species (Naylor 2010) provides them with an important role in the transference of matter and energy from the upper primary productive layers of the ocean, where these species tend to feed, down to the continental slope epibenthic community, where they dwell during day-time hours (Cartes 1993c, Herring and Roe 1988, Naylor 2010). Nektobenthic/benthopelagic decapod crustaceans constitute a fundamental food item to fish, other crustaceans and cephalopods with epibenthic habits on the continental slope and deep sea (Garrison and Link 2000, Fanelli and Cartes 2008). Some of these species have a relevant ecological importance as well as an exploitation potential as commercial target species (Nanjo and Ohtomi 2009). Relevant studies have however been mainly conducted only in a few regions, such as the Mediterranean Sea (e.g. Casanova and Judknis 1977, Orsi-Relini and Relini 1990, Cartes *et al.* 1993, Company *et al.* 2001, 2003, Ramirez-Llodra *et al.* 2007), the Japan Sea (Nanjo 2007, Nanjo and Ohtomi 2009), or the Northeast and Southeast Atlantic Ocean (Matthews and Pinnoi 1973, Gibbons *et al.* 1994, Kensley and Schotte 2006).

The genus *Pasiphaea* has a worldwide distribution and over sixty species are known to date (Hayashi 1999, Tavares and Cardoso 2006, De Grave and Fransen 2011). Members of this family show a strong lateral compression of both cephalotorax and abdomen (Zariquiey Álvarez 1968), which may improve their swimming and dispersal abilities (Cartes *et al.* 1993, Aguzzi *et al.* 2009). While up to 18 species of the family Pasiphaeidae are known to occur in the northeast Atlantic and Mediterranean waters (eight of them belonging to the genus *Pasiphaea* (d'Udekem d'Acoz, 1999), only two of them are present in the Mediterranean Sea, namely *Pasiphaea sivado* and *Pasiphaea multidentata* (Zariquiey Álvarez 1968, Casanova and Judkins 1977, Koukouras 2000).

Pasiphaea sivado is a benthopelagic caridean shrimp commonly caught by demersal trawling on the upper slope across the East Atlantic and Mediterranean Sea (González-Gurriarán and Olaso 1987, Abelló *et al.* 2002). Biological studies on this species have been mainly performed in the western Mediterranean, where it has been reported to reproduce continuously throughout the year, although peaking in autumn-winter (Company *et al.* 2001). The smallest ovigerous female sampled was 14.7 mm CL and its longevity has been estimated to be around 2 years. *P. sivado* is a night feeder on pelagic crustaceans such as euphausiids and calanoid copepods (Lagardère 1972), as well as on mobile epibenthic organisms such as peracarid crustaceans (Cartes 1993b).

Pasiphaea multidentata inhabits benthic boundary layers in the middle and lower slope down to 2000 m depth (Cartes 1993a, Abelló *et al.* 2002, Tecchio *et al.* 2011), with juveniles inhabiting shallower waters than adults (Company *et al.* 2001). It also performs night vertical migrations into upper water layers, although apparently mainly restricted to juvenile individuals (Aguzzi *et al.* 2007, Cartes 1993c). In the northwestern Mediterranean the species shows a marked seasonality in reproduction with ovigerous females being only present from September to February, and with an estimated maximum longevity of around 3.5 years (Company *et al.* 2001, Ramirez-Llodra *et al.* 2007). It is an active nocturnal feeder on pelagic crustaceans such as *Gennadas elegans*, *P. sivado*, *P. multidentata*, sergestid shrimps and little mesopelagic fish as myctophids and *Cyclothone* spp. (Cartes 1993b).

Comparative studies of both species have addressed different life history processes. Company *et al.* (2001) suggested that the greater fecundity output of *P. sivado* supports its higher population densities when compared with *P. multidentata.* Both species perform daily vertical migrations, with the apparent exception of the adults of *P. multidentat*a (CL > 30 mm), which are assumed to perform epibenthic depth displacements (Cartes *et al.* 1993, Aguzzi *et al.* 2007). The dietary overlap between these species is low due the different size spectra of their respective prey items (Cartes 1993b).

This paper aims to analyze the main characteristics of the bathymetric and geographic distribution, population size structure and some reproductive characteristics of both *Pasiphaea sivado* and *P. multidentata* along the Mediterranean coasts of the Iberian Peninsula, and to relate the patterns obtained with geomorphological and hydrographic characteristics. The data base was obtained in a series of annual demersal fisheries research surveys performed throughout the continental shelf and upper slope of the western Mediterranean (Bertrand *et al.* 2002).

1.2. Material and methods

1.2.1. Study area and oceanographic context

The study area encompassed the continental shelf, upper and middle slope down to 800 m along the Iberian Peninsula Mediterranean coasts from Gibraltar in the SW to Cape Creus in the NE (Figure 1). Overall, the continental shelf is very narrow in the Alboran Sea and Vera Gulf, south of Cape Palos, and widens to the north, reaching a maximum width of up to 70 km in the Ebro Delta - Columbretes area. North of Barcelona, the continental shelf is heavily indented by several submarine canyons.

The Western Mediterranean is influenced by the inflow of Atlantic water through the Strait of Gibraltar (Hopkins 1985, Millot 2005), where lighter Atlantic water inflows towards the Mediterranean on surface waters, and higher density Mediterranean water outflows towards the Atlantic Ocean in depth. This surface inflow of Atlantic waters generates two anticyclonic gyres between the Strait of Gibraltar and Cape Gata, and adjacent upwelling cells in the vicinity of the

Strait (Vargas-Yáñez and Sabatés 2007). The main current of inflowing Atlantic waters is directed from Cape Gata towards the North African coast, generating the Almeria-Oran front (AOF), therefrom continuing its inflow along the North African coasts towards the Central and Eastern Mediterranean. The AOF is a strong thermohaline density front confined to the upper layers of the water column and shows important seasonal and interanual variability in strength (Tintoré *et al.* 1988). The Atlantic water also flows north-eastwardly, due to the detachment of anticyclonic gyres which reach the Balearic Islands and generate a second density front along the northeastern part of the archipelago, associated to the NE flowing Balearic Current (Tintoré *et al.* 1988, López-Jurado et al, 2008, Monserrat *et al.* 2008). The interaction between the strong Northern Current, flowing southwestwards along the continental slope from the Gulf of Lions, and the Balearic Current in the Ibiza Channel region (between Cape La Nao and the island of Ibiza) generates a cyclonic gyre over the Balearic basin enclosing the older resident waters in its center (Salat 1995, Sabatés *et al.* 2007).

Figure 1: Iberian Peninsula Mediterranean coast showing the study regions: Western Alboran (WALB); Eastern Alboran (EALB); Alboran Island (ALBO); Vera Gulf (VERA); Alicante (ALIC); Ibiza Island (IBIZ); Valencia (VALE); Ebro Delta region (DELT); Central Catalonia (CCAT) and North Catalonia (NCAT). 200 m and 1000 m isobaths are shown.

1.2.2. Sampling and Analysis

The material analyzed in the present work was obtained from the annual MEDITS_ES surveys performed between 1994 and 2008. The aim of the Mediterranean International Trawl Surveys project (MEDITS) is to obtain density, biomass and recruitment indexes of the main target species exploited by the demersal fishery throughout the European Union and adjacent Mediterranean countries, based in a common sampling protocol (Bertrand *et al.* 2000, 2002). The Spanish surveys were performed on board R/V 'Cornide de Saavedra'. These cruises took place in spring, centered in the month of May and had duration of about 30 days. All hauls were performed during day-time hours. Overall, samples were taken at depths from around 40 m down to 800 m depth based on a sample design randomly stratified by geographical sector and depth stratum. Ten geographic sectors were established according to geomorphology characteristics of the overall area and previous biogeographic results (Abelló *et al.* 2002, Rufino *et al.* 2005): Western Alboran (WALB) from Gibraltar to Cape Sacratif, Eastern Alboran (EALB) from Cape Sacratif to Cape Gata, Alboran Island (ALBO), Vera Gulf (VERA) from Cape Gata to Cape Palos, Alicante (ALIC) from Cape Palos to Cape La Nao, Ibiza Island (IBIZ), Valencia (VALE) from Cape La Nao to Castelló/Columbretes Islands, Ebro Delta region (DELT) from Castelló/Columbretes Islands to Cape Salou/Tarragona, Central Catalonia (CCAT) from Cape Salou/Tarragona to Barcelona, and North Catalonia (NCAT), from Barcelona to Cape Creus. The bottom trawl used was a GOC-73 model (Fiorentini *et al.* 1999). It has a large, up to 3 m vertical opening allowing the capture of epibenthic and benthopelagic fish and crustaceans, and a codend stretched mesh size of 20 mm. The trawls were performed at a speed of 3 knots with duration of one hour, except for the hauls performed above the 200 m isobath that had duration of 30 minutes. A total of 1741 valid hauls were performed along the studied period (Table 1).

On board, after each haul, the total amount of each species sampled was weighed and counted. Random sub-samples were taken whenever necessary. Density and biomass were later standardized by swept area, to obtain number of individuals and weight (in g) per square kilometer. The swept area was calculated taking into account the horizontal opening of the trawl, measured with Scanmar devices, and distance from starting point of the haul (net on the bottom) to end of the effective haul (net off the bottom), measured from GPS latitude and longitude readings. The frequency of occurrence, density, biomass and size structure was analyzed in terms of bathymetrical and geographical distribution in the two species. Mean density and biomass values were calculated by averaging positive haul density values over total number of hauls made within each geographic sector – depth interval cell. One-way analysis of variance was used to test for the occurrence of significant interannual differences in density (natural logarithmic transformation), after testing for normality of data and homogeneity of variances. If no significant differences were detected, a two-way ANOVA was used considering geographical sectors and 100 m depth strata as factors to test for the occurrence of significant differences among these factors. As a requisite for ANOVA, only factor cells with positive values were used in the analyses, so that only the most relevant sectors and strata were used.

Table 1: Number of hauls taken by depth stratum and geographic sector (1994-2008): Western Alboran (WALB); Eastern Alboran (EALB); Alboran Island (ALBO); Vera Gulf (VERA); Alicante (ALIC); Ibiza Island (IBIZ); Valencia (VALE); Ebro Delta region (DELT); Central Catalonia (CCAT); North Catalonia (NCAT).

Depth stratum (m)	WALB	EALB	ALBO	VERA	ALIC	IBIZ	VALE	DELT	CCAT	NCAT	Total
0-50	22	15	-	8	31	-	13	43	26	6	164
51-100	47	35	2	23	86	-	76	172	62	33	536
101-150	23	11	3	5	46	6	35	44	24	49	246
151-200	9	10	-	18	23	-	5	5	4	7	81
201-250	10	11	-	-	7	5	-	-	10	6	49
251-300	7	6	-	20	20	8	6	1	3	9	80
301-350	21	10	2	6	12	4	10	1	5	15	86
351-400	14	5	5	1	4	2	2	1	5	3	42
401-450	16	14	-	8	14	1	-	2	6	10	71
451-500	1	3	1	2	19	11	-	-	3	11	51
501-550	21	14	4	9	18	4	-	-	6	11	87
551-600	13	10	3	3	19	10	3	-	6	5	72
601-650	22	15	2	1	5	-	2	-	5	11	63
651-700	12	1	2	3	1	11	-	-	6	8	44
701-750	15	1	1	7	-	2	10	-	2	2	40
751-800	13	7	3	-	-	-	5	-	1	-	28
Total	266	168	28	114	305	64	167	269	174	186	1741

From 1998, all individuals, or a subsample of up to 60 individuals, of each species, *Pasiphaea sivado* and/or *P. multidentata*, for each haul, were sexed and measured (carapace length, CL), with an accuracy of 0.1 mm. Ovigerous females were also noted down. Size frequency distributions (SFD), weighted by the density of the corresponding sample, were obtained for each combination of geographic sector and depth stratum. SFD based on <15 individuals per cell have not been shown. Normal-distributed components were identified in SFD using the Bhattacharya method implemented in Fisat II (Gayanillo *et al.* 2005). Mean sizes identified with this method for each depth stratum were later pooled in a frequency distribution by sector in order to identify the commonest mean size classes within each geographic sector. This would provide greater precision on the actual number of age classes present in each geographic sector and on their average size (Yamasaki 1988, Queiroga 1993). The cumulative frequency of ovigerous females per sector was adjusted to a logistic distribution in order to calculate the mean size of occurrence of ovigerous females of *P. sivado* per sector. These were tested with a t-test.

Table 2: Mean bottom temperature (ºC) and salinity ± SD in each 50 m depth stratum in the five most relevant geographic sectors for *Pasiphaea* spp. Light blue cells show the preferential depth strata for *P. sivado*; dark blue cells show the preferential depth strata for *P. multidentata*

TEMPERATURE						SALINITY				
Depth stratum (m)	WALB	EALB	ALIC	CCAT	NCAT	WALB	EALB	ALIC	CCAT	NCAT
001-050	13.96±0.75	14.60±1.28	14.15±0.45	14.75±1.42	14.61±1.08	38.01±0.45	37.78±0.58	38.08±0.17	38.00±0.15	37.91±0.17
051-100	13.57±0.28	14.09±0.84	13.61±0.61	13.94±0.47	13.31±0.26	38.15±0.16	37.94±0.45	38.14±0.11	38.03±0.12	38.07±0.19
101-150	13.32±0.09	13.41±0.22	13.15±0.24	13.35±0.41	13.25±0.29	38.31±0.07	38.26±0.05	38.24±0.13	38.10±0.11	38.13±0.10
151-200	------	13.30±0.13	13.08±0.12	13.61±0.43	------	------	38.35±0.09	38.28±0.12	38.06±0.10	
201-250	13.27±0.03	13.38±0.17	13.24±0.39	13.15±0.16	13.18±0.14	38.37±0.12	38.29±0.10	37.58±1.33	38.23±0.07	38.34±0.05
251-300	------	13.72±0.95	13.14±0.07	13.24±0.01	------	------	38.34±0.22	38.38±0.06	38.32±0.09	------
301-350	13.26±0.02	13.29±0.07	13.14±0.07	13.04±0.03	13.30±0.14	38.45±0.07	38.41±0.09	38.42±0.07	38.36±0.02	38.39±0.15
351-400	13.27±0.01	13.25±0.08	------	13.26±0.00	------	38.50±0.02	38.42±0.01	------	38.35±0.04	------
401-450	13.24±0.02	13.26±0.03	13.22±0.09	13.20±0.03	13.31±0.00	38.50±0.02	38.50±0.01	38.50±0.03	38.43±0.06	38.50±0.02
451-500	------	13.36±0.09	13.20±0.15	------	13.24±0.14	------	38.39±0.17	38.45±0.13	------	38.47±0.06
501-550	13.22±0.05	13.20±0.08	13.16±0.09	------	13.29±0.06	38.48±0.04	38.49±0.02	38.42±0.14	------	38.51±0.04
551-600	13.23±0.03	13.20±0.07	13.12±0.09	13.12±0.22	------	38.50±0.03	37.86±1.37	38.44±0.12	38.35±0.15	------
601-650	13.18±0.04	13.25±0.086	14.33±2.45	------	13.17±0.13	38.48±0.04	38.38±0.12	38.37±0.16	------	38.50±0.02
651-700	------	------	------	13.13±0.07	------	------	------	------	38.50±0.01	------
701-750	13.14±0.04	------	------	13.14±0.18	------	38.48±0.02	------	------	38.40±0.09	------
751-800	------	13.15±0.06	------	------	------	------	38.49±0.02	------	------	------

Information on mean temperature and salinity on the bottom during each trawl was recorded with a CTD SBE-37 placed at the float-line of the net (Table 2). Data from the 2001-2006 cruises were used to calculate mean temperature and salinity by depth stratum for each geographic sector. In order to assess the optimal temperature and salinity window for each species, the range, 25 and 75 percentiles and median values were calculated. For each species each sample was additionally categorized as juvenile or adult depending on its mean CL: For *P. sivado*, samples with mean CL≤16 mm were considered as juvenile samples, and those with CL >16 mm as adult; for *P. multidentata*, a size break at 20 mm CL was considered (see below).

1.3. Results

1.3.1. *Pasiphaea sivado*

1.3.1.1. Occurrences

The overall depth distribution of *P. sivado* along the study area ranged between 141 and 765 m, however the highest frequency of occurrence was located between the 350 and 500 m depth strata (Table 3a). At depths both shallower and deeper the occurrence of *P. sivado* was progressively rarer (Figure 2a).

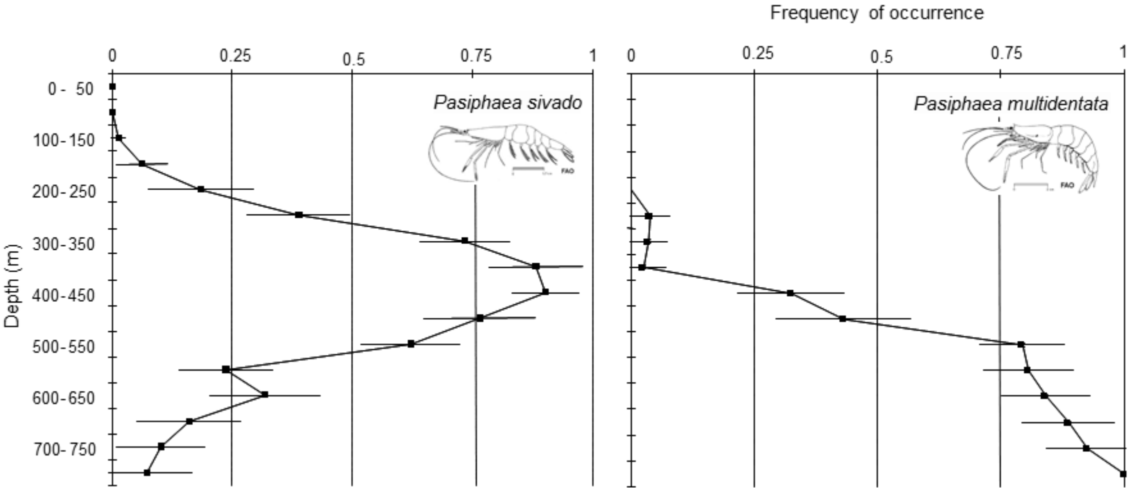


Figure 2: Overall percentage occurrence (± 95% confidence intervals) of *Pasiphaea sivado* (a) and *P. multidentata* (b) by depth intervals along the Iberian Peninsula Mediterranean coast.

Taking into account both the frequency of occurrence within the preferential depth interval of the species (350-500 m, Table 3a), and the sampling effort within each geographic sector (i.e excluding those sectors where the number of samples taken in the preferential depth strata of the species were < 20, Table 1), the highest frequencies of occurrence of *P. sivado* were located in Western and Eastern Alboran, Alicante and Northern Catalonia (Table 3a).

Table 3: Frequency of occurrence of (a) *Pasiphaea sivado* and (b) *Pasiphaea multidentata* (in %) within each geographic sector and depth stratum. Occurrences larger than 75% are highlighted in bold. Cells with (*) correspond to data with less than 3 hauls (see Table 1), not shown since considered not enough informative due to low sampling size. Values in the lowest line ((350 to 500 m in *P. sivado* and >500 m in *P. multidentata*) correspond to the overall percentage occurrence by sector restricted to the depth strata with overall percentage occurrence higher than 75%.

(a) *Pasiphaea sivado*											
Depth stratum (m)	WALB	EALB	ALBO	VERA	ALIC	IBIZ	VALE	DELT	CCAT	NCAT	Total
0-50	0.0	0.0	-	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0
50-100	0.0	0.0	0.0	0.0	0.0	-	0.0	0.0	0.0	0.0	0.0
100-150	4.3	0.0	0.0	0.0	4.3	0.0	0.0	0.0	0.0	0.0	1.2
150-200	22.2	0.0	-	11.1	0.0	-	20.0	0.0	0.0	0.0	6.2
200-250	60.0	0.0	-	-	14.3	0.0	-	-	20.0	0.0	18.4
250-300	57.1	50.0	-	30.0	45.0	0.0	66.7	*	33.3	33.3	38.8
300-350	81.0	90.0	*	50.0	75.0	0.0	70.0	*	100.0	80.0	73.3
350-400	100.0	100.0	40.0	*	75.0	*	*	*	100.0	100.0	88.1
400-450	93.8	100.0	-	62.5	92.9	*	-	*	83.3	100.0	90.1
450-500	*	100.0	100.0	50.0	73.7	100.0	-	-	66.7	54.5	76.5
500-550	90.5	50.0	0.0	44.4	61.1	25.0	-	-	50.0	81.8	62.1
550-600	69.2	0.0	0.0	0.0	5.3	20.0	0.0	-	50.0	40.0	23.6
600-650	50.0	26.7	0.0	*	0.0	-	50.0	-	0.0	27.3	31.7
650-700	41.7	*	*	33.3	*	0.0	-	-	16.7	0.0	15.9
700-750	6.7	*	*	0.0	-	*	0.0	-	*	*	10.0
750-800	15.4	0.0	0.0	-	-	-	0.0	-	*	-	7.1
No. samples (350-500 m)	31	22	6	11	37	14	2	3	14	24	164
Mean frequency (350 to 500 m)	96.8	100.0	50.0	63.6	81.1	85.7	100.0	100.0	85.7	79.2	85.4

(b) *Pasiphaea multidentata*											
Depth stratum (m)	WALB	EALB	ALBO	VERA	ALIC	IBIZ	VALE	DELT	CCAT	NCAT	Total
200-250	0.0	0.0	-	-	0.0	0.0	-	-	0.0	0.0	0.0
250-300	14.3	0.0	-	5.0	0.0	0.0	0.0	*	0.0	0.0	3.8
300-350	0.0	0.0	0.0	0.0	8.3	0.0	20.0	*	0.0	0.0	3.5
350-400	0.0	20.0	*	*	0.0	*	0.0	*	0.0	0.0	2.4
400-450	6.3	35.7	-	37.5	35.7	*	-	*	50.0	50.0	32.4
450-500	*	66.7	*	*	52.6	18.2	-	-	100.0	36.4	43.1
500-550	71.4	71.4	100.0	100.0	88.9	100.0	-	-	83.3	54.5	79.3
550-600	61.5	90.0	66.7	100.0	84.2	90.0	66.7	-	66.7	100.0	80.6
600-650	77.3	100.0	*	*	100.0	-	*	-	60.0	81.8	84.1
650-700	91.7	*	*	100.0	*	100.0	-	-	66.7	75.0	88.6
700-750	86.7	*	*	100.0	-	*	100.0	-	*	*	92.5
750-800	92.3	100.0	100.0	-	-	-	100.0	-	*	-	100.0
No. samples (>500 m)	96	48	15	23	43	27	20	0	26	37	334
Mean frequency (>500 m)	79.2	87.5	86.7	100.0	88.4	96.3	95.0	-	73.1	75.7	85.1

Table 3a also clearly shows that *P. sivado* in Western Alboran, contrasting with the rest of geographical sectors, presented both a much shallower and a deeper distribution, since the species occurred from the 100-150 m stratum down to the deepest sampled stratum (750-800 m), whereas in the rest of areas the distribution of *P. sivado* usually started from the 200-250 m stratum down to maximum depths of around 600-700 m.

1.3.1.2. Density and biomass

Positive densities of *P. sivado* per haul ranged between 8 and 186,109 ind.km^{-2}. No significant differences were found concerning interannual variability in densities (ANOVA, $F_{14,340}=0.7313$, p>0.5). The interaction between depth and geographical sector was not significant. Two-way ANOVA showed that densities across both geographical sectors ($F_5=4.331$, p<0.001) and depth strata ($F_3=20.4$, p<0.0001) differed significantly among them. No significant interaction was found (p>0.1). The highest mean density values were found at depths between 250-500 m, i.e. within the preferential occurrence depth strata for the species (350-500 m) and also at the two adjacent upper strata. The Western Alboran population showed the widest density distribution range in depth for all sampled populations. Sizeable densities were found in this sector at depths between 150 and 600 m. In the rest of sampled areas, densities rose sharply only from 250 m downwards to around 500-600 m. Overall, the areas with highest mean density values were Western and Eastern Alboran and Central and North Catalonia. Ibiza also showed very high mean densities, but the relatively low sample size and the large variability in densities in this area did not allow to take these values as fully representative (Table 3a). Both density and biomass (Tables 4a and 5a) followed a similar bathymetric and geographic pattern along the studied geographic area.

The relationship between percentage occurrence and mean density per depth strata (Figure 3) showed that high densities were associated to depths with a high percentage occurrence of the

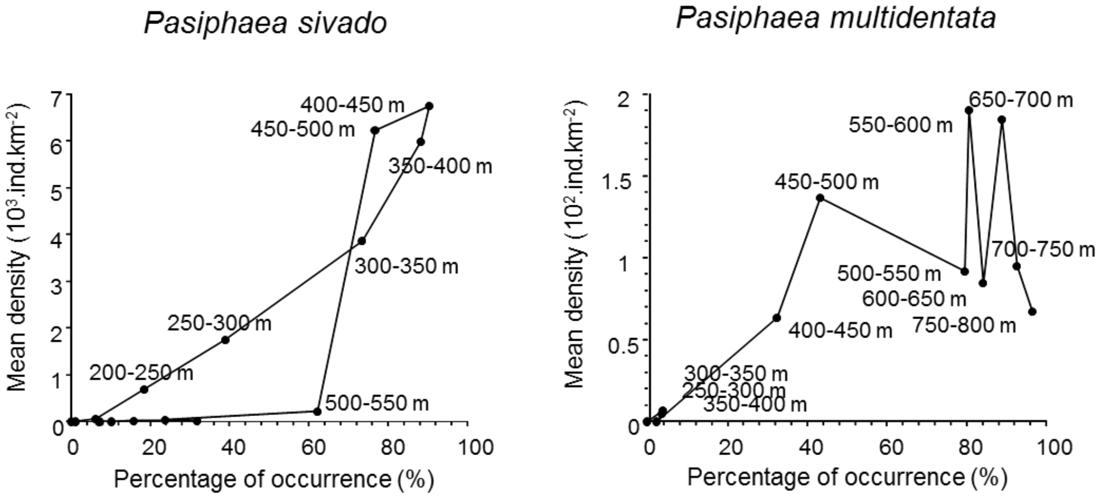


Figure 3: Relationship between **mean** density and percentage occurrence in *Pasiphaea sivado* and *P. multidentata*.

species, especially so in depth strata comprised between 350 and 500 m, which could therefore be categorised as the optimal depth ranges for *P. sivado* in the study area for both occurrence and density. It also showed that densities gradually increased with increasing depth and percentage occurrence up to the 350-400 m depth stratum, and sharply decreased deeper than 500 m. Also, the ratio between biomass and density (i.e. an index of mean weight per individual within each depth stratum) followed a significant increasing trend with depth ($P < 0.01$), clearly indicating the occurrence of a marked size increase with depth (Figure 4).

Table 4: Mean density (ind.km-2) of (a) *Pasiphaea sivado* and (b) *Pasiphaea multidentata* within each depth stratum and geographic sector along the Iberian Peninsula Mediterranean coast. Mean density per geographic sector corresponds to densities within the 350-500 m depth strata for *P. sivado* and >500 m for *P. multidentata* (i.e. those with the overall highest frequency of occurrence).

(a) *Pasiphaea sivado*

Depth stratum (m)	WALB	EALB	ALBO	VERA	ALIC	IBIZ	VALE	DELT	CCAT	NCAT	Total
0-50	0	0	-	0	0	-	0	0	0	0	0
50-100	0	0	0	0	0	-	0	0	0	0	0
100-150	1	0	0	0	3	0	0	0	0	0	1
150-200	411	0	-	3	0	-	4	0	0	0	47
200-250	3347	0	-	-	9	0	-	-	11	0	687
250-300	7146	1048	-	750	3145	0	295	3167	61	56	1748
300-350	8663	3453	0	119	4288	0	1027	176	3328	2400	3857
350-400	9200	2847	81	2350	426	1588	458	436	18358	2483	5983
400-450	13614	7387	-	187	1782	0	-	108	6879	8916	6737
450-500	5867	110	588	296	442	26368	-	-	299	987	6227
500-550	494	55	0	158	110	3		-	61	380	220
550-600	156	0	0	0	0	3	0	-	23	40	33
600-650	17	4	0	657	0	-	5	-	0	7	19
650-700	10	0	0	18	0	0	-	-	128	0	21
700-750	2	0	0	0	-	0	0	-	44	44	5
750-800	3	0	0	-	-	-	0	-	0	-	1
No. samples (350-500 m)	31	22	6	11	37	14	2	3	14	24	164
Mean density (350-500 m)	9560	3448	335	944	883	9319	458	272	8512	4129	6316

(b) *Pasiphaea multidentata*

Depth stratum (m)	WALB	EALB	ALBO	VERA	ALIC	IBIZ	VALE	DELT	CCAT	NCAT	Total
0-50	0	0	-	0	0	-	0	0	0	0	0
50-100	0	0	0	0	0	-	0	0	0	0	0
100-150	0	0	0	0	0	0	0	0	0	0	0
150-200	0	0	-	0	0	-	0	0	0	0	0
200-250	0	0	-	-	0	0	-	-	0	0	0
250-300	74	0	-	0	0	0	0	11	0	0	7
300-350	0	0	0	0	11	0	25	0	0	0	4
350-400	0	2	0	0	0	0	0	0	0	0	0
400-450	4	22	-	56	117	0	-	199	141	80	63
450-500	0	80	0	50	97	18	-	-	142	379	137
500-550	28	42	92	281	124	94	-	-	199	11	92
550-600	34	37	28	59	281	502	58	-	127	263	190
600-650	124	38	70	213	178	-	102	-	16	46	85
650-700	24	48	99	1029	439	295	-	-	21	85	185
700-750	87	0	66	101	-	95	93	-	158	138	95
750-800	36	35	37	-	-	-	186	-	154	-	67
No. samples (>500 m)	96	48	15	23	43	27	20	0	26	37	335
Mean density (>500 m)	56	33	65	337	256	247	110	-	113	109	119

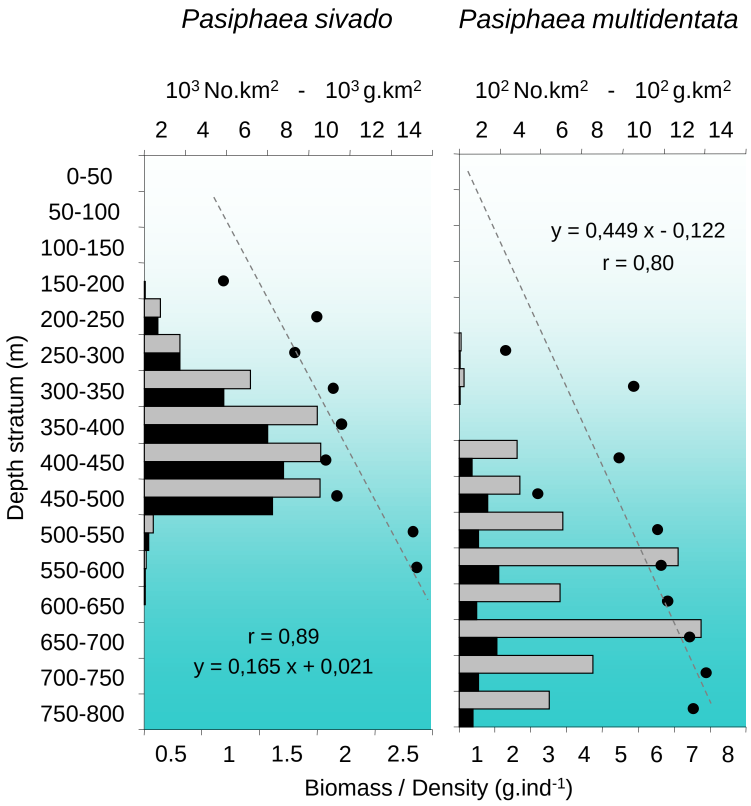
Table 5: Mean biomass (g km-2) of (a) *Pasiphaea sivado* and (b) *Pasiphaea multidentata* within each depth stratum and geographic sector along the Iberian Peninsula Mediterranean coast. Mean biomass per geographic sector corresponds to values within the 350-500 m depth strata for *P. sivado*) and >500 m for *P. multidentata* (i.e. those with the overall highest frequency of occurrence)

(a) Pasiphaea sivado

Depth stratum (m)	WALB	EALB	ALBO	VERA	ALIC	IBIZ	VALE	DELT	CCAT	NCAT	Total
0-50	0	0	-	0	0	-	0	0	0	0	0
50-100	0	0	0	0	0	-	0	0	0	0	0
100-150	1	0	0	0	4	0	0	0	0	0	1
150-200	150	0	-	4	0	-	4	0	0	0	18
200-250	3979	0	-	-	17	0	-	-	18	0	818
250-300	9174	1020	-	524	2656	0	155	4642	29	32	1749
300-350	13853	2961	0	139	4023	0	1423	301	3146	2811	5140
350-400	12988	4967	131	1224	490	2299	664	185	25558	2739	8396
400-450	22070	7192	-	287	2021	0	-	155	5306	9323	8588
450-500	15496	178	1098	477	568	35611	-	-	436	1159	8523
500-550	1141	91	0	266	184	3	-	-	94	650	444
550-600	343	0	0	0	0	2	0	-	29	54	68
600-650	34	6	0	1137	0	-	11	-	0	6	33
650-700	22	0	0	25	0	0	-	-	72	0	17
700-750	3	0	0	0	-	0	0	-	67	44	7
750-800	6	0	0	-	-	-	0	-	0	-	3
No. samples (350-500 m)	31	22	6	11	37	14	2	3	14	24	164
Mean biomass (350-500 m)	16851	4112	614	663	1026	12637	664	170	10433	4407	8502

(b) *Pasiphaea multidentata*

Depth stratum (m)	WALB	EALB	ALBO	VERA	ALIC	IBIZ	VALE	DELT	CCAT	NCAT	Total
0-50	0	0	-	0	0	-	0	0	0	0	0
50-100	0	0	0	0	0	-	0	0	0	0	0
100-150	0	0	0	0	0	0	0	0	0	0	0
150-200	0	0	-	0	0	-	0	0	0	0	0
200-250	0	0	-	-	0	0	-	-	0	0	0
250-300	88	0	-	2	0	0	0	22	0	0	8
300-350	0	0	0	0	33	0	145	0	0	0	21
350-400	0	14	0	0	0	0	0	0	0	0	2
400-450	26	130	-	361	693	0	-	945	370	102	281
450-500	0	501	0	317	466	88	-	-	266	209	295
500-550	167	198	577	2009	575	449	-	-	773	59	507
550-600	230	252	165	386	1551	2823	338	-	726	1351	1069
600-650	696	256	413	1314	999	-	577	-	114	271	492
650-700	165	402	577	7568	2718	1779		-	118	351	1183
700-750	672	0	485	584	-	587	656	-	917	944	653
750-800	245	281	861	-	-	-	1064	-	1121	-	437
No. samples taken (> 500 m)	96	48	15	23	43	27	20	0	26	37	335
Mean biomass (>500 m)	362	231	513	2372	1461	1410	659	-	628	595	723



Figue 4: Mean density (black bars), biomass (grey bars) and biomass/density ratio (dots) of *Pasiphaea sivado* and *P. multidentata* by depth strata.

1.3.1.3. Size structure and size at maturity

Overall, sizes of *P. sivado* ranged from 9.2 to 26.1 mm CL. Size frequency distributions per 100 m depth strata and geographic sector (Figure 5) showed that the populations in the Alboran Sea reached larger sizes than those in the northwestern Mediterranean and the intermediate Alicante sector. The western Alboran population showed a clear size-increasing trend with depth, with juvenile individuals being restricted to the upper 100-300 m depth strata; the largest individuals were recorded in the two deepest strata (500-700 m). Populations along the Catalan coast did not show such a marked increasing trend, but rather showed a similar size structure with depth, except for the dominant presence of juveniles in the upper depth occurrence stratum (200-300 m).

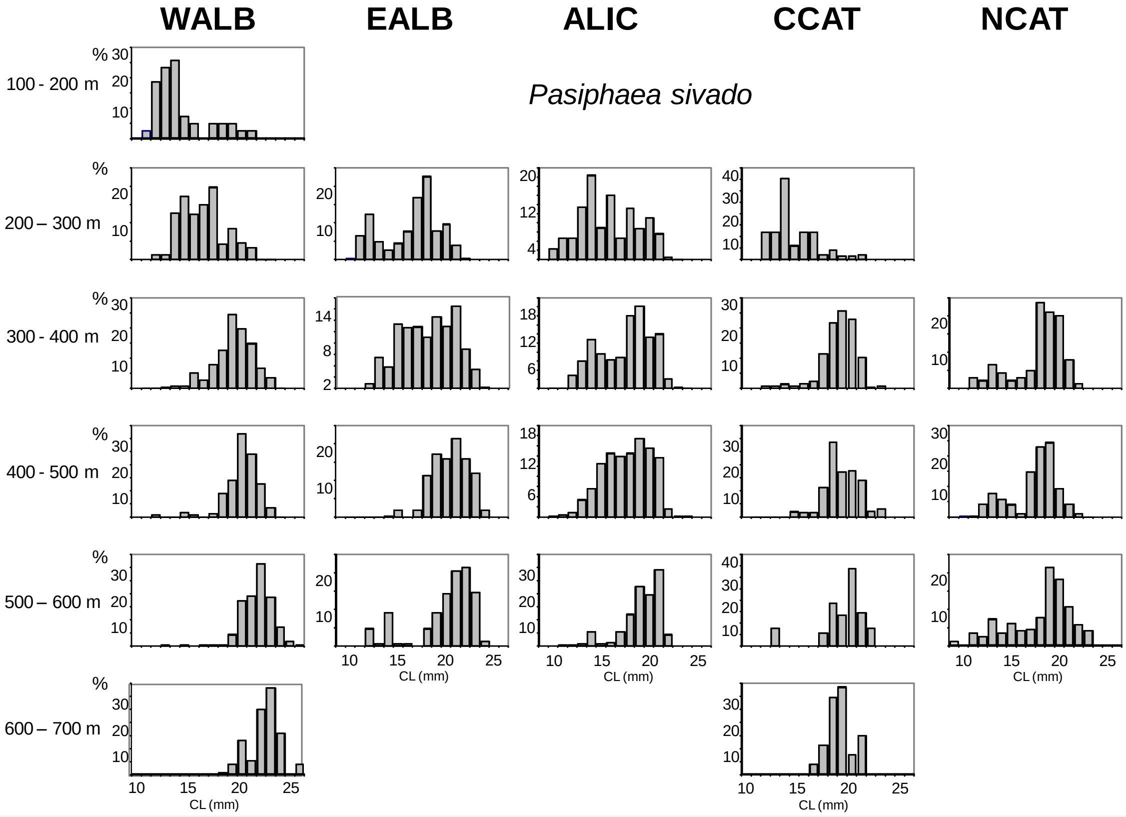


Figure 5: Size frequency distributions of *Pasiphaea sivado* per depth interval in the five best sampled geographic sectors. Total N: WALB = 1502; EALB = 592; ALIC = 999; CCAT = 653; NCAT = 462)

Based on the analysis of significant normally-distributed cohorts on the several SFD by depth stratum using the Bhattacharya method (Figure 7), the frequency distribution of the mean sizes of the normally-distributed identified cohorts per sector (Figure 8) showed that most populations appeared to be structured in two main cohorts, broadly corresponding to juvenile (mainly placed around 14 mm CL) and adult individuals (located mainly around 19 mm CL); the populations in Alboran Sea also showed additional significant cohorts around 22 mm CL which were not identified in the rest of sampled sectors.

To calculate the mean size of ovigerous females per geographic sector, a logistic curve was fitted to the cumulative frequency of ovigerous females per sector (Figure 9). This showed that the populations from western and eastern Alboran significantly overlapped (Table 6). These sizes were significantly larger than those in the rest of sampled regions, which distributions also broadly and significantly overlapped.

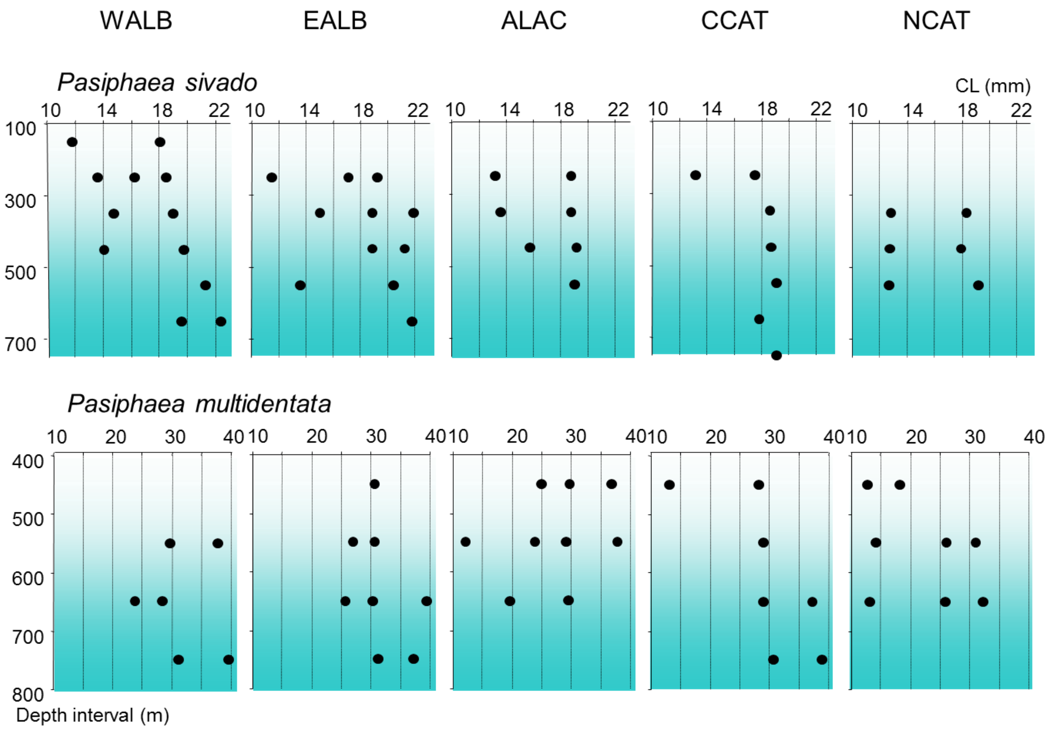


Figure 7: Mean size of the different normally-distributed identified components (using the Bhattacharya method implemented in Fisat II) in the size frequency distributions of *Pasiphaea sivado* and *P. multidentata* by geographic sector (NCAT: North Catalonia; CCAT: Central Catalonia; ALIC: Alicante; EALB: Eastern Alboran; WALB: Western Alboran)

Table 6 (a) Minimum, maximum, and mean (SD and N) (obtained by fitting a logistic curve) sizes of ovigerous female *Pasiphaea sivado* per geographic sector. (b) Pairwise comparisons of mean ovigerous female sizes by geographic sector (t-test values).

(a)

Carapace Length	WALB	EALB	ALAC	CCAT	NCAT
Min	14.5	17.2	17.5	16.4	17.2
Max	24.8	23.2	21.5	22.1	22.7
Mean	20.7	20.5	19.1	19.6	19
SD	1.8	1.7	1.2	1.6	1.6
N	243	59	49	37	20

(b)

	EALB	ALAC	CCAT	NCAT
WALB	n.s.	***	***	***
EALB		***	*	***
ALAC			n.s.	n.s.
CCAT				n.s.

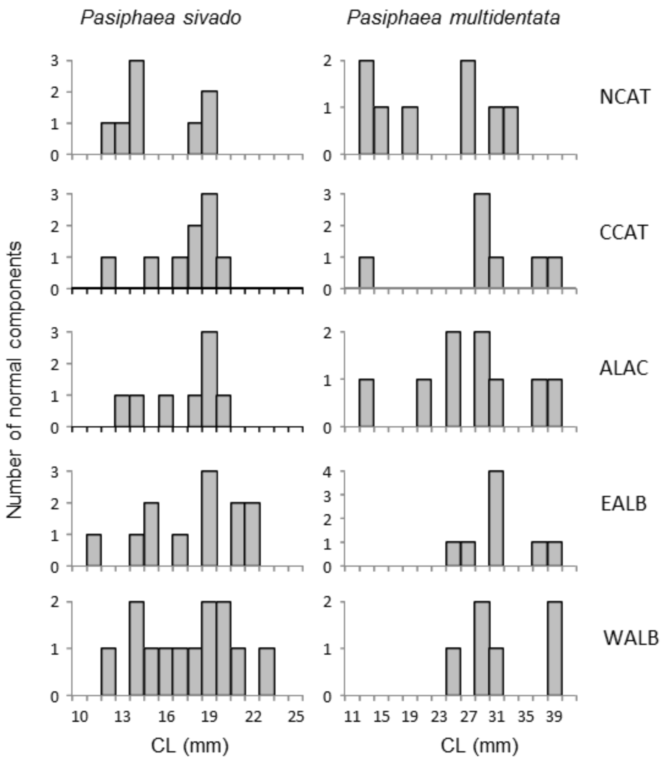


Figure 8: Frequency distribution of normally-distributed components identified in the size frequency distributions of *Pasiphaea sivado* and *P. multidentata* by geographic sector (NCAT: North Catalonia; CCAT: Central Catalonia; ALIC: Alicante; EALB: Eastern Alboran; WALB: Western Alboran). Size class intervals of 1 mm in *P. sivado* and 2 mm in *P. multidentata*.

1.3.1.4. Temperature and salinity

Occurrences of *P. sivado* took place at bottom temperatures comprised between 12.98 and 13.42°C and salinities between 38.18 and 38.54. The temperature-salinity (TS) window was narrower for adults than for juveniles (Figure 10). In particular, adults occurred (percentiles 25-75) at temperatures between 13.17 and 13.27°C and salinities of 38.43 and 38.51, while juveniles occurred at temperatures between 13.04 and 13.28°C and salinities between 38.36 and 38.48.

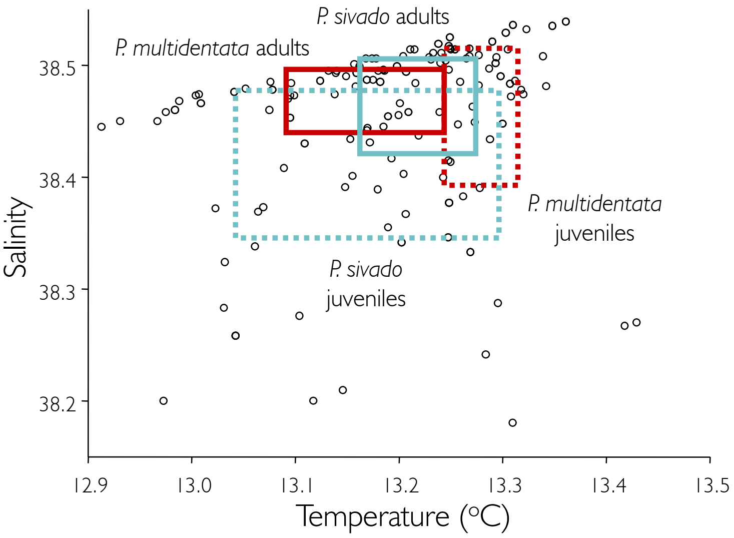


Figure 10 Temperature and salinity windows (25-75 percentiles) for juvenile (dashed lines) and adult (solid lines) of *Pasiphaea sivado* (grey) and *P. multidentata* (black). Only temperature/salinity data from CTD samples taken in the depth range 200–800 m are shown.

1.3.2. *Pasiphaea multidentata*

1.3.2.1. Occurrences

The overall depth distribution of *P. multidentata* along the study area ranged between 265 and 799 m. Its frequency of occurrence was null at strata shallower than 250 m and very low down to 400 m. Then it increased sharply down to 500 m, and reached occurrences higher than 75% from 500 m down to the deepest sampled stratum (750-800 m) (Figure 2b), clearly showing that the sampling strategy did not fully encompass the overall bathymetric range of the species.

As in *P. sivado*, the upper occurrence limit of *P. multidentata* in WALB and VERA took place at depths shallower (250-300 m) than in the rest of Mediterranean geographic sectors sampled (Table 2). At depths greater than 500 m (where the frequency of occurrence was > 75%) the species was common throughout the geographic area studied. However, and differing from *P. sivado*, the overall frequency of occurrence values were lower (73-76%) in the Balearic basin (excluding the Gulf of Valencia sector) than in the rest of geographic sectors sampled, all of them located in the Algerian basin, where overall frequencies of occurrence were markedly higher (most of them being higher than 85%).

1.3.2.2. Density and biomass

Positive density values of *P. multidentata* ranged between 7.5 and 3,696 ind.km^{-2}. No significant differences were found concerning interannual variability in densities (ANOVA, $F_{14,321}$=1.266, p>0.1). The interaction between depth and geographical sector was not significant. Two-way ANOVA showed that densities across geographical sectors (F_6=7.489, p<0.0001) differed significantly among them but not among depth strata (F_3=0.5484, p>0.5). Significant interaction was detected (p<0.01).The highest mean density values were generally found deeper than 500 m in those sectors belonging to the Algerian basin (from Western Alboran to Ibiza), whereas in the northernmost sectors the highest mean densities where found slightly shallower (400-500 m) (Table 3b). Overall, the highest mean densities were found in the intermediate sectors, from Vera to Ibiza; densities where also high in the northernmost sectors (Central and Northern Catalonia), while the lowest density values were detected in the Alboran Sea sectors. Biomass however showed a slightly different pattern, since the depth of highest biomass values was found below 500 m in all sampled sectors (except Eastern Alboran), including those in the Balearic basin, a fact related to the different size structure found in the two basins, with a higher occurrence of juveniles, occurring in shallower waters, in the northern sectors, as compared to the rest of sampled sectors (see below).

The relationship between percentage occurrence and mean density per depth strata (Figure 3b) showed that high densities were associated to depths with a high percentage occurrence of the species, especially so in strata deeper than 450 m, which would accordingly be categorised as the optimal depth ranges for *P. multidentata* in the study area for both occurrence and density, taking always into account that the depth distribution range of the species was not fully covered by the samplings. Occurrences of the species at depths shallower than 400 m were always associated to very low densities.

Concerning the ratio between biomass and density by depth (i.e. an index of mean weight per individual within each depth stratum), it also showed, as in *P. sivado*, a significant increasing trend with depth ($P < 0.01$), clearly suggesting the occurrence of a marked size increase with depth (Figure 4b).

1.3.2.3. Size structure

Sizes of *P. multidentata* ranged between 7.7 and 47.9 mm CL. The most remarkable feature that size frequency distributions per depth and sector showed (Figure 6) is that the occurrence of juveniles was practically restricted to the sectors of the Balearic basin (CCAT and NCAT), where the population size structure was clearly bimodal, with the juvenile cohort being present at sizes comprised between 8 and 16 mm CL and adults found at sizes between around 24 and 32 mm CL. In these northern sectors, the occurrence of individuals with sizes around or larger than 40 mm CL was practically anecdotical. In contrast, very few or hardly any juvenile was present in the populations from the Alboran Sea and Alicante, the sectors belonging to the Algerian basin. In these sectors, most of the population was comprised of adult individuals of sizes ranging between 24 and 32 mm CL, but also a third, larger, cohort was discernible at sizes of around 40 mm in most samples.

The frequency distribution of the mean sizes of the cohorts identified in the SFD by depth strata (Figures 7 and 8), showed that most populations were structured in 2-3 main cohorts, broadly corresponding to juvenile (placed around 13-19 mm CL) and adult individuals, with two main cohorts, one around 30 mm CL, and another at around 38 mm CL, this last not present in NCAT. Juvenile cohorts were only identified in the Balearic basin (NCAT, CCAT) and in Alicante (ALIC); they were not identified in the Alboran Sea.

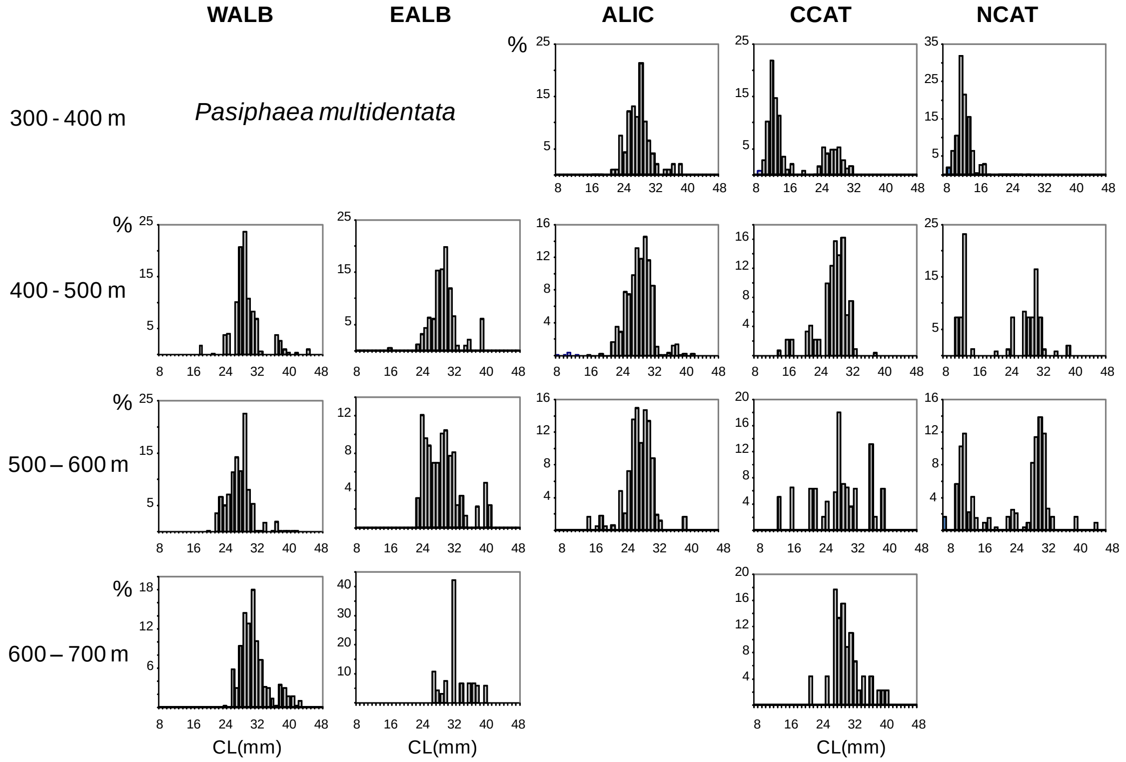


Figure 6: Size frequency distributions of *Pasiphaea multidentata* per depth interval in the five best sampled geographic sectors. Total N: WALB = 337; EALB = 118; ALIC = 668; CCAT = 225; NCAT = 205)

1.3.2.4. Temperature and salinity

Occurrences of *P. multidentata* took place at bottom temperatures comprised between 12.91 and 13.43ºC and salinities between 38.20 and 38.54. The temperature window was narrower for juveniles than for adults (Figure 10), while concerning salinities the range for juveniles was slightly broader. In particular, adults occurred (percentiles 25-75) at temperatures between 13.10 and 13.25ºC and salinities of 38.44 and 38.50, while juveniles occurred at temperatures between 13.25 and 13.30 and salinities between 38.42 and 38.50.

1.4. Discussion

The information obtained during the studied series of trawl surveys has allowed the analysis of the distribution patterns of density and biomass of the two species of the genus

Pasiphaea present in the western Mediterranean throughout the southern and eastern coasts of the Iberian Peninsula down to depths of around 800 m. Furthermore their population size structure was also described. While the overall depth range distribution of both *P. sivado* and *P. multidentata* found in the present study fall within the ranges described in the literature for these species (Cartes 1993a, Koukouras *et al.* 2000, Fanelli *et al.* 2007), several patterns could be studied in detail given the broad geographic range of the surveys (Bertrand *et al.* 2002). This has allowed delimiting up to 10 smaller geographic sectors, based on the heterogeneous geomorphological and oceanographic characteristics of the western Mediterranean.

Most of the information available on distribution patterns, size structure and other population characteristics of the two species of *Pasiphaea* studied herein was restricted to the northernmost sampled area, the Catalan coasts (Abelló *et al.* 1988, 2002, Cartes 1993a,b,c, Company *et al.* 2001, 2003, Aguzzi *et al.* 2007, Ramirez-Llodra *et al.* 2007). Orsi-Relini and Pinca (1990) and Orsi-Relini and Relini (1990) respectively provided information on reproduction and trophic interaction in the Ligurian Sea (NE of the western Mediterranean basin). No biological or population information was to date available from other areas within the distribution range of the two species. Present results have shown the occurrence of marked differences in both distribution and population characteristics between the southwestern areas in the Alboran Sea, located in the Algerian basin of the western Mediterranean, and those present in the northeastern area, the Catalan Sea in the Balearic basin.

Thus, the geographic distribution of densities of *P. sivado* has firstly shown a heterogeneous distribution pattern, with two main nuclei, one in the Alboran Sea, and especially in its western area, and another along the Catalan coasts in the Northeast. Additionally, the bathymetric distribution in the West Alboran sector was markedly different to that found in the rest of geographic sectors. In this area, the species occurred in both much shallower and deeper waters reaching markedly higher densities at depths between 100 and 250 m, where this species was practically absent from the rest of sectors. The bathymetric distribution of *P. multidentata* reached also shallower waters in the western Alboran sector. This pattern found in both *Pasiphaea* species, although more marked in *P. sivado,* is in agreement with the occurrence of temporal upwellings located along the northwesternmost region of the Alboran Sea, in the area around Malaga (Vargas-

Yáñez and Sabatés 2007). These upwellings are responsible for the occurrence of high primary production cells which have been shown to allow delayed coupling of epibenthic shrimp populations with respect of plankton blooms associated to these upwelling cells (Fanelli and Cartes 2004). The occurrence of these upwelling cells close to the coast is a consequence of the interaction of the permanent strong eastward inflow of Atlantic water into the Mediterranean through the nearby Strait of Gibraltar with the steep continental slope of the southern Iberian continental margin, and intense local westerly winds (Millot 2005, Vargas-Yáñez and Sabatés 2007). Moreover, densities of *P. sivado* have been shown to be much higher than those of *P. multidentata* in accordance with Company *et al.* (2001) who suggested that the greater fecundity output of *P. sivado* would support its higher population densities when compared with *P. multidentata.*

Both juvenile and adult *P. sivado* are known to perform daily vertical migrations into the pelagic realm during night-time hours, while remaining on the bottom or close to it (epibenthic layer) during the day (Cartes1993c, Aguzzi *et al.* 2007), in this way, they can be considered as bentopelagic or nektobenthic species. Night-time daily migrations into the water column have also been reported for *P. multidentata*, but mainly restricted to juveniles, with the apparent exception of the adults (CL > 30 mm), which are assumed to perform epibenthic depth displacements (Cartes1993c, Cartes *et al.* 1993, Aguzzi *et al.* 2007). The day-time sampling schedule of the present trawl surveys would accordingly be adequate to sampling both juvenile and adults of the two species.

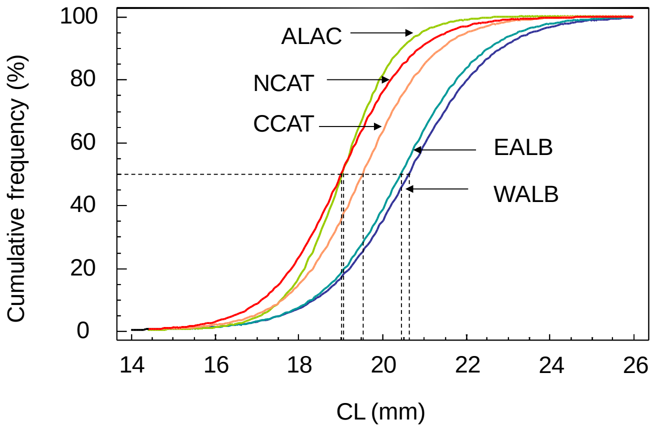


Figure 9: Logistic curves adjusted to the cumulative distribution by size of ovigerous female Pasiphaea sivado by geographic sector (NCAT: North Catalonia; CCAT: Central Catalonia; ALAC: Alacant; EALB: Eastern Alboran; WALB: Western Alboran)

The relationship between mean density and percentage occurrence by depth stratum allowed delimitating those depth strata showing high figures of both density and occurrence, which could be assumed to be the optimal depth ranges for the species. These were clearly located between 300 and 500 m in *P. sivado*, and deeper than 500 m (down to the deepest sampled depth, 800 m) in *P. multidentata.* It must be emphasized that the sampling schedule clearly encompassed the whole bathymetric distribution range of *P. sivado* in the study area (Abelló *et al.* 1988, 2002, Cartes *et al.* 1994), whereas in *P. multidentata* it did not reach the deepest distribution of the species, which has been reported to occur down to 2261 m depth (Cartes 1993a) in the Mediterranean.

Both species showed a marked size increasing trend with depth, as shown in previous papers (Mauchline 1972, Company *et al.* 2001), with juveniles being found in much shallower waters than adults. In *P. sivado*, populations in the Alboran Sea reached larger sizes than those present in the northwestern Mediterranean and in the intermediate Alicante sector. This implies that population dynamics are different in these areas, probably linked to the high productivity of the Alboran Sea (Fanelli and Cartes 2004). Recruitment in *P. sivado* was detected throughout the study area in the present surveys, performed in spring, in accordance with the main autumn-winter reproductive season of the species (Company *et al.* 2001), and was mainly located at depths shallower than 300-400 m. On the contrary, recruitment in *P. multidentata* was not present in the Algerian basin sectors (Alboran Sea and Alicante regions), while it was marked in the Balearic basin. The reproductive season in *P. multidentata*, from studies made in the Catalan Sea (Company *et al.* 2001, Ramirez-Llodra *et al.* 2007), is centered in late atumn – winter. The absence of recruitment in spring in the geographic sectors belonging to the Algerian basin may imply that seasonality of reproduction in this species significantly differs between the populations inhabiting the two basins.

The analysis of the environmental information available clearly detected differences between the occurrence patterns of the two species with respect to temperature and salinity on the bottom, as well as between juveniles and adults of the two species. Temperature and salinity windows (25-75 percentiles) of adults of the two species clearly overlapped, but the salinity range of *P. multidentata* was narrower than that of *P. sivado*, and much narrower than that shown by juveniles of the species, while the temperature range was contiguous but non-overlapping at a limiting temperature of 13.25 °C. In contrast, the temperature-salinity window of *P. sivado* juveniles was much larger, and widely overlapping, than that of the adults, which reached, however, higher salinities. This clearly showed that in both species, but particularly in *P. sivado,* juveniles showed a wider thermohaline window, being able to cope with a wider variability in temperature and salinity conditions. The ability of juveniles to inhabit a wider range of salinity is common to other marine species, a physiological feature that has been mainly studied in coastal waters (McGaw and Naylor 1992, Abelló *et al.* 1997, Reid *et al.* 1997) related to the ability to cope with osmotic stress and the energetic balance involved. A close dependence on a restricted salinity range has also been shown in deep-sea and continental slope crustaceans (Guijarro *et al.* 2008, 2009, Cartes *et al.* 2011).

The differential distribution pattern between juveniles and adults of both *P. sivado* and *P. multidentata* (Company *et al.* 2001) could be related, as in other continental slope caridean shrimps (Company and Sardá 1997, Carbonell and Abelló 1998), to trophic resource partitioning between the two ontogenetic phases, which would allow a lesser degree of intraspecific competition. The dietary overlap between these species has been shown to be low due the different size spectra of their respective prey items (Mauchline 1972, Cartes 1993b). Adults of *P. sivado* and juvenile *P. multidentata* share a similar size spectrum and they are both important food items to *P. multidentata* adults (Cartes 1993b). Size segregation, although implying a higher degree of osmotic regulation and its concomitant higher energetic expenses, would also be helpful to avoid intraspecific predation by juvenile *P. multidentata*.

The present study showed that in both*P. sivado* and *P. multidentata* Alboran Sea populations, their bathymetric distribution, density, maturity size, and population size structure were clearly differentiated from those in the Balearic basin. The populations in the intermediate Alicante sector showed more affinities with the Alboran Sea populations, especially so in *P. multidentata*, than with the northern populations, in agreement with their belonging to the Algerian basin.

Chapter 2

Sexual dimorphism in the benthopelagic shrimp *Pasiphaea sivado* (Crustacea: Caridea: Pasiphaeidae)

Sexual dimorphism in the benthopelagic shrimp *Pasiphaea sivado* (Crustacea: Caridea: Pasiphaeidae)

2.1 Introduction

Pasiphaeid shrimps are a group of benthopelagic and pelagic species that presently comprises a total of 98 species grouped in seven genera: *Alainopasiphaea* (2), *Eupasiphae* (4), *Glyphus* (1), *Leptochela* (15), *Parapasiphae* (4), *Pasiphaea* (67), and *Psathyrocaris* (5) (De Grave and Fransen 2011, Komai *et al.* 2012). In particular, within the genus *Pasiphaea* up to four morphologically-related species groups have been recognized, among them the *Pasiphaea sivado* group, to which a total of nine species belong (Hayashi, 1999, 2006). Species of this family are distributed along a wide latitudinal range, from tropical to arctic and subantarctic waters, mainly on and above continental slopes and in the open sea, including deep-sea habitats (Clarke and Holmes 1987, Kaartvedt *et al.* 1988, Gibbons *et al.* 1994, d'Udekem and d'Acoz 1999, Tavares and Cardoso 2006). Morphological features in this family, such as lateral compression of the body and pleopods, are considered to be the result of selective pressures on adaptation to the pelagic environment (Cartes *et al.* 1993a, Johnsen 2005, Aguzzi *et al.* 2007). Notwithstanding the large amount of systematics and faunistics work on this family (e.g. Stephensen 1923, Zariquiey Álvarez 1957, Iwasaki 1990, Hayashi 1999, Tavares and Cardoso 2006) there are still few detailed studies on biological aspects of species of the family, and most of them refer mainly to the European species *P. sivado* and *P. multidentata* (Cartes 1993b, Company and Sardá 2000, Frank 2000, Company *et al.* 2001, Ramirez-Llodra *et al.* 2007), but also to Pacific Ocean species such as *Pasiphaea japonica* and *Leptochela gracilis* (Nanjo 2007, Oh *et al.* 2006).

No reference is made to the occurrence of macroscopic differences in shape in relation to gender in this family (Stephensen 1923, Zariquiey Álvarez 1968, Iwasaki 1990, Hayashi 1999, Tavares and Cardoso 2006), except for the noted occurrence of differences in pleopod shape in *Pasiphaea japonica* (Doi 1975, Nanjo and Ohtomi 2009). Concerning size dimorphism, males have been reported to be slightly larger than females in *P. sivado*, and slightly smaller in *P. multidentata* (Company *et al.* 2001), based in overall size frequency distributions, but sexual dimorphism was so far considered to be not significant in neither *P. sivado* nor *P. multidentata*.

After careful macroscopic examination of previously sexed individuals of *P. sivado* (based on the presence of appendix masculina in the second pleopods of males), marked differences in pleopod shape were observed between adult male and female individuals of the species. We herein aim to report the occurrence of this morphological dimorphism in *P. sivado*, as well as to quantify it, based in both traditional and geometric morphometrics, which may help to further evidence and characterize the occurrence of this dimorphism (Rufino *et al.* 2006). Size-related population structure and sex-ratio are also analyzed in relation to geographic and bathymetric characteristics.

2.2 Material and Methods

2.2.1 Pleopod morphology

Ten adult specimens of *Pasiphaea sivado* (five males and five females) were selected from a sample taken by demersal trawling in the western Mediterranean Sea (Alboran Sea: 36.4908 N, -4.2885 W) at 424-428 m depth during daytime on 17/05/2010. The haul was performed on board R/V "Cornide de Saavedra" at a speed of 3 knots during 1h using a demersal GOC-73 otter trawl with a 2.5-3.0 m vertical opening and a codend stretched mesh size of 20 mm (Fiorentini *et al.* 1999). The cruise was part of the MEDITS_ES project (Bertrand *et al.* 2000, 2002).

The selected specimens had a cephalothorax length (CL) > 18 mm and were clearly morphologically considered as adults (Company *et al.* 2001; present observations). CL was measured using a calliper rule with a precision of 0.01 mm. Sizes of the examined specimens ranged between 18.8–23.5 mm CL in females, and between 21.6-24.0 mm CL in males. The individuals were sexed by checking the presence/absence of appendix masculina on the endopod of the second pleopod, as usual in caridean shrimps.

In a first step, the five pleopod protopods of one of the males and one of the females of similar size were figured in order to graphically show the a priori differences observed between adult males and females. Then, with the aid of a "Leica MZ12" magnifier provided with ocular micrometer, the length and maximum width of all right pleopod protopods of all ten specimens studied were measured to obtain a first index of pleopod shape based on the ratio between length and width of this segment.

Pictures were also taken of all right second pleopods (external view) of all five males and females studied using the same magnifier (which has an integrated video camera Leica DC100 and DC200 digital imaging systems) to further assess the occurrence of sexual dimorphism using geometric morphometric techniques. The first landmark was placed on the dorsal maximum convex curvature of the protopod, the second and fourth were placed at the anterior maximum curvature where the exopod and endopod insert on the protopod, the third at the maximum concavity between these two landmarks, the fifth at the maximum perpendicular width of the protopod in relation to the first landmark, the sixth at the ventral maximum curvature in the posterior part of the protopod and the seventh at the maximum concave curvature on the posterior dorsal margin of the protopod (Figure 1).

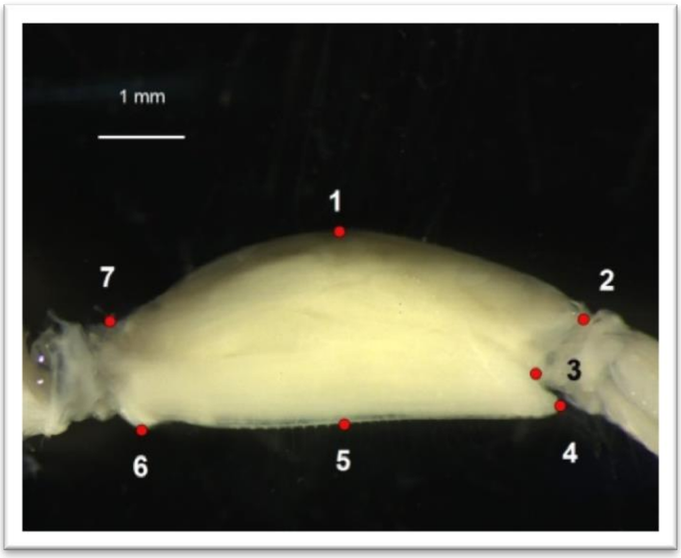


Figure 1: Landmark positions in the digital images obtained with stereoscope using a 16x magnifier with optical zoom. The width and length of each *Pasiphaea sivado* pleopod protopod were measured from the scalar distance (in mm) between the landmarks 1-5 and 4-6, respectively.

Using Tps.Dig2 v2.16 software (Rohlf 2010a), the *x,y* coordinates of the landmark dataset were obtained. Generalized procrustes superimposition analysis (GPA) was performed to align and to eliminate scale and orientation distortions before proceeding to shape analysis using tpsRelw v1.49 software (Rohlf, 2010b). Statistical analysis of shape variance was assessed using the MorphoJ software (Klingenberg 2011). Then procrustes ANOVA was applied, which is a two factorial ANOVA that performs analysis of both size (univariate) and shape (multivariate). This procedure therefore is a useful method to discover localized variation (Klingenberg and McIntyre 1998). Finally a principal component analysis (PCA) was applied to quantify the contribution of the principal factor of landmark displacement that explains the shape variance between genders. This PCA was run using the covariance matrix generated from shape variation components, which were previously derived from the coordinates landmark dataset, pooled within-group with sex as covariate (Viscosi and Cardini 2011).

2.2.2 Gender differences in size. Sex-ratio

In order to assess whether there occurred significant differences between males and females concerning overall size dimorphism, data were obtained from a total of 4010 *P. sivado* individuals. The samples were collected during a series of demersal fisheries research cruises (MEDITS_ES) between 2004 and 2011 at depths from around 40 to 800 m along the Mediterranean coasts of the Iberian Peninsula (Bertrand *et al.* 2002). In these cruises, *P. sivado* was only collected at depths between 150-750 m.

All specimens were sexed as described above and males were classified as juveniles when the appendix masculina was not fully formed, which usually appeared as small buds. This was evident in individuals with sizes ranging between 9 and 16 mm CL, which were therefore classified as juvenile males. However, the proportion of individuals showing appendix masculina buds was very small at sizes smaller than 11-12 mm CL. This implies that based only in this character the correct assignation of gender was not possible at these small sizes, there probably being a larger proportion of males with still undeveloped appendix masculina. Therefore, sex-ratio analysis was restricted to individuals exceeding 12 mm CL.

Sex-ratio, as proportion of males, was calculated within each 1 mm CL size class, separately for samples taken in the Alboran Sea (the westernmost basin of the Mediterranean Sea), the intermediate Alacant region (between Cape Palos and Cape La Nao) and the Catalan Sea (in the NW Mediterranean). These sectors were established according to previous results (Simão *et al.*

submitted; see Chapter 1 on population characteristics of pasiphaeid shrimps) in which differences in size at maturity were observed between Alboran Sea populations and the rest of sampled populations along the Iberian Peninsula Mediterranean coasts. Sex-ratio was not calculated in size classes with less than 25 individuals. The significance of the deviation from an expected 1:1 sex-ratio was calculated using a heterogeneity G-test (Sokal and Rohlf 1981, Abelló *et al.* 1990). The relationship of sex-ratio with depth was also calculated; only samples ≥25 sexed individuals larger than 15 mm CL were considered for this analysis; this cutting size was chosen to ensure that sex-ratio was calculated over adult specimens to minimize error in gender assessment (see above), and also to encompass the whole adult cohort as visually assessed through size frequency distributions.

In order to verify whether size frequency distributions (SFD) differed between geographic populations and between depth strata, the Kolmogorov-Smirnov test within VITMAN fisheries software (Lleonart and Salat 1992) was used. Combinations of geographic area and depth strata resulting in less than 100 individuals measured (i.e. at both extremes of the bathymetric range of the species in each area) were not used in the analyses.

2.3 Results

2.3.1 Pleopod morphology

The difference in protopod morphology between genders of *Pasiphaea sivado* can be easily identified by sight (Figure 2). Overall, in all five pleopod pairs, adult female protopods were clearly much thinner than those in adult males. Additionally, the anterior edge of the male protopods was clearly much more convex than in females, while the posterior edge was approximately straight in both males and females. As shown in Figure 3, the ratio between pleopod protopod length and width (L/W), which can be considered a protopod thinness index, was much higher in females than in males, thus characterizing the thinner, rectangular, elongated shape in females versus the more robust shape present in males. A significant difference between genders was obtained for all five pleopod pairs (Table 1). Additionally, and concerning overall morphology, no setae were present on the anterior edge of the first pleopod protopod in any of the adult males examined. Also, in the rest of pleopods, anterior setae were much scarcer in males than in females.

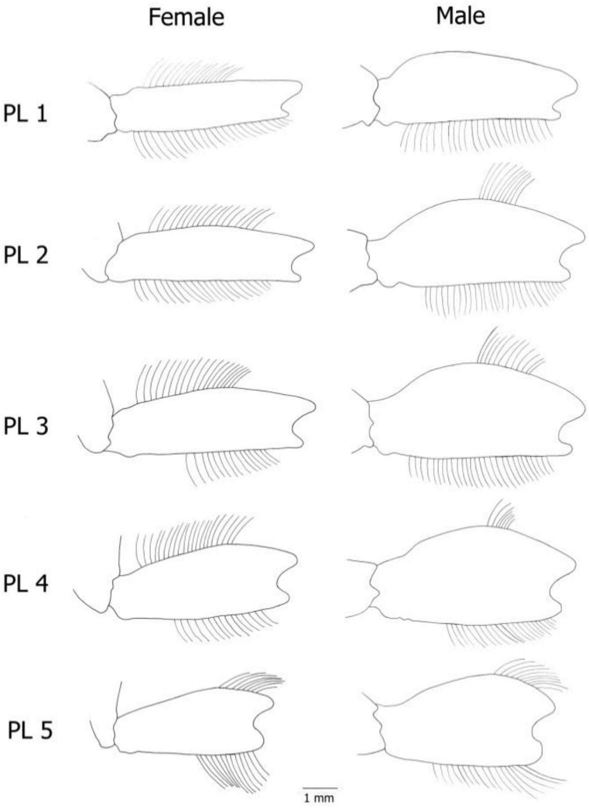


Figure 2: Lateral view of the protopods of each five pleopods of a 22.7 mm CL *Pasiphaea sivado* female and of a 22.6 mm CL male.

The use of geometric morphometrics techniques further evidenced the differences between genders in pleopod protopod shape. Thus, both *P. sivado* males and females grouped as two well-differentiated clusters when analyzing the shape of their second pleopod protopods (Figure 4a). The overall consensus shape (Figure 4b) of the pleopod protopods, which is based on the geometric mean position of each landmark, clearly differentiated between the thinner, elongated shape of the females versus the heavier found in males. Principal Component Analysis showed that the first principal component (PC1), which accounted for 56.1% of the total shape variability, clearly differentiated males from females. The shape variance between genders explained by the PC1 was significantly different (ANOVA $F_{10,80}$ = 27.24 $p < 0.0001$) and was not affected by the centroid size effect (ANOVA $F_{1,8}$= 1.87 $p = 0.209$). The next five principal components explained 16.5%, 10.4%, 6.2%, 3.5% and 1.26% of the total shape variance, respectively.

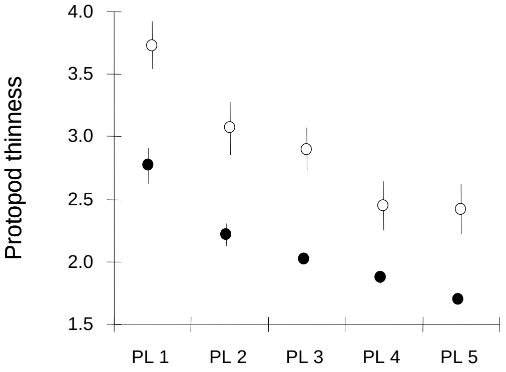


Figure 3: Mean pleopod (PL) protopod thinness index (length/width) (±SEM) of female (white circles) and male (black circles) *Pasiphaea sivado*.

Table 1: Number of individuals examined (N), mean, standard deviation, maximum and minimum length (L) and width (W) of the pleopod protopod (PL1-5), and mean ratio L/W of adult *Pasiphaea* sivado females (CL_{range}= 18.8–23.5 mm) and males (CL_{range}= 21.6-24.0 mm). Significance of differences of the L/W ratio between genders is also shown.

Females		Length				Width				Mean L/W	*t* Test
	N	Mean	+-SD	Max	Min	Mean	+-SD	Max	Min	ratio	p
PL 1	4	4.65	0.38	5.0	4.1	1.25	0.14	1.4	1.1	3.73	p< 0.05
PL 2	5	5.13	0.47	5.8	4.7	1.73	0.38	2.3	1.3	3.07	p< 0.02
PL 3	5	4.97	0.38	5.5	4.5	1.79	0.41	2.4	1.4	2.90	p< 0.05
PL 4	5	4.73	0.43	5.2	4.2	1.99	0.38	2.6	1.5	2.44	p< 0.05
PL 5	4	4.19	0.40	4.5	3.6	1.73	0.20	1.9	1.4	2.42	p< 0.001
Males		Length				Width				Mean L/W	
	N	Mean	+-SD	Max	Min	Mean	+-SD	Max	Min	ratio	
PL 1	5	4.89	0.22	5.2	4.6	1.79	0.22	2.0	1.4	2.76	
PL 2	5	5.09	0.09	5.2	5.0	2.33	0.22	2.5	2.0	2.21	
PL 3	5	5.08	0.36	5.6	4.8	2.52	0.16	2.8	2.4	2.01	
PL 4	5	4.86	0.30	5.3	4.5	2.60	0.09	2.7	2.5	1.87	
PL 5	5	4.16	0.23	4.4	3.8	2.46	0.16	2.7	2.3	1.70	

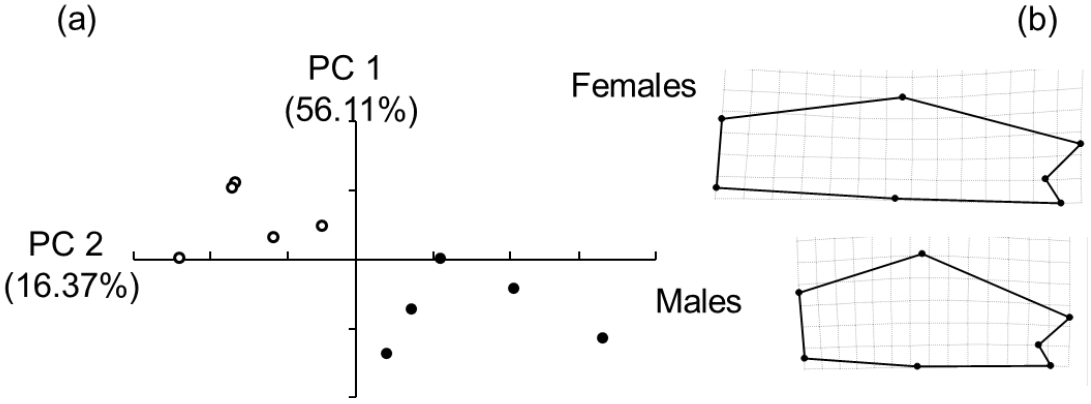


Figure 4: Shape variation analysis (a) and thin plate consensus shapes (b) for adults *Pasiphaea sivado*: females (white circles) and males (black circles). The contribution percentages of the two principal components are presented in the correspondent axis.

2.3.2 Comparison of sizes and size-related sex-ratio among geographic areas

The overall size frequency distributions of sexed individuals by geographic area (Figure 5) were biased towards adult individuals in all populations. The Alboran Sea population showed a higher proportion of individuals in the largest sizes, when compared with those obtained in the other areas: Alacant and Catalan Sea. The comparison between male and female SFDs within each area showed that they were not significantly different in any case (main diagonal in Table 2). Comparison of male or female SFDs among areas also showed non-significant values (Table 2).

The analysis of sex-ratio by size class showed, in all sectors, that females were dominant at sizes smaller than around 18-19 mm CL, while the proportion of males was significantly higher at larger sizes, therefore evidencing the occurrence of a slight sexual size dimorphism in *P. sivado* (Figure 5). When paying especial attention to the precise size at which the dominance of males occurred, a decreasing gradient was found along the study area, with the proportion of males being significantly higher at sizes larger than 21 mm CL in the Alboran Sea, 20 mm in Alacant and 19 mm in the Catalan Sea (Figure 5).

Table 2: Comparison of SFD between geographic populations (Kolmogorov-Smirnov test). The *p* values for the comparison between females are presented above the diagonal, those between males below the diagonal, and those comparing males and females within each population along the main diagonal.

Kolmogorov-Smirnov (p value)

		Alboran	Alacant	Catalan	
Alboran		0.869	0.308	0.537	♀
Alacant		0.522	0.263	1.000	
Catalan	♂	0.182	1.000	0.243	

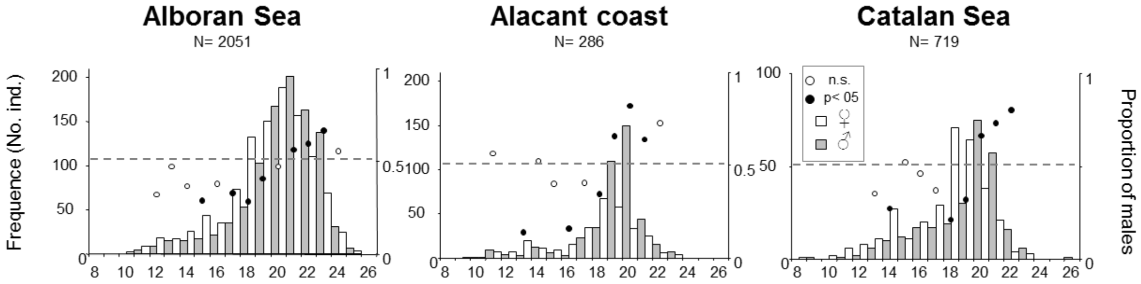


Figure 5: Size frequency distributions by sex in *Pasiphaea sivado* from the Alboran Sea, Alacant coast and Catalan Sea geographic sectors. The Sex-ratio, as proportion of males is represented by circles, with indication of values showing a significant (p<0.05, dark circles) or non-significant (*n.s.*, light circles) deviation from an expected 1:1 ratio. The total number of individuals analysed by geographic area is presented.

2.3.3 Size structure with depth by geographic area

When the shape of the SFDs was analyzed by depth, a high frequency of juveniles in relation to adults was evident in the shallowest depth stratum (200-300 m), especially so in the Alboran Sea (Figure 6). In this area, the population showed a marked SFD displacement towards larger sizes with increasing depth; no noticeable variation in SFD displacement was evident in the Alacant and Catalan Sea populations (Figure 6 and Table 3). The comparison of the SFDs with the Kolmogorov-

Smirnov test showed the occurrence of significant differences only in the Alboran Sea population, in both males and females (Table 4). No significant differences of SFD variation with depth were detected in the Alacant and Catalan Sea populations.

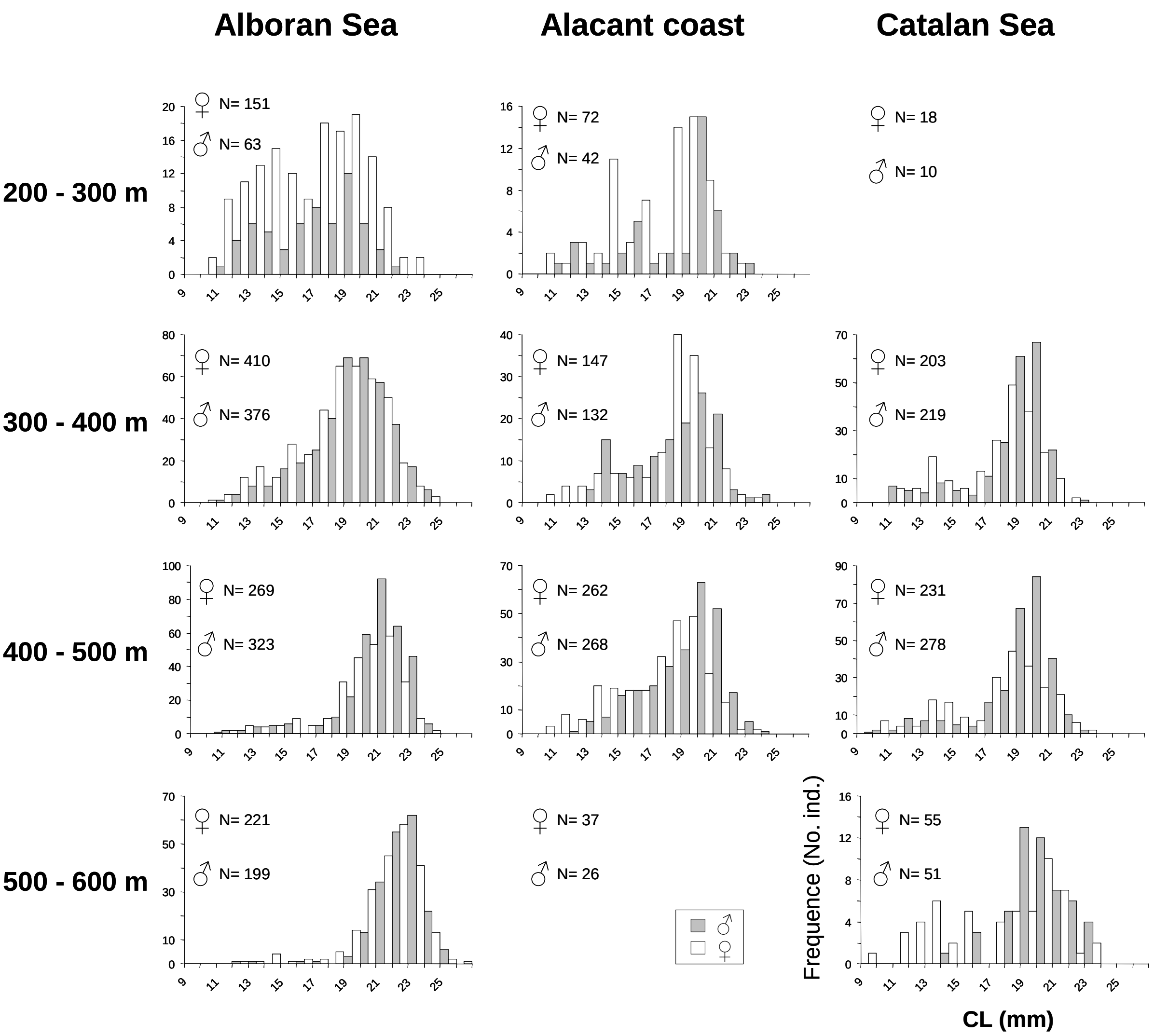


Figure 6: Size frequency distributions of male and female *Pasiphaea sivado* by 100 m depth strata. Strata with less than 100 measured individuals were not taken into account. The number of measured individuals by gender (N) is shown.

Table 3: Comparison of median sizes among 100 m depth strata within each geographic population. The obtained Mann-Whitney *p* values for the comparison between females are presented above the diagonal, between males below the diagonal and between genders within each depth stratum along the diagonal.

	Median CL ♂	Depth Strata (m)		200-300	300-400	400-500	500-600		Median CL ♀
Alboran Sea	17.0	200-300		0.301	0.000	0.000	0.000	♀	16.8
	19.4	300-400		0.000	0.000	0.000	0.000		18.4
	21.0	400-500		0.000	0.000	0.000	0.000		19.9
	22.3	500-600	♂	0.000	0.000	0.000	0.000		22.4

	Median CL ♂	Depth Strata (m)		200-300	300-400	400-500	500-600		Median CL ♀
Alacant Sea	19.9	200-300		0.001	0.294	0.970	-	♀	17.8
	19.0	300-400		0.298	0.004	0.083	-		18.1
	19.7	400-500		0.626	0.008	0.000	-		17.6
	20.0	500-600	♂	-	-	-	-		20.0

	Median CL ♂	Depth Strata (m)		200-300	300-400	400-500	500-600		Median CL ♀
Catalan Sea	21.8	200-300		-	-	-	-	♀	12.9
	19.1	300-400		-	0.000	0.412	0.030		17.8
	19.4	400-500		-	0.036	0.000	0.959		17.9
	19.8	500-600	♂	-	0.001	0.023	0.000		17.8

Table 4: Comparison of size frequency distributions by 100 m depth strata within each geographic population. The Kolmogorov-Smirnov *p* values for the comparison between females are presented above the diagonal, between males below the diagonal, and between genders along the diagonal (values in italics); significant values are shown in bold.

			200-300	300-400	400-500	500-600	
Alboran Sea	200-300		*1.000*	0.650	0.061	0.000	♀
	300-400		0.278	*0.931*	0.685	0.013	
	400-500		0.001	0.272	*0.517*	0.184	
	500-600	♂	0.000	0.004	0.171	*0.830*	

			200-300	300-400	400-500	500-600	
Alacant coast	200-300		*0.105*	0.978	1.000	-	♀
	300-400		0.957	*0.687*	1.000	-	
	400-500		0.997	1.000	*0.208*	-	
	500-600	♂	-	-	-	-	

			200-300	300-400	400-500	500-600	
Catalan Sea	200-300		-	-	-	-	♀
	300-400		-	*0.240*	1.000	0.772	
	400-500		-	1.000	*0.261*	0.992	
	500-600	♂	-	0.736	0.985	*0.199*	

2.3.4 Sex-ratio with depth

A total of 84 samples were used to analyze the sex ratio across the bathymetric distribution of the species (Figure 7). Most of the samples (70.2%) did not show a significant deviation from a 1:1 sex-ratio. However, significant sex segregation was found in the rest of samples, with 11.9% of them being biased toward females and 17.9% towards males. The highest proportion of significant deviation toward females was found at the shallowest extreme of the bathymetric distribution of the species but also in the deepest strata. On the contrary, samples with a significantly higher proportion of males were located in the central depth strata. Overall, no significant tendency of gender segregation across the bathymetric distribution was found (r=0.0803, p=0.4677).

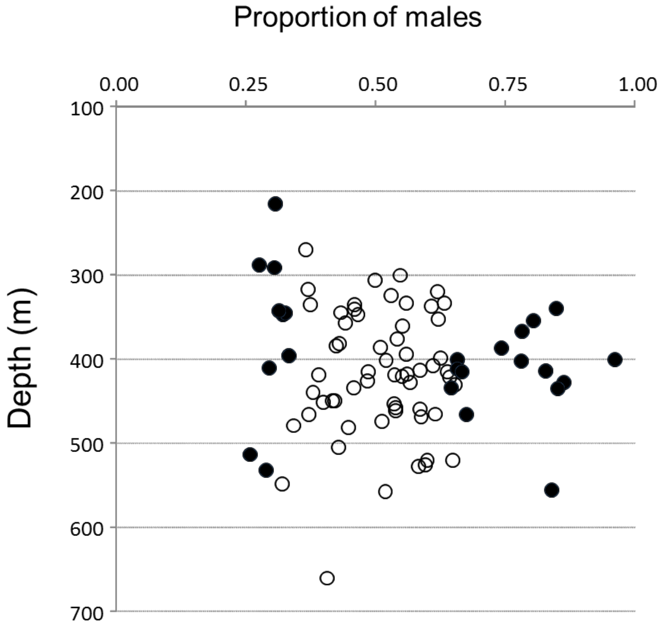


Figure 7: Sex-ratio distribution of *Pasiphaea* sivado as function of depth by geographic area: Alboran Sea (circles), Alacant coast (squares) and Catalan Sea (triangles). The full symbols correspond to samples significantly different to the expected 1:1 sex-ratio, while the empty circles indicate no significant difference in sex-ratio.

2.4 Discussion

2.4.1 Pleopod morphology

The occurrence of sexual dimorphism in pleopod shape was detected in *Pasiphaea sivado* by both the traditional linear measurement method and by the geometric morphometric analysis. An important, useful application of the present observations and description of the occurrence of sexual dimorphism in pleopod shape in adult *P. sivado* is, from now on, the ability to quickly assess the gender of individuals when performing population biology studies.

A similar sexual dimorphism in pleopod shape has also been reported in *Pasiphaea japonica*, but it also seems to occur in *Alainopasiphaea nudipeda* as evidenced by figures 1 and 2d in Hayashi (1999), as well as in *Pasiphaea semispinosa* (authors personal observations based in specimens deposited in collections: CBR at ICM-CSIC in Barcelona and CCDE at IEO-Cádiz). Other personal observations, not yet quantified, on the congeneric species *Pasiphaea multidentata* - a species that coexists with *P. sivado* across the Mediterranean Sea (Company *et al.* 2001, Abelló *et al.* 2002)- indicate that this species does not show such dimorphism.

The phylogenetic relationships within the family *Pasiphaeidae* are far from being properly understood (Hayashi 1999, 2006, Matzen da Silva *et al.* 2011; see also the section on genetics in this thesis). Based on present findings, the occurrence of sexual dimorphism in pleopod shape may well have a significant role in determining phylogenetic relationships within the family *Pasiphaeidae*, in particular within the *Pasiphaea sivado* group (Hayashi 1999, 2006), since both *P. sivado* and *P. japonica* (who also belongs to this group) have been shown to present this morphological characteristic (Doi 1975, Nanjo and Ohtomi 2009). Not enough information is available concerning other species in the genus or the family.

2.4.2 Size dimorphism and sex ratio

The present study provides also evidence on the occurrence of significant size dimorphism in *P. sivado*, a fact previously unreported in this species. In all geographic areas and depth strata, males were significantly larger than females. *P. sivado* presented a marked size structure pattern where females accumulated in size classes smaller than around 20 mm CL while males dominated in the largest size classes. Indeed, no strong size dimorphism is usually found in species with pelagic habits, while strong differences in size usually occur in strictly benthic/epibenthic species such as brachyuran crabs or lobsters.

In *P. sivado*, sex-ratio as a function of size clearly suggests the occurrence of differential growth rates between the genders, as reported in many species of crustaceans (Wenner 1972, Abelló and Cartes 1992, Guerao *et al.* 1994, Ben Mariem 2004, Oh *et al.* 2002, Kim 2005, Kevrekidis and Thessalou-Legaki 2006, Oh *et al.* 2006), a fact that is usually attributed to a higher investment of females on reproduction rather than on growth. This pattern appears to follow the so-called anomalous pattern described by Wenner (1972), in which no differences in sex-ratio would occur in juveniles, then a predominance of females would take place due to a lower female growth rate, with males finally accumulating their frequency in the largest sizes. Several explanations may account for this pattern, with different growth rates between the genders from the puberty moult or size at maturity being the most likely (Ben Mariem 2004, Fantucci *et al.* 2009). Some biological characters estimated by Company *et al.* (2001) for *P. sivado* in the Catalan Sea support the sex-ratio pattern with size reported in the present study. In that work males showed a faster growth rate (*k*= 0.62), a larger asymptotic size (L_∞=27.5 mm) and a higher growth performance index (Φ=0.13) than females (0.55, 26 mm and 0.06, respectively). Reproductive aspects of the species such as the continuous reproduction throughout the year and the higher volumetric index of females (2.92) compared to males (2.76) (Company *et al.* 2001) also suggest the smaller amount of energy investment in size growth by *P. sivado* females in relation to males. Another factor that may also limit the growth in females is the moulting delay of ovigerous females in order to avoid loss of attached embryos, a phenomenon characteristic of crustacean species with egg-carrying biological features (Guerao *et al.* 1994, Oh *et al.* 1999, Viegas *et al.* 2007).

Differential mortality rates between the genders may also contribute to reaching the observed pattern of sex-ratio variation with size (Abelló and Macpherson 1992). It is however so far not possible to assess the occurrence of different mortality rates between the sexes given the lack of behavioural observations on *P. sivado*. The size at which differences in the sex-ratio become significant has also been suggested to be an indirect indicator of the size at puberty moult and/or maturity size/age of the species (Abelló *et al.* 1990). In the present study the precise size at which males outnumbered females progressively decreased from the Alboran to the Catalan Sea through the Alacant area, corroborating the size and size at maturity pattern observed in Chapter 1, and suggesting the occurrence of environmental and ecological processes affecting growth rates.

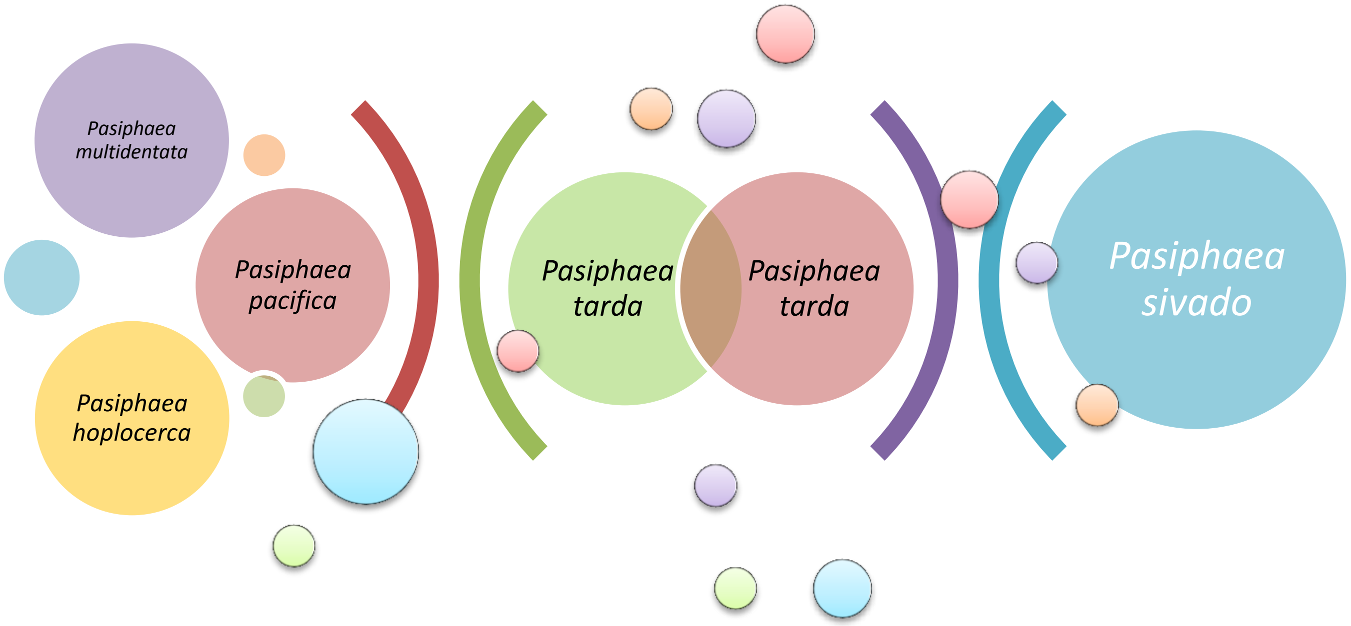
Overall, no significant gender segregation in *P. sivado* populations as a function of depth was verified, suggesting that this species does not perform differential migration between sexes over the sea bottom. In the Catalan Sea the absence of gender segregation by depth was also reported by Company *et al.* (2001) throughout the year, which could be related to the gregarious behaviour of macroplankton/micronekton organisms (McManus and Woodson 2012). During the day time, *P. sivado* inhabits the epi-/suprabenthic environment over the upper and medium slope, with high densities recorded when sampled by demersal trawl nets during fishery research surveys (see Chapter 1) (Fariña *et al.* 1997; Fanelli *et al.* 2007; Abad *et al.* 2007; Cartes *et al.* 2007b; García-Rodríguez *et al.* 2011), while during the night the species has been reported to migrate several hundred meters up the water column to waters near the surface to feed on plankton (Froglia and Giannini 1982, Cartes 1993a, Aguzzi *et al.* 2007, see also Chapter 4 on vertical migrations in this thesis).

Controversially, a relatively high number of samples showed significantly deviated sex ratios, both towards a higher proportion of males and of females. In the absence of direct observations, sex-ratio deviated groups, indicative of aggregative behaviour within genders, are suggestive of differential reproductive behaviour and/or selective feeding in relation with the energetics and quality needs depending on gonadal developmental stages. Thus, an increase in stomach fullness and dietary energy intake has been recorded in pre-reproductive females of the deep-sea shrimp *Aristeus antennatus*, parallel to a significant increase of the gonado-somatic index and fecundity (Cartes *et al.* 2008). Differences in diet composition between genders and maturity stages have also been found in other shrimps such as *Plesionika heterocarpus* and *Plesionika martia* (Fanelli and Cartes 2008). Aggregative behaviour within genders has also been related to other causes, such as the need to look for appropriate habitat for moulting (Reid *et al.* 1994, 1997, Sampedro and González-Gurriarán 2004) or for hatching (i.e females look for adequate sites to improve dispersal and survival of recently hatched larvae) (Hicks 1985; Zeng and Naylor 1997; Carr *et al.* 2004).

In attention to the concept of operational sex ratio (OSR), defined as the proportion of receptive females to sexually active males (Correa and Thiel 2003), the results of the present study suggest that the populations of *P. sivado* may overall be close to the OSR. The histograms of Figure 6 show that the overall shape of the SFDs of males and females is very similar, but males are displaced towards the right (largest sizes) by just one size class. If we quantify the total adult males and females bigger than the size class precedent of the one where the sex ratio starts to be biased towards males, we will find a sex-ratio very similar to the equilibrium. Even with the existence of a size sexual dimorphism or some samples with biased sex-ratio, no overall sex segregation at population level was found.

Chapter 3

Population genetics and phylogeny of pasiphaeid shrimps from the western Mediterranean Sea



Population genetics and phylogeny of pasiphaeid shrimps from the western Mediterranean Sea

3.1. Introduction

Pasiphaeid shrimps are a worldwide distributed family of pelagic and benthopelagic shrimps. They comprise so far a total of 97 species grouped in seven genera: *AlainoPasiphaea* (2), *Eupasiphae* (4), *Glyphus* (1), *Leptochela* (15), *Parapasiphae* (4), *Pasiphaea* (66), and *Psathyrocaris* (5) (De Grave & Fransen, 2011). Most pasiphaeid species are benthopelagic species occurring on the continental slope and show important diel vertical movements, being present on the bottom or benthic boundary layer during the day, while rising several hundred meters into the water column during the night (Aguzzi *et al.* 2007, Naylor 2010). The wide geographical and bathymetrical distribution as well as their important role in linking the pelagic and benthic trophic webs confer to the *Pasiphaeidae* a wide presence on marine community and trophic studies (Fashan and Foxton 1979, Cartes 1993a, Vereshchaka 1995, Company & Sardá 2000, Frank 2000, Company *et al.* 2001, Oh *et al.* 2006, Ramirez-Llodra *et al.* 2007, Nanjo and Otomi 2009). However there still lacks substantial information about the population biology and genetic dynamics of this family.

In the Mediterranean Sea the Pasiphaeoidea superfamily is represented by just two, but very common species *Pasiphaea sivado* and *Pasiphaea multidentata*. *Pasiphaea sivado* is a widely distributed species along the Mediterranean Sea, northeastern Atlantic and western African coasts being commonly sampled by trawling during demersal fisheries research surveys (Politou *et al.* 2000, Monteiro *et al.* 2001, Ungaro *et al.* 2005, Fanelli *et al.* 2007, García-Rodríguez *et al.* 2011). The species has a marked diel vertical migration occurring up to the first 50 m below the surface during the night, while occurring on the upper and middle slope bottom during the day (Omori 1974, see also the Chapter 3 of this thesis), where it inhabits preferentially on bottom depths of around 350-500 m (Abelló *et al.* 1988, 2002, Company *et al.* 2001, Muñoz *et al.* 2012). In the

Mediterranean *P. sivado* reproduces continuously around the year, a pattern common in tropical and shallow water species (Orsi-Relini and Pinca 1990, Company *et al.* 2001).

Pasiphaea multidentata, on the contrary, is a much scarcer species, with a much deeper habitat (deeper than 500 m) (Cartes *et al.* 1993, Biagi *et al.* 2002, Company *et al.* 2004) and more linked to the benthic environment (Cartes 1993b, Cartes *et al.* 2007, 2009) than *P. sivado*. Its pelagic behaviour has been mainly reported for juvenile individuals, which show a shallower distribution than adults (Company *et al.* 2001, Aguzzi *et al.* 2007). However adults are also able to perform wide vertical movements but their distance range is more restricted to the benthic boundary layer (BBL), being absent in pelagic strata shallower than 400 m depth (see the Chapter 3 of this thesis). Moreover the species presents seasonal reproduction placed during the boreal autumn and winter, producing fewer but bigger yolked eggs than *P. sivado* (Company *et al.* 2001), in the Mediterranean Sea, indicating that *P. multidentata* is a typical demersal / temperate species (Ramirez-Llodra *et al.* 2007).

Considering the daily night occurrence of both species in the pelagic realm and their relatively wide vertical distribution, their populations would a priori tend to present panmitic characteristics. However, the pelagic environment is not a homogeneous system. Different water masses, with their own temperature, salinity and density characteristics are present in the ocean basins and species can evolutionarily evolve to preferentially associate with different physico-chemistry characteristics (Cartes *et al.* 2011). Also, the occurrence of geographic and geomorphologic barriers may prevent population mixing and dispersion (Palero *et al.* 2008, 2011, García-Merchán *et al.* 2012). Strong oceanographic currents, usually associated to water mass fronts, may also play both a population restriction role across the front, and a dispersion effect along their preferential direction (Patarnello *et al.* 2007, Carreras *et al.* 2011, Schunter *et al.* 2011).

In order to investigate the genetic characteristics along the Atlantic-Mediterranean transition, the two congeneric species of pelagic shrimps, *Pasiphaea sivado* and *Pasiphaea multidentata,* were selected to verify the occurrence of any population genetic differentiation between eastern Atlantic and Mediterranean populations which could be linked to oceanographic and/or geographic characteristics. Intraspecific population variability is also linked to interspecific variability in this wide group of species.

The precise placement of the family Pasiphaeidae within the Caridea, as well as the phylogenetic relationships among the genera and species within the family are not yet fully understood (Christoffersen 1990, Bracken *et al.* 2009, Chan *et al.* 2010, Matzen da Silva *et al.* 2011, Li *et al.* 2011). Recent taxonomic studies of Hayashi (1999) proposed differentiation of the *Pasiphaea sivado* group, composed by *P. sivado* (Risso 1816) and nine other species from the rest of the *Pasiphaeidae* family which present branchial reduction (see Hayashi 1999, 2006). Detailed studies on phylogeny and molecular aspects of *Pasiphaeidae* are still missing. In this way the selection of these two congeneric species may also allow to perform phylogenetic studies. We therefore aimed to present additional information on the genetic phylogeny of the Atlantic-Mediterranean species of Pasiphaea, taking into account the populations inhabiting both the Mediterranean and eastern Atlantic basins.

3.2. Material and Methods

3.2.1. Sampling

The material analysed in the present work was obtained from two demersal fishery research surveys run by the Instituto Español de Oceanografía (IEO). The Mediterranean Sea material was sampled across the Spanish coast during the 2009 and 2010 MEDITS_ES surveys (Bertrand *et al.* 2002) while the Atlantic Ocean material was obtained from the 2009 ARSA survey (Lopez de la Rosa 1997, Silva *et al.* 2011) in the Gulf of Cadiz. The Mediterranean samples were obtained from three previously defined zones established according to previous oceanographic and biogeographic results (Abelló *et al.* 2002, Rufino *et al.* 2005, Palero *et al.* 2009, García-Merchán 2012): Northern Alboran Sea (between the Strait of Gibraltar and Cape Gata), Alacant coast (from Cape Palos to Cape La Nao), and Catalan Sea (from Barcelona to Palamós).

On board, after each haul, the species were identified according to Zariquiey Álvarez (1968). The number of sampled individuals by species and geographic sector is shown in Table 1. For each individual a section of the abdominal muscle (of approximately 20 mg) was preserved in absolute ethanol at 4 °C for posterior genetic analysis in the laboratory on land.

Table 1: Sampled areas and number of COI sequences used for the intra-population variability analysis. Five sequences from the Gulf of Cadiz, and those from western Scotland and Gulf of St. Lawrence were obtained from GenBank.

Sampled area	Sea	*P. sivado*	*P. multidentata*
Catalan Sea	W Mediterranean	16	3
Alacant coast	W Mediterranean	6	-
Alboran Sea	W Mediterranean	16	4
Gulf of Cadiz	NE Atlantic	11	-
Western Scotland	NE Atlantic	-	2
Gulf of St. Lawrence	NW Atlantic	-	3
Total		49	12

3.2.2. DNA isolation, amplification and sequencing

The mitochondrial DNA was extracted by the Estoup *et al.* (1996) method that consists in incubating the dried tissue (ca. 2 µg) in a mix of 200 µl of Chelex 10% with 2 µl of K proteinase (20 mg/µl). The minimum incubation time was 3 hours, blending the samples each 30 minutes. Fragments of cytocrome oxidase I (COI) gene from the mitochondrial DNA were amplified using the universal primers designed by Folmer *et al.* (1994): LCOI-1490 (GGT CAA CAA ATC ATA AAG ATA TTG) and HCOI-2198 (TAA ACT TCA GGG TGA CCA AAA AAT CA). The amplification reactions were performed using 40 ng of mitochondrial DNA in a solution of 1U of polimerase Taq (Amersham), 1x PCR buffer (Amersham), 0.2 mM of each primer, 0.12 mM of dNTPs and 1.5 mM of $MgCl_2$ in a final volume of 13 µl. After the DNA denaturalization at 94ºC during 4 minutes, the PCR program starts 36 cycles of incubations: 1 minute at 94ºC, 1 minute at 54ºC, 1 minute at 72 ºC and a final extension of 7 minutes at 72ºC. Two microliters of the amplification product were purified using the EXO-SAP enzymatic method with 0.34 µl of Exonuclease I (ThermoScientific 20U/µl) and 0.66 µl of S.A.P. (Shrimp Alkaline Phosphatase) (PROMEGA 1U/µl) by two incubation steps:

first 15 minutes at 37ºC, then another 15 minutes at 80ºC. The sequences were obtained through the sequencing kit Big-Dye v3.1 (Applied Biosystems) by the assumptions of the automatic sequencing ABI PRISM® 3770 from the technical and scientific service of the Universitat de Barcelona: 0.75 µl of BigDye, 1.25 µl of BigDye´s Buffer, 1.0 µl of the forward primer (0.2 µM), 3.0µl of the purified product in final volume of 10 µl.

Prior to perform population and phylogenetic analysis the obtained sequences of COI fragments were manually checked for ambiguous base calls, assembled and aligned in BioEdit v7.0.1 software (Hall 1999). In the DnaSP v5 (Librado and Rozas 2009) software the aminoacid translation of COI fragments were examined to ensure that no gaps or stop codons were present.

3.2.3. Intraspecific genetic diversity

To investigate the effect of the Gibraltar Strait in the genetic population structure of the benthopelagic *Pasiphaeidae* dwelling across the Atlantic-Mediterranean gradient, a total of 44 *Pasiphaea sivado* individuals from four different populations were sequenced: Gulf of Cadiz in the eastern central Atlantic, and Alboran Sea, Alacant, and Catalan, coasts along the western Mediterranean Sea (Figure 1). Five additional sequences from GenBank were added to our data from Atlantic waters off Algarve, Portugal, in the western part of the Gulf of Cadiz (GenBank access numbers: JQ306261-65) (Matzen da Silva *et al.* 2011).

Figure 1: Atlanto-Mediterranean transition showing the sampling locations of *P. sivado* (circles) and *P. multidentata* (squares): Gulf of Cadiz, Alboran Sea, Alacant coast and Catalan Sea.

Samples of the scarcer *Pasiphaea multidentata* were obtained from the two Mediterranean extremes of the sampled area, namely Catalan Sea and Alboran Sea (seven sequences); two additional sequences from the NE Atlantic (Scotland) (Matzen da Silva *et al.* 2011), and three more from the NW Atlantic (Gulf of St. Lawrence) (Radulovici *et al.* 2009) were obtained from Genbank (access numbers: JQ305977-78 and FJ581853-55, respectively). No *P. multidentata* could be sampled in any of the surveys performed in the Gulf of Cadiz.

The haplotype and nucleotide diversities, as well as the gene flow measurements (Gamma$_{st}$, Kst and Snn) and the neutral tests (Tajimas´s D and Ramos-Onsins and Rozas, R^2) were estimated. The Snn statistic was obtained by Hudson's method (2000). The haplotype networks were constructed using the "median joining network" method by using the Network v.4.5.1.6 (Fluxus Thecnology) program.

3.2.4. Phylogenetic analysis

To provide an approach to the phylogenetic reconstruction of the *Pasiphaea* genus we used one sequence for each known haplotype of the genus, taken from our own present data (from the above population genetics analysis) and the information available in GeneBank (25/04/2013): *Pasiphaea hoplocerca*, *Pasiphaea multidentata, Pasiphaea pacifica*, *Pasiphaea sivado* and *Pasiphaea tarda*. As outgroup, the closely related caridean shrimps *Acanthephyra pelagica*, *Alvinocaris muricola* and *Glyphocrangon neglecta* (Li *et al.* 2011) were selected, with sequences obtained either from GeneBank, or from the University of Barcelona Genetics Department Database (Table 2). The caridean species were selected as outgroup to avoid the noise due to the high substitution rates of the COI fragment compared to another genes (Xia *et al.* 2013). In those cases when due to methodological requisites only one outgroup could be selected *G. neglecta* was used as such. The nucleotide substitution saturation and transitions over transversions ratios were analysed by using the DAMBE5 software (Xia *et al.* 2013).

To elucidate the phylogenetic relationships within the *Pasiphaea* genus two methods were used to reconstruct the phylogenetic trees: the Maximum likelihood estimation (ML) performed in MEGA v. 5.1Beta3 (Tamura *et al.* 2011) and the Bayesian Inference (BI) method by using MrBayes program (Ronquist *et al.* 2012). The best fitted evolutive method GTR+G+I, adjusted by using the jModelTest (Posada 2008), was used in both phylogenetic reconstructions with 1000 bootstraps. Eleven independent ML reconstructions were performed to better identify the tree topology and the branch sustentation probability. The BI method was conducted using the Markov chain Monte Carlo (MCMC) algorithm with four Markov chains run for 100000 generations and sampled every 100 generations. The resulting phylogenetic data generated was visualized and edited in the FigTree v.1.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Table 2: Species and locality used to reconstruct the phylogeny tree of Pasiphaea genus. UB – University of Barcelona.

Species	Sampled area	Haplotype	Geographic coordinates (Lat.Long.)	GenBank Access number
Pasiphaea sivado	Mediterranean – Alacant	PS_hap1	38°08'N -0°04'W	UB (unpublished)
	Mediterranean – Alboran Sea	PS_hap3	36°35'N -4°15'W	UB (unpublished)
	Central E Atlantic – Gulf of Cadiz	PS_hap2	36°15'N -6°43'W	UB (unpublished)
	Atlantic – south Portugal	PS_hap4	36°80'N 7°77'W	JQ306265.1
Pasiphaea multidentata	Mediterranean – Alboran Sea	PM_hap1	36°16'N -4°56'W	UB (unpublished)
	Mediterranean – Catalan Sea	PM_hap3	41°23'N 3°18'W	UB (unpublished)
	Mediterranean – Alboran Sea	PM_hap4	36°16'N -4°56'W	UB (unpublished)
	NE Atlantic – Scotland	PM_hap2	58°29'N 9°W	JG305978.1
	NW Atlantic - Gulf of St. Lawrence	PM_hap2	49°90'N 65° 06'W	FJ581854.1
Pasiphaea pacifica	NE Pacific - Gulf of Alaska	PP_hap1	51°54'N 128°21'W	DQ882134.1
		PP_hap2		DQ882132.1
		PP_hap3		DQ882133.1
Pasiphaea tarda	NE Pacific - Gulf of Alaska	PT_hap1	53° 42'N 133° 24'W	DQ882136.1
		PT_hap2		JQ305937.1
	NW Atlantic - Gulf of St. Lawrence	PT_hap3	59°21'N 10°W	DQ882134.1
		PT_hap4		DQ882139.1
		PT_hap5		JQ305981.1
		PT_hap6		JQ305979.1
Pasiphaea hoplocerca	Central E Atlantic - south Portugal	Unique	36°55'N 9°07'W	JQ306169.1
Acanthephyra pelagica	NE Atlantic – Scotland	Outgroup	58°29'N 9°W	JQ305961
Alvinocaris muricola	not available	Outgroup	not available	EU031814.1
Glyphocrangon neglecta	Caribbean Sea	Outgroup	not available	UB (unpublished)

Pairwise comparison among significant haplotype groups identified by the phylogenetic tree analysis was performed to analyse the degree of difference (as a proportion) among the species/haplotype groups. Within groups p-distances were computed as the proportion of nucleotide sites at which two sequences being compared are different (MEGA v5, Tamura *et al.* 2011).

3.3. Results

3.3.1 Population genetic structure

The successfully amplified COI fragments presented around 550 and 600 bp for *P. sivado* and *P. multidentata,* respectively. After the joining of the present sequenced data with the extra sequences from GenBank the final COI dada set obtained had 518 and 576 bp for *P. sivado* and *P. multidentata* respectively (Table 3).

Table 3: Haplotype frequencies of *Pasiphaea sivado* and *P. multidentata* by sampled area: Catalan Sea, Alacant coast, Alboran Sea (westernmost Mediterranean Sea), Gulf of Cadiz (includes samples taken in Portugal and in Spain).

Pasiphaea sivado (518 bp)	Psiv_01	Psiv_02	Psiv_03	Psiv_04	Total
Catalan Sea - NW Mediterranean	15	1	-	-	16
Alacant coasts - Central W Mediterranean	6	-	-	-	6
Alboran Sea - W Mediterranean	15	-	-	1	16
Gulf of Cadiz - Central E Atlantic	-	10	1	-	11
Total	36	11	1	1	49

Pasiphaea multidentata (576 bp)	Pmul_01	Pmul_02	Pmul_03	Pmul_04	Total
Catalan Sea - NW Mediterranean	2	-	1	-	3
Alboran Sea - W Mediterranean	3	-	-	1	4
Eastern Scotland - NE Atlantic	-	2	-	-	2
Gulf of St. Lawrence - NW Atlantic	-	3	-	-	3
Total	5	5	1	1	12

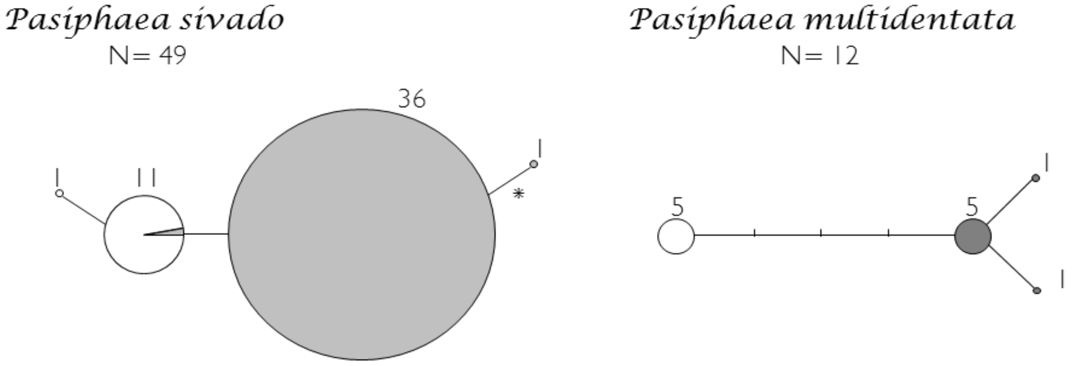


Figure 1: Atlanto-Mediterranean transition showing the sampling locations of *P. sivado* (circles) and *P. multidentata* (squares): Gulf of Cadiz, Alboran Sea, Alacant coast and Catalan Sea.

In *P. sivado*, four different haplotypes were found, of which three sites were polymorphic. The main haplotype was representative of the Mediterranean population (81.82% of total individuals) while the second most frequent haplotype (13.64%) was practically only found in the Atlantic population sampled. The other two haplotypes were unique and derived through a variation in just one polymorphic position from each main haplotype, i. e. one from the Mediterranean haplotype and the other from the Atlantic population (Table 3, Figure 2). Overall, the three populations sampled within the Mediterranean Sea were genetically homogeneous and no gradient was found across the Mediterranean Sea. The gene flow in the western Mediterranean Sea, between Alboran and Catalonia populations, was high and characterized by low values of Fst and no significant values of Snn (0.484 and 0.763 respectively) (Table 4). However it is important to point out that the nucleotide change observed in the Mediterranean haplotype does translate into an aminoacid change. The nucleotide shift occurred in the second position base and generated a translation from alanine to valine.

Table 4: Population genetic variability of *Pasiphaea sivado* populations across the Atlantic-Mediterranean transition. The statistical significance of $p < 0.001$ is expressed as (***), $p < 0.01$ (**), $p < 0.05$ (*) and non-statistical significance (ns).

Pasiphaea sivado	Within Mediterranean	Within Atlantic	Between Atlantic-Mediterranean
Genetic diversity			
N. of sequences	38	11	49
Sequence size, bp	518	518	518
No. of haplotypes	3	2	4
No. polymorphic sites	2	1	3
Haplotype diversity, Hd (± standard deviation)	0,107 (±0.07)	0,182 (±0.14)	0.424 (±0.70)
Nucleotide diversity, Pi	0.0002	0.0004	0.0009
Number of nucleotide differences, k	0.108	0.1818	0.466
Neutral tests			
Tajima's D	-1.493 ns	-1.129 ns	-0.647 ns
Ramos-Onsins and Rozas, R^2	0.113	0.288	0.092
Gene flow estimates			
$Gamma_{st}$			0.74
Snn*			0.956
Chi^2			42.854 ***
F_{st}			0.867
K_{st^*}			0.727

The deeper dwelling *P. multidentata* presented four different haplotypes segregated in six polymorphic sites. The North Atlantic populations, from Scotland (East) and Gulf of St. Lawrence

(West), shared a unique haplotype. The Mediterranean populations concentrated the genetic diversity in three haplotypes, one main and two unique haplotypes derived from two different polymorphic sites (Table 3). However, among the nucleotide changes observed in *P. multidentata* none translated into an aminoacid change. The gene flow among the Mediterranean populations was marked with low values of Fst and no significant values of Snn (0.0000, 0.4286 and 0.6890 respectively) (Table 5).

Table 5: Population genetic variability of *Pasiphaea multidentata* populations across Atlantic-Mediterranean transition. The statistical significance of $p < 0.001$ is expressed as (***), $p < 0.01$ (**), $p < 0.05$ (*) and non-statistical significance (ns).

Pasiphaea multidentata	Within Mediterranean	Within Atlantic	Between Atlantic-Mediterranean
Genetic diversity			
N. of sequences	7	5	12
Sequence size, bp	572	572	572
No. of haplotypes	3	1	4
No. polymorphic sites	2	0	6
Haplotype diversity, Hd (± standard deviation)	0.524 (±0.21)	0 (±0.00)	0.697 (±0.09)
Nucleotide diversity, Pi	0.001	0	0.004
Number of nucleotide differences, k	0.571	0	24.546
Neutral tests			
Tajima's D	-1.237 ns	-	0.898 ns
Ramos-Onsins and Rozas, R^2	0.226 ns	-	0.187
Gene flow estimates			
Gamma$_{st}$			0.873
Snn*			1
Chi^2			12.000**
F_{st}			0.933
K_{st*}			0.762

The genetic differentiation between Atlantic and Mediterranean populations was significant for both *P. sivado* (Gamma$_{ST}$ = 0.741 $p < 0.0001$) and *P. multidentata* (Gamma$_{ST}$ = 0.873 $p < 0.0001$) showing that the Gibraltar strait represents a barrier to the genetic flux in these benthopelagic shrimps (Tables 4 and 5). Moreover, no haplotype frequency cline was observed across the Atlantic-Mediterranean gradient in either pasiphaeid species. The neutrality tests of Tajima and Ramos-Onsins and Rozas showed that the populations of *P. sivado* and *P. multidentata* did not follow a neutral evolving model, indicating the influence of the natural selection in the genetic differentiation of Atlantic and Mediterranean populations.

3.3.2. Phylogeny of *Pasiphaea*

The fragment sequence size obtained for the phylogenetic study had a total of 558 bp. The aligned data presented 175 polymorphic sites, from which 170 were parsimony informative. The pasiphaeid species presented unequal base composition (25.99% A, 19.78% C, 18.74% G and 35.49% T (X^2= 663.9566 p< 0.0001). With a substitution rate of 0.4723% of invariable sites (taking just *Glyphocrangon neglecta* as outgroup) the data presented little saturation according to the substitution saturation analysis of Xia *et al.* (2013) (Iss= 0.482, Iss.c= 0.724, p<0.0001). Concerning the nucleotide substitution pattern there was no transitions over transversions bias as indicated by the low ts:tv ratio of 0.403. The proportion of total variable sites in the first and second position were almost equal (43.7 and 43.5 respectively) and greater than those in the third position (12.8) where no transition was detected by Xia's D method.

Both the Maximum Likelihood and Bayesian Inference methods were straightforward in placing all the sequences of different haplotypes together in their respective species clade. Moreover the estimated trees properly grouped the haplotypes according to pasiphaeid species and the oceanic region sampled. Both methods also showed the same grouping pattern and similar topologies (Figure 3). Two main groups were identified: a first one segregating all *Pasiphaea sivado* haplotypes from the rest of *Pasiphaea* haplotypes. Within this second group, all haplotypes significantly grouped by species, except in the case of *P. tarda* in which two significant subgroups clustered according locality (NE Atlantic vs NE Pacific).

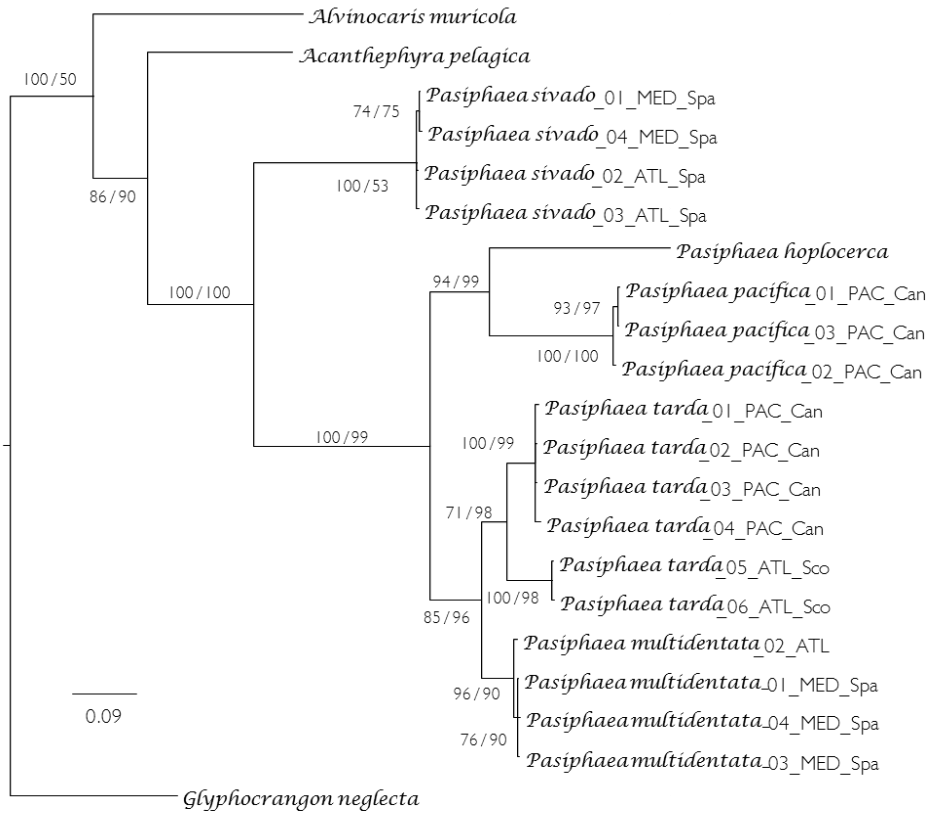


Figure 3: Phylogenetic treeresolved by Bayesian Inference analysis from cytochrome oxydase I (COI) DNA information on Pasiphaea species. A similar tree was reached by using Maximum Likelihood analysis. Small numerals show support levels based on 1000 bootstrap replicates/Bayesian posterior probabilities (ML/BI) expressed as percent.

Pairwise comparison among significant haplotype groups identified by the phylogenetic tree analysis (Table 6) showed that the high divergence of *P. sivado* with the rest of *Pasiphaea* species is comparable with the distance between outgroups and all pasiphaeid species, suggesting that, given probable more future information, the genus *Pasiphaea* could be splitted in two or more genera.

The segregation of *P. tarda* haplotypes in two different clades suggests that species can actually comprise two sibling species, as already shown by (Matzen da Silva *et al.* 2011). *P. tarda*, taking into account both the Atlantic and Pacific populations, was the species that presented the highest intraspecific divergence (4.3%), one order of magnitude greater than the following species, *P. pacifica* (0.6%), *P. multidentata* (0.4%) and *P. sivado* (0.3%). The p-distances between *P. tarda* populations from Pacific (0.4%) and Atlantic (0.2%) oceans were comparable to the above intraspecific values remarking the divergent character of the two populations of *P. tarda*.

Table 6: Pairwise comparison in phylogenetic distance (expressed as a proportion) among significant haplotype groups identified by the phylogenetic tree analysis estimated from COI with GTR+G+I model in (A) Pasiphaea species and outgroups, and (B) Pasiphaea (all species), Pasiphaea excluding *P. sivado*, and outgroups.

(A)

Between p-distance	*P. multidentata*	*P. tarda_1*	*P. hoplocerca*	*P. tarda_2*	*P. pacifica*	*P. sivado*	*Alvinocaris*	*Acanthephyra*	*Glyphocrangon*
P. multidentata	-								
P. tarda_1	0.07	-							
P. hoplocerca	0.18	0.19	-						
P. tarda_2	0.08	0.07	0.18	-					
P. pacifica	0.17	0.18	0.19	0.18	-				
P. sivado	0.2	0.21	0.23	0.2	0.23	-			
Alvinocaris	0.25	0.25	0.24	0.24	0.26	0.25	-		
Acanthephyra	0.21	0.21	0.25	0.21	0.25	0.21	0.2	-	
Glyphocrangon	0.22	0.22	0.24	0.22	0.24	0.21	0.22	0.19	-

(B)

	Pasiphaea sivado	*Alvinocaris*	*Acanthephyra*	*Glyphocrangon*
Pasiphaea (all)		0.249	0.217	0.22
Pasiphaea (excluding *P. sivado*)	0.215	0.249	0.22	0.225

3.4. Discussion

3.4.1. Population genetics

The genetic structure of both *P. sivado* and *P. multidentata* showed a marked break characterized by the almost complete separation of haplotypes between Atlantic and Mediterranean populations, with the absence of a common haplotype. This discontinuity was placed in the Gibraltar Strait area, affecting the populations sampled in the Gulf of Cadiz and Western Alboran Sea. This region has already been reported as a genetic barrier for several species of crustaceans, fishes, cephalopods and other invertebrates (Carreras-Carbonell *et al.* 2005, Schunter *et al.* 2011, Perez-Losada *et al.* 2002, Reuschel and Schubart 2006, Fernández *et al.* 2011). Another evidence of the Gibraltar Strait acting as a genetic barrier to Pasiphaeidae concerns the biogeography of the family, with the presence of only two species in the Mediterranean Sea, when compared with a much richer presence of pasiphaeid shrimps in nearby Atlantic areas. Thus, d'Udekem d'Acoz (1999) reports a minimum of 14 Pasiphaeidae species in NE Atlantic waters, of which 6 belong to the genus *Pasiphaea*. As a geographically close exemple, *Pasiphaea hoplocerca* has been recorded in south Portugal close to the Gulf of Cadiz but the species is absent in the Mediterranean Sea (Dos Santos and Moreira 2003).

Circulation patterns in the Gibraltar Strait area and throughout the Mediterranean are mainly driven by the water deficit in the Mediterranean basin induced by high evaporation levels due to high atmospheric temperatures in summer and strong, dry, cold northerly winds in winter. Evaporation is especially high in the eastern Mediterranean. Water loss by evaporation also implies a strong salinity increase and associated changes in density. These density differences created by the thermal and salinity changes imply that cooler, lower-salinity water from the Atlantic enter the Mediterranean through the Gibraltar Strait through surface layers (shallower than around 250 m depth) to compensate evaporation and Mediterranean denser water outflux through deeper waters. Atlantic waters entering the Mediterranean basin become modified as they travel east through the Alboran Sea, Almeria-Oran Front and along the North African coasts, from where they travel east and north. They sink in the eastern Mediterranean as salinity increases and then circulate westward through deep layers as Levantine Intermediate waters, also mixing with Mediterranean Deep Water, to finally spill over the Gibraltar Strait sill. This denser Mediterranean Water outflows into the Atlantic while very slowly remixing with Atlantic water masses (Tizperman and Malanotte-Rizzoli 1991, Cacho *et al.* 1999, Millot and Taupier-Letage 2005). However, despite the relatively good knowledge of Mediterranean hydrography, few works have used crustaceans, or particularly pelagic crustaceans, to identify possible oceanographic barriers for the genetic flux and distribution of species (Pannacciulli *et al.* 1997, Zane *et al.* 2000, Reuschel and Schubart 2006, Luttikhuizen *et al.* 2008, Sala Bozano *et al.* 2009, García-Merchán *et al.* 2012).

Morphological differences between Atlantic and Mediterranean populations of pasiphaeid shrimps were already reported by Zariquiey Álvarez (1957) and Omori (1976). When comparing the description of *Pasiphaea japonica* with the very similar *P. sivado*, Omori (1976) detected the occurrence of significant morphological differences between the newly described *P. japonica* and both Atlantic (Gulf of Cadiz and adjacent waters) and western Mediterranean populations of *P. sivado*. In particular, the total number of spinules on the merus of the first and second pereiopods was higher in *P. japonica* vs. both Atlantic and Mediterranean *P. sivado*. Also, significant differences between Atlantic and Mediterranean *P. sivado* were detected, with Mediterranean specimens bearing a higher number of spinules than the Atlantic specimens.

In *P. multidentata*, Zariquiey Alvarez (1957) also described some morphological differences between western Mediterranean and Atlantic (North Sea) populations concerning postrostral spine shape (with Mediterranean specimens showing a longer and more pointed rostrum), and, as in *P. sivado*, concerning also the number of spinules on the merus of the first pereiopod, as well as and on the basis and merus of the second pereiopod. The Mediterranean form of *P. multidentata* was even described as a different species, under the name *Pasiphaea* (*Phye*) *sicula* Riggio, 1896, later on considered a subspecies of *P. multidentata* (Zariquiey Álvarez 1946). Sivertsen & Holthuis (1956) did not accept the species status of *P. sicula*. Zariquiey Alvarez (1957) reported that his father R. Zariquiey Cenarro had a paper in press detailing the differences between Mediterranean and Atlantic *P. multidentata* populations that was unfortunately lost during an air raid that destroyed the press during the Spanish civil war (1936-1939). He concluded that Mediterranean specimens could be assigned to *P. multidentata*, but clearly remarked the occurrence of these morphological differences that could in the future assign the Mediterranean specimens to a different "race", as then reported by him, or to significantly different populations using present nomenclature. Casanova and Judkins (1976) reported that all the pelagic decapods that occurred in the Mediterranean were also present in the Atlantic. These authors also detected an interruption in the distribution of several deep sea shrimps with pelagic habits, namely *P. multidentata*, *Gennadas elegans* and *Acanthephyra pelagica*, west of the Gibraltar Strait, thus indicating that geographical isolation could lead to morphological divergence between Atlantic and Mediterranean populations of these species. The absence of *P. multidentata* in the trawlable bottoms of the Cadiz Gulf (López de la Rosa 1997) is in agreement with the distributional gap also reported by Casanova and Judkins (1976).

The occurrence of these phenotype differences already reported by morphologists and taxonomists, together with the occurrence of geographic differences in population biology (see Chapters 1 and 2) are in agreement with the high genetic differences observed in the present study between Atlantic and Mediterranean populations of both *P. sivado* and *P. multidentata*. These morphological and genetic characteristics may probably be adaptive to the different oceanographic conditions found in the two seas, with salinity being markedly higher in the Mediterranean than in the Atlantic (Millot and Taupier-Letage 2005).

In most decapod crustaceans, and particularly in benthic and epibenthic species, connectivity among populations is assumed to occur during the relatively long planktonic larval stage (Pineda *et al.* 2007, Galarza *et al.* 2009, Schunter *et al.* 2011). However, adult pelagic and benthopelagic shrimps can also be involved in long displacements during the pelagic phase of their activity rhythms (Aguzzi *et al.* 2007, Naylor 2010). To date not much is known on the early life stages of pasiphaeid species (Omori 1974). *P. sivado* late stage larvae were captured near the sea bottom around the 100 m isobaths by Williams (1960) in the Irish Sea. Nanjo and Konishi (2009) observed that *P. japonica* larvae reached the decapodid stage in 12 days when kept at surface temperatures, but full development was not accomplished at much lower temperatures, such as those inhabited by the adults, suggesting that larvae would have to develop in shallower waters as state Omori (1974). Considering that juvenile *P. sivado* occur on the upper slope, in much shallower waters than the adults (see Chapter 1 of this thesis) and that they can reach shallow epipelagic waters during the night (see Chapter 3 of this thesis) we can suppose that dispersion of *P. sivado* along the Iberian coast of the Mediterranean Sea would be mainly guided by the oceanographic processes taking place in epipelagic and intermediate waters.

Nanjo and Konishi (2009) also remarked that the larval development of *P. japonica* is lecitrotophic, and that the larvae could go through the entire larval stages utilizing their yolk. These authors also suggested that larvae of *P. sivado* and *P. tarda* could also be able to grow without feeding due to the large amount of yolk in the egg, as reported by Gurney (1942) and Williamson (1960). Ramirez-Llodra *et al.* (2007) also showed that egg size in *P. multidentata* is indicative of lecithotrophic development with a reduction in the number of larval stages. Company *et al.* (2001)

also showed that *P. sivado* eggs are remarkably large. This would imply a smaller residence time in the plankton with the consequent much weaker dispersion ability of the larvae and its retention within intermediate and deep waters. Taking into account that larger larvae would tend to feed on a wider diversity of praise, they would depend less on direct epipelagic sources, while remaining in deeper waters (Mauchline 1972). This would also imply that the genetic flux of the species is governed by the deep Mediterranean circulation, ie. by the displacement of the Levantine Intermediate Water (LIW) from the eastern Mediterranean towards the Gibraltar Strait. This lack of connectivity between Mediterranean and Atlantic basins due to the existence of the Gibraltar sill at around 300 m is probably the cause of the marked population genetic differences between the two basins in both *P. sivado* and *P. multidentata*.

According to Casanova and Judkins (1976), the deep-sea sergestid benthopelagic shrimp *Sergia robusta*, in contrast with *P. multidentata*, is distributed continuously throughout the Mediterranean and neighbouring Atlantic. Two different morphotypes have been reported in this species, one present in Atlantic waters, and another in Mediterranean (Crosnier and Forest 1973, Casanova and Judkins 1976). The distribution of these two morphotypes, based in petasma morphology, in the vicinity of Gibraltar Strait was studied by Casanova and Judkins (1976), reporting that the Mediterranean form occurred throughout the Mediterranean with the westernmost record located in the Gibraltar Strait area slightly west of Tarifa, not entering the Gulf of Cadiz, while the Atlantic form was sampled in the Atlantic not being located east of Gibraltar. These authors also noted that, although the two morphotypes co-occurred in the Gibraltar Strait, no intermediate morphologies were detected, thus excluding interbreeding between these morphs in this zone of contact. This pattern suggests that the connectivity of *S. robusta*, as that of *P. sivado* and *P. multidentata*, is governed by larval depth distribution in deep water layers in a way that it is not possible for them to enter the Mediterranean through epipelagic waters incoming from the Atlantic, and it is not also possible to exit the Mediterranean through the Gibraltar sill via intermediate waters. Larval distribution in these species should be therefore deeper than the Gibraltar sill.

Present evidences on marked genetic differences between Atlantic and Mediterranean populations of both *P. sivado* and *P. multidentata*, together with assumptions on relatively deep-sea larval location and behaviour, and present patterns of circulation through the Gibraltar Strait, would be suggestive of a limited genetic flux at present. However, hydrographic patterns during Holocene glaciation cycles have necessarily had to change and allow, with much cooler waters in most of the NE Atlantic including the Gibraltar Strait area and NW Africa, a much higher degree of connectivity between the Atlantic and Mediterranean (Cacho *et al.* 1999, Hewitt 2000, Maggs *et al.* 2008, Palero *et al.* 2008). More knowledge on larval ecology and behaviour is necessary to be able to understand the movements and connectivity of these species. The precise location of the larvae in the water column and their daily vertical movements, if any, are a keystone to understand both connectivity and population dynamics of these species.

3.4.2 Phylogeny

Pasiphaeidae is a worldwide family, with a relatively large number of species: 97 grouped in seven genera. The most representative genus of the family is the *Pasiphaea,* composed by 66 species, while the resting 31 species of the family is distributed in 6 other genera (De Grave & Fransen, 2011). Present results provide evidences on the internal phylogenetic structuring within the genus *Pasiphaea*, with especial emphasis on the northeastern Atlantic and Mediterranean species, although making use of the scarce available information on other species of the family within GenBank database (null concerning other genera of the family). Therefore, while the total number of species used in the analysis is relatively low, the results have shown significant evidences on the internal structuring of the genus *Pasiphaea*. Two main groups have been recognized, one comprising all *P. sivado* haplotyes, the other grouping the rest of pasiphaeids analysed. Within this second group, all haplotypes significantly clustered by species, except in the case of *P. tarda*, where, as already shown by Matzen da Silva *et al.* (2011), two significant groups were obtained, one corresponding to NE Atlantic haplotypes and the other to NE Pacific haplotypes.

Taxonomists have already reported on the occurrence of several species groups within the genus, the most important one concerning the *P. sivado* species group. Hayashi (1999, 2006) proposed the differentiation of the *Pasiphaea sivado* group, composed by *P. sivado* (Risso 1816) and ten other species from the rest of the *Pasiphaea* genus, based mainly on branchial reduction characteristics. Within this group, two additional subgroups could be identified according to branchial reduction: those species having four pleurobranchiae (*P. debitusae* Hayashi, 1999, *P. fragilis* Hayashi, 1999, *P. gracilis* Hayashi, 1999, *P. laevis* Hayashi, 1999, *P. marisrubri* Iwasaki, 1989, *P. philippinensis* Hayashi, 1999, and *P. mclaughlinae* Hayashi, 2006), and those with five pleurobranchiae, although the last pleurobranchia is small and rudimentary (*P. sivado* (Risso,1816), *P. propinqua* De Man, 1916, and *P. japonica* Omori, 1976).

Present results, although lacking information on the rest of species of the *P. sivado* group, provided relevant information concerning the significant phylogenetic differences of the species *P. sivado* versus the rest of other available species of the *Pasiphaea* genus. The highly morphologically similar *Pasiphaea japonica* Omori, 1976 was rather recently described as a new species, but remarking the very high similarities with *P. sivado*, with which it was previously confounded, as clarified by Omori (1976). Unfortunately no information on DNA COI haplotypes is yet available for the species, so it could not be used for the phylogenetic analyses. Although these findings may suggest that *P. sivado* could correspond to a distinct genus within the *Pasiphaeidae*, new phylogenetic analisis adding additional pasiphaeid species from the *P. sivado* group, as well as from as many other *Pasiphaea* species is needed to confirm the internal phylogenetic relationships within the genus and family.

P. multidentata has shown its closer affinities with the *P. tarda* complex, both with the Pacific and Atlantic probable species, than with the rest of species used, *P. pacifica* and *P. hoplocerca*. Within the species, the three Mediterranean haplotypes clustered together, while the Atlantic haplotype joined this subgroup, although at a non-significant value, however revealing and confirming the occurrence of differences between the two basis, as also detected by morphological and taxonomic studies (Zariquiey Álvarez, 1957, 1968).

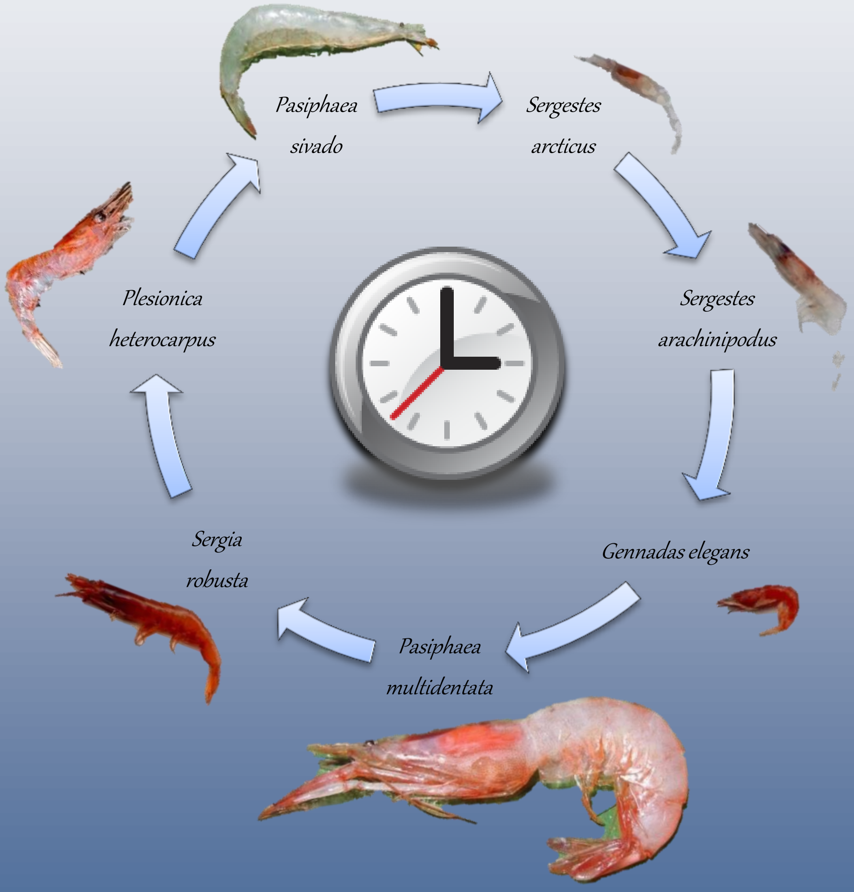
The identification of two significant clades within *P. tarda* was already reported by Matzen da Silva *et al.* (2011), as present results confirm. It must be also taken into account that the taxonomic history of *P. tarda* is complex. Sund (1913) considered that *Pasiphaea princeps* Smith 1884*, P. principalis* Sund 1913, and *P. tarda* Kroyer 1845 were three distinct species, while Sivertsen & Holthuis (1956) concluded that these three species were actually synonyms for one species, *P. tarda.* According to Williams (1960) few authors distinguished between *P. multidentata, P. tarda* and *P. principalis* and consequently the name *P. tarda* had been generally used to include any of these species. More recently Iwasaki (1990) defended the species status of *P. princeps* based on relevant morphological characteristic. In this scenario a careful taxonomic revision is needed at morphological level together with clarification from the geneticists point of view.

From the phylogenetic point of view, the precise placement of the family *Pasiphaeidae* within the Caridea, as well as the relationships among the genera and species within the family, are not yet fully understood (Christoffersen 1990, Bracken *et al.* 2009, Chan *et al.* 2010, Matzen da Silva *et al.* 2011, Li *et al.* 2011). In the recent work of Li *et al.* (2011), using nuclear genes, the *Pasiphaeidae* were shown to be monophyletic, and they belonged, together with *Rhynchocinetidae, Oplophoridae, Nematocarcinidae, Alvinocarididae, Campylonotidae,* and *Eugonatonotidae* to one of the two major clades identified within the Caridea. In two also recent molecular studies (Bracken *et al.* 2009, Chan *et al.* 2010) the *Pasiphaeidae* were shown not to be basal lineages within the Caridea, as were sometimes considered based on some morphological characteristics (Thompson 1967, Chace 1992), but rather they were found to be nested within a large caridean clade.

Chapter

4

Vertical and seasonal distribution of pelagic decapod crustaceans over the shelf-break and middle slope in two contrasting zones around Mallorca (western Mediterranean Sea)



Vertical distribution of pelagic decapod crustaceans over the shelf-break and middle slope in contrasting seasons and zones around Mallorca (western Mediterranean Sea)

4.1. Introduction

Pelagic decapod shrimps have a widespread geographic and bathymetric distribution from high latitudes in both hemispheres to intertropical waters (Casanova and Judkins 1977, Fasham and Foxton 1979, Clarke and Holmes 1987, Burghart *et al.* 2007). Provided of high locomotion abilities, some species are able to maintain themselves at particular water masses as well as to migrate to other water masses or layers (Omori 1974, Kaartvedt *et al.*1988, Karuppasamy *et al.* 2006). This displacement is usually cyclic and associated to the photoperiod as shown in some pasiphaeid and sergestid shrimps (Froglia and Giannini 1982, Cartes *et al.*1993, Aguzzi *et al.*2007). The vertical daily migration ability present in the behaviour of these crustaceans (Naylor 2010) provides them with an important role in the transference of matter and energy from the upper primary productive layers of the ocean, where these species tend to feed at night, down to the middle slope epibenthic community, where many of them dwell during day-time hours (Cartes 1993a, Herring and Roe 1988, Naylor 2010). Some of these pelagic shrimps have a relevant ecological importance as well as an exploitation potential as commercial target species (Nanjo and Ohtomi, 2009). Relevant studies faunistics, biology and ecology of pelagic shrimps have however been mainly conducted only in a few regions, such as the Mediterranean Sea (Mauchline 1972, Casanova and Judkins 1977, Orsi-Relini and Relini 1990, Company *et al.* 2001, 2003, Ramirez-Llodra *et al.* 2007), the Japan Sea (Nanjo 2007, Nanjo and Ohtomi 2009), or the Northeast and Southeast Atlantic Ocean (Matthews and Pinnoi 1973, Gibbons *et al.* 1994, Kensley and Schotte 2006).

From the energetics point of view, in oligotrophic areas, such as most of the Mediterranean Sea (Estrada *et al.* 1985, Estrada 1996), the secondary production of deep-sea species depends, in a practically exclusive way, on the role of the vertical migrators throughout the water column (Macquart-Moulin and Patriti 1993, Klages *et al.* 2001). Pelagic shrimps constitute a fundamental food item to fish, other crustaceans and cephalopods with epibenthic habits on the continental slope and deep sea (Dalpadado *et al.* 1998, Garrison and Link 2000, Fanelli and Cartes 2008). Some of them can be considered as benthopelagic since they spend most of the daytime in close contact with the bottom (Cartes 1993a, 2001, Aguzzi *et al.* 2007), and accordingly play, as stated above, a relevant role in the energetics dynamics of the continental slope and bathyal ecosystems.

The present work aims at studying the pelagic decapod crustacean assemblages inhabiting two hydrographically differentiated areas around the island of Mallorca in the western Mediterranean. Faunistic and size composition, differential distribution in relation to oceanographic water masses, geomorphology (shelf break versus deep continental slope), time of the day and seasonality were particularly the specific targets of this study.

4.2. Material and Methods

4.2.1. Study area and hydrographic setting

Mallorca is the main island of the Balearic archipelago, in the western Mediterranean. It is located east of the Iberian Peninsula and is separated from the continent by a wide hydro-geographical basin with maximum depths of 2200 m (Massutí *et al.* 2008). This archipelago is located in an area showing high hydrographical variability, both in space and time, which is mainly conditioned by the circulation of water masses through their channels (López-Jurado *et al.* 2008). Surface waters are usually formed by lower salinity Atlantic Water entering from the south through the channels between the islands, and between them and the continent. Below 100 m depth more saline Mediterranean water masses predominate. The Western Mediterranean Intermediate Waters

(WIW) usually occupies the 100-300 m depth stratum, the Levantine Intermediate Waters (LIW) are placed between 200-700 m and finally the Western Mediterranean Deep Water (DW) is found on the bottom (Pinot *et al.* 2002).

The geomorphology of the Balearic Basin, coupled to the climatological variability of the region, confers to this Sea a very dynamic hydrographical scenario, which is conditioned mainly by the amount of WIW occupying the submarine channels. During mild winters the Northern current (NC) from the Gulf of Lions flows southward along the continental slope, reaching the Ibiza Channel and bifurcates creating the north-eastern Balearic current (BC) when reaching surface low salinity Atlantic Water, while part of the main current flows southwest along the continental margin. However during severe winters the high amount of WIW occupying the channels block partially the NC current reinforcing the BC, recirculating the waters in the NW basin without a significant transport of Mediterranean waters through the channels into the Algerian Basin (Monserrat *et al.* 2008).

The hydrographical characteristics actually observed during the sampling cruises in the study area (Olivar *et al.* 2012, Torres *et al.* 2013) showed that surface waters were of recent Atlantic origin. During the December cruise, both temperature and salinity were homogeneous within the first 60-80 m of the water column and a highest gradient was located from there to 120 m depth. In the summer the mixing layer was restricted to the first 10 m and from there a gradient zone was placed until 100 m depth. Below the thermocline layer down to 400 m temperature was approximately constant at around 13 ^{o}C, while salinity gradually increased up to 38.5 until reaching 300-400 m depth.

4.2.2. Sampling

On board the R/V "Sarmiento de Gamboa" two cruises were conducted around the Mallorca Island: one in December 2009 (late autumn) and the other in July 2010 (early summer) (Olivar *et al.* 2012). Two geographic regions were selected according to previous knowledge on water productivity characteristics: one off Cabrera Island (in the SE of Mallorca, Algerian sub-basin) and the other off the town of Sóller, along the western coasts of Mallorca (Balearic sub-basin) (Figure 1). Within each area samples were taken over (1) the continental shelf-break and (2) the middle slope. Hydrographical data were obtained by means of CTD casts from the surface to the bottom with a CTD SBE911 in two regular grids of stations covering from 100 to 1200 m isobaths.

Aggregations of pelagic organisms were detected by acoustic backscatter using a Simrad EK60 echosounder and then the sampling was performed with midwater trawls at different depths of the water column inside the acoustic layers that presented the strongest and widest echo-reverberation, i.e. the Deep Scattering Layers (DSL) in mid waters (ca. 400 m) and the Benthic Boundary Layer (BBL), ca. 50 m above the bottom.

Nekton and micronekton were sampled by two different pelagic gears: a Pelagic Trawl net (PT) and an Isaaks-Kidd Midwater Trawl (IKMT). Trawl gear geometry and depth were controlled using Scanmar sensors. The Pelagic Trawl net used was a double-warp, modified commercial mid-water trawl, with standard pelagic otter board doors and a codend mesh size of 10 mm; the effective duration of the trawls was of one hour at a speed of 4 knots (nautical miles h^{-1}). PT densities were calculated as number of individuals per hour standardized to 100 m^2 of net mouth surface. The IKMT net had a length of 3 m, 3 m^2 of mouth opening and 3 mm codend mesh size, the trawls had duration of 30 minutes at a speed of 3 knots. IKMT densities were calculated as number of individuals per 104 m3 filtered. More detailed

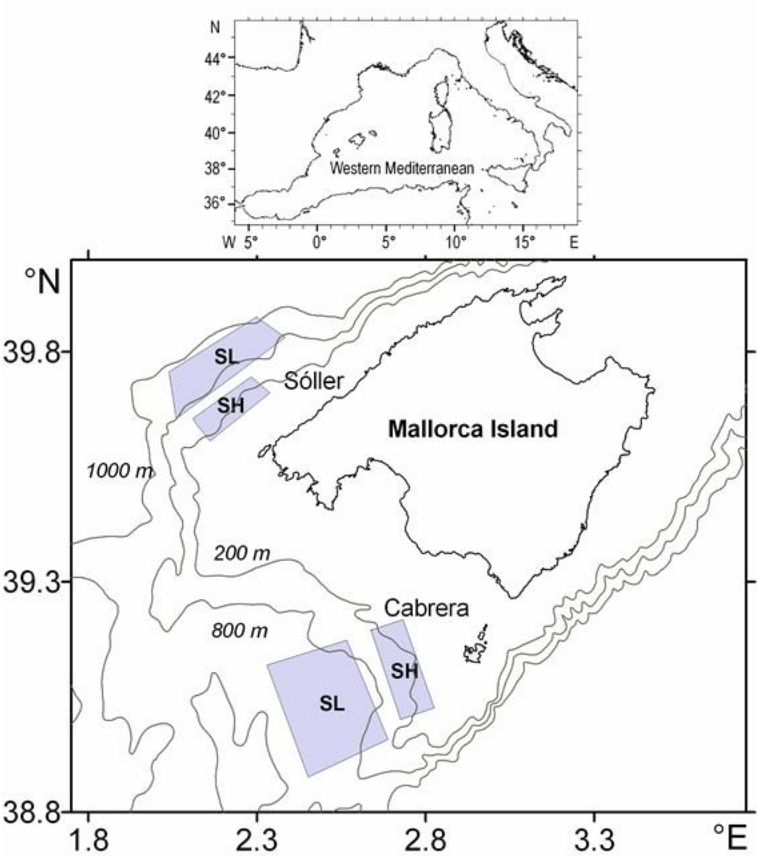


Figure 1: Map of Mallorca Island (western Mediterranean) showing the study areas (shelf-break (SH) and medium slope (SL) of Sóller and Cabrera).

sampling information is available in Olivar *et al.* (2012). Table 1 shows the number of samples taken within each Sampling Cell (SC), i.e. combination of season (December/July), area (Cabrera/Sóller), shelf/slope and daylight strata by sampling gear (PT and IKMT). A total of 36 valid PT samples were obtained, 12 in Cabrera and 24 in Sóller. In the December cruise all the PT hauls were performed at night time; while in July the shelf of Cabrera could not be sampled with PT. A total of 65 valid IKMT samples were obtained, 37 in Cabrera and 28 in Sóller. Additionally, each sample was assigned to one of the three defined positions in the water column, i.e. Epipelagic (samples taken in near surface waters well above the DSL), DSL (which was usually located around 400 m depth in both study periods) or BBL (samples taken at around 50 m above the bottom).

Pelagic decapod crustaceans were sorted out and identified to species level. Total fresh weight and number of specimens per species and haul were obtained from PT hauls. All individuals, or a representative subsample of a minimum of 50 individuals, were sexed and measured (carapace length (CL), in mm) with a 0.01 mm precision using a digital calliper. The samples from IKMT were fixed in buffered 5% formalin. After sorting out and species identification and quantification, each specimen was sexed and CL measured using a 'Leica MZ12' magnifier provided with ocular micrometer.

Size frequency distributions (SFD) (carapace length, CL, in mm) were generated by pelagic layers (Epipelagic, Deep Scattering Layers - DSL, and Benthic Boundary Layers – BBL) for the most abundant species for both Pelagic Trawl and IKMT samples. Additionally, samples obtained from the epibenthic/demersal survey taking place in parallel with the pelagic survey (Massutí *et al.* 2013) were also used to generate the corresponding SFD for the population fraction of each species sampled in the epibenthic environment during day time. The gears used to collect demersal fauna were a "Huelvano tirantero" trawl with an 16 m horizontal width, opening height of 2.7-3.2 m and 20 mm codend mesh size, and an epibenthic beam trawl (horizontal and vertical openings: 3.5 m and 0.6 m respectively; codend mesh size: 10 mm).

4.2.3. Data analysis

Due to the different dimensions and mesh-size of the sampling gears, which affect the efficiency of sampling of particular species or size classes, data from each gear were analysed separately. The abundance values of pelagic hauls (PT) were shown as number of individuals per hour, standardized to an area of 100 m^2 of sampler mouth opening, while the abundance values from the IKMT were standardized to number of individuals per 10.000 m^3 of filtered water. Prior to applying the statistical tests the data were normalised by a double square root transformation. Since samples taken during the day were very scarce concerning decapod crustacean occurrences, only night samples were analyzed for comparative studies.

The faunistic structure of the decapod community sampled by each net was analysed through multivariate two-way clustering and non-metric multidimensional scaling (MDS) analysis. All species matrices were obtained by calculating Bray-Curtis distances similarity. Significance of the cluster branches was tested using the SIMPROF subroutine within the Primer v.6 software using a significance level of 0.05 in the case of the PT samples and of 0.10 in the IKMT samples. The percentage contribution of each species to the dissimilarity between two groups was quantified using SIMPER analysis. All the statistics were performed by using the software PAST v.2.11 (Hammer *et al.* 2001). To specifically test for the occurrence of significant differences between areas (Cabrera/Sóller) and seasons (December/July) an ANOSIM test was performed within epipelagic and DSL layers. Samples without decapod crustaceans were not used in the analyses. Species richness, Shannon-Weaver diversity (H'; using $\log_2$) and Pielou's equitability (J) indexes were used to analyze the species diversity of the samples.

4.3. Results

4.3.1. Faunistics

Overall, a total of 14 species were present in the samples taken (both PT and IKMT) (Tables 1 and 2): five species of dendrobranchiate shrimps, eight caridean shrimps and one scyllarid lobster (nisto postlarval stage). Figure 2 shows the overall depth distribution of the different species sampled, taking into account both PT and IKMT samplings, excluding those taxa not identified to species level and the scyllarid nisto stage. *Chlorotocus crassicornis* and *Plesionika heterocarpus* were only sampled in the shelf-break; *Pasiphaea sivado* and *Sergestes vigilax* were sampled throughout the sampled depths, but especially so in the shelf-break; *Sergestes arcticus* was also found throughout, but especially so in the middle slope region; *Gennadas elegans* reached shallow depths, but was especially present on deep samples; *Pasiphaea multidentata* and *Sergia robusta* were only located in deeper waters, never reaching the epipelagic layers.

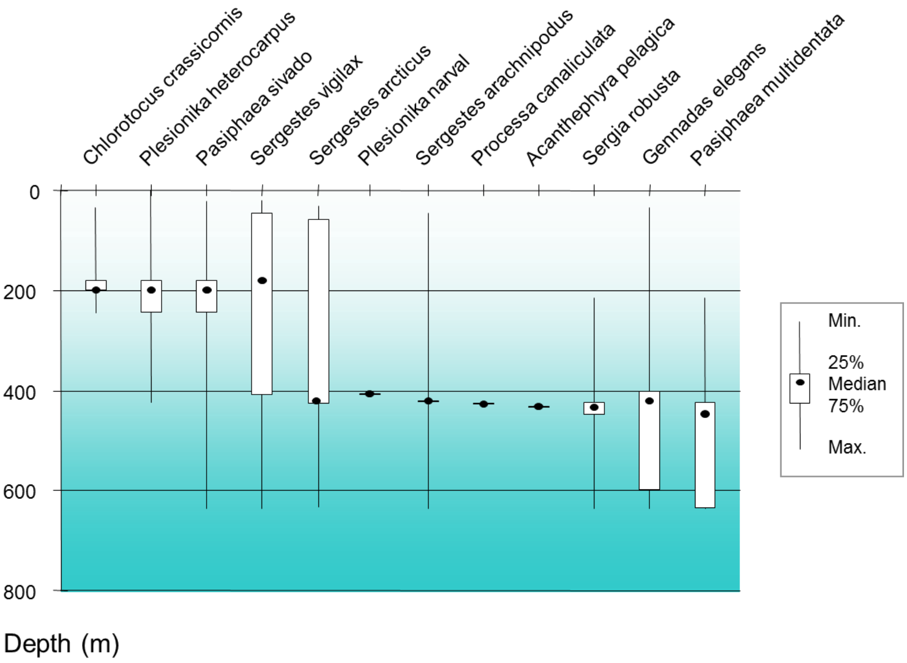


Figure 2: Depth distribution of the decapod crustacean species sampled with both the Pelagic Trawl and IKMT nets: depth range, 25 and 75 percentiles and median depth and of the overall species occurrences.

Table 1: Mean density (number of individuals h-1) of decapod crustacean species sampled by the pelagic trawl gear (PT). The number of samples taken by season (December/July), geographic area (Cabrera/Sóller), bathymetric zone (Shelf/Slope) and time of the day (Day (D)/Night(N)) are shown on the bottom line.

Pelagic samples	December								July							
	Cabrera				Sóller				Cabrera				Sóller			
	Shelf		Slope		Shelf		Slope		Shelf		Slope		Shelf		Slope	
	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N
Chlorotocus crassicornis		4.1		0		1.6		0			0	0	0	17.9	0	0
Gennadas elegans		0		2.5		0		22.5			0	5.1	0	0	0	0.7
Pasiphaea multidentata		0		1.1		0		4			0	0.9	0	0	0	15.0
Pasiphaea sivado		31.2		1.9		16.4		1.4			0	2.7	0	36.6	0.5	9.4
Plesionika heterocarpus		0.6		0		1.0		0			0	0	0	2.2	0	0
Plesionika narval		0		0		0		0			0	0	0	0	0.3	0
Plesionika sp.		0		0		0		0.1			0	0	0	0	0	0
Processa canaliculata		0		0		0		0			0	0	0	0	0	0.9
Scyllarus arctus		0		0.1		0		0			0	0	0	0	0	0
Sergestes arachnipodus		0		1.8		0.1		8.0			0	0	0	0	0.3	0.9
Sergestes arcticus		0		7.9		0.3		33.8			0	30.4	0	0	2.3	84.8
Sergestes vigilax		0		0		0		0			0	1.8	0	0	0.1	4.7
Sergia robusta		0		0.4		0		4.9			0	1.5	0	0	0.1	6.9
No. Samples	0	2	0	5	0	3	0	5	0	0	2	3	3	2	7	4

Table 2: Mean density of decapod crustaceans sampled by the IKMT net presented in number of individuals per 104 m3. The number of samples taken by season (December/July), geographic area (Cabrera/Sóller), bathymetric zone(Shelf/Slope) and photoperiods (Day (D)/Night(N)) are indicated on the bottom of the correspondent column.

IKMT samples	December								July							
	Cabrera				Sóller				Cabrera				Sóller			
	Shelf		Slope		Shelf		Slope		Shelf		Slope		Shelf		Slope	
	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N
Acanthephyra pelagica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gennadas elegans	0	0	0	6	0	0	0	4.2	0	0	0	11	0	0	30	4
Pasiphaea multidentata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Pasiphaea sivado	0	0	0	0	0	0	0	0.6	0	0	0	1.2	0	1	2	1
Plesionika heterocarpus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sergestes *arachnipodus*	0	0	0	0	1	5	0	0	0	0	0	0	0	0	0	1
Sergestes arcticus	0	0	0	4	0	0	15	10	0	0	0	21	0	0	3	4
Sergestes vigilax	0	0	0	5	10	0	1.2	0	0	0	0	1.8	0	0	0	1
Sergia robusta	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0
No. Samples	3	4	1	6	1	1	1	2	9	8	4	2	8	6	3	6

4.3.2. Day/night depth distribution

The depth and time of the day distribution of the occurrences of the main sampled species (Figure 3) clearly showed that all decapod crustacean species sampled avoided the depths shallower than around 400 m during the central hours of the day. A clear U-shaped pattern, with occurrences reaching epipelagic waters during the night, was found in *P. sivado*¸*S. arcticus* and *S. arachnipodus*, species witch reach 30, 30, and 50 m respectively. However this pattern was not so evident in *G. elegans; P. multidentata* that did not reach epipelagic waters found just below 400 m; as well in *P. heterocarpus* and *C. crassicornis* that were only sampled during night-time hours.

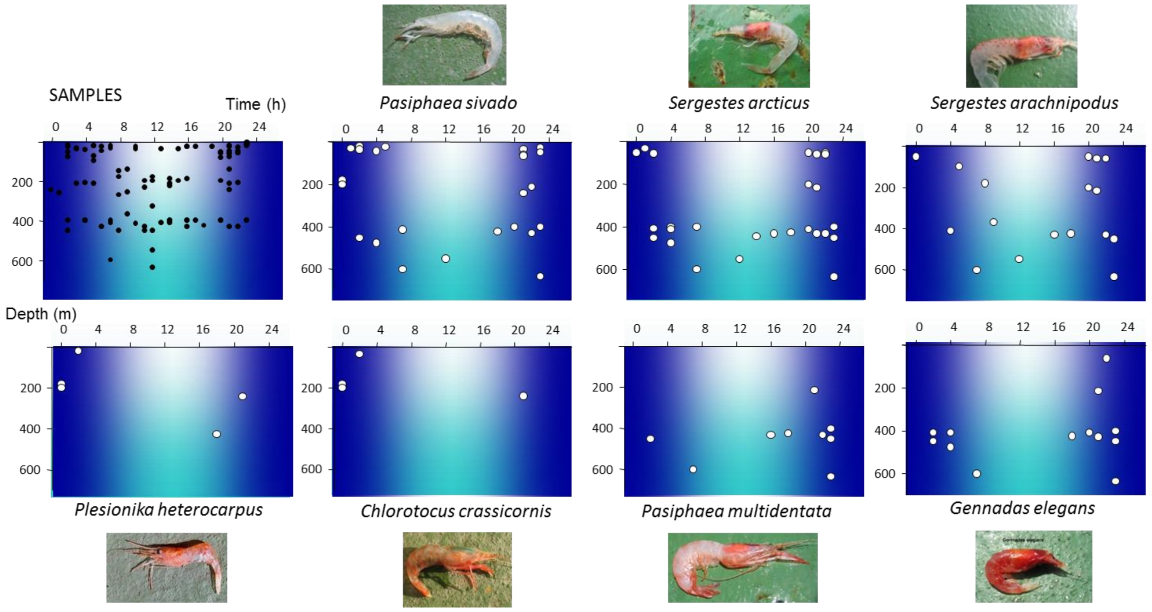


Figure 3: Depth and time of the day distribution of the occurrences of the main sampled species. The first figure shows the overall arrangement of the samples taken, including both Pelagic Trawl and IKMT sampling gears. The images of the fresh sampled individuals are presented.

4.3.3. Pelagic Trawl samples

A total of 13 decapod crustacean species were present in Pelagic trawls (PT) samples (Table 1). The species showing the highest densities were *Sergestes arcticus* and *Pasiphaea sivado*, followed by *Gennadas elegans*, *Chlorotocus crassicornis* and *Pasiphaea multidentata*.

The species rank plots show that the dominance pattern of pelagic decapod species varied between sampling cells (Figure 4). The highest dominance was found in the December shelf-break cells in both Sóller and Cabrera, which were dominated by a single species (*P. sivado*) accounting for >85% of the total abundance; only 3-4 species occurred in these SCs. The lowest dominance was found in the December slope cells, where the number of species found was higher, seven in both areas. The summer samples showed an intermediate pattern.

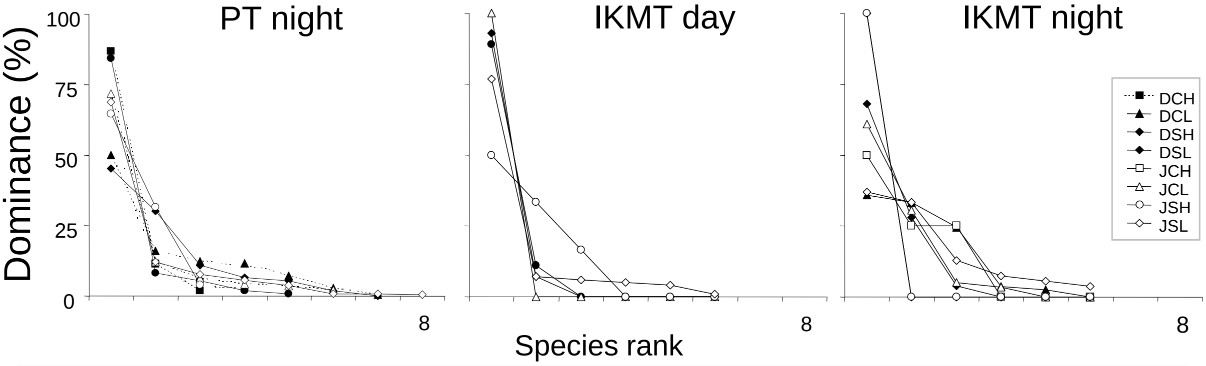


Figure 4: Dominance plots for the Pelagic Trawls (PT) and IKMT day and night samples. The values represent the percentage of the total abundance of the ranked species within each Sampling Cell, i.e sampled combination of season (December, D/July, J), area (Sóller, S/Cabrera, C) and shelf (H)/slope (L) factors. Key symbols indicate the combination of factors: black symbols represent December samples; white, July samples: squares, Cabrera-shelf; triangles, Cabrera-slope; circles, Sóller-shelf; diamonds, Sóller-slope.

Cluster and MDS analyses (Figures 5 and 6) showed the occurrence of five significant (according to SIMPROF subroutine) groups of samples, arranged according to a depth gradient, clearly evident in the MDS analyses, in addition to one single isolated epipelagic sample characterized by the occurrence of a single species, *Sergestes vigilax*. Table 3 shows the main characteristics of the groups of samples identified by cluster analysis, such as depth of the samples, distance from the bottom, depth of the bottom, percentage of the samples taken in each season, area, depth stratum and day time period. Clusters A and B, comprised the samples taken over the shelf-break; in

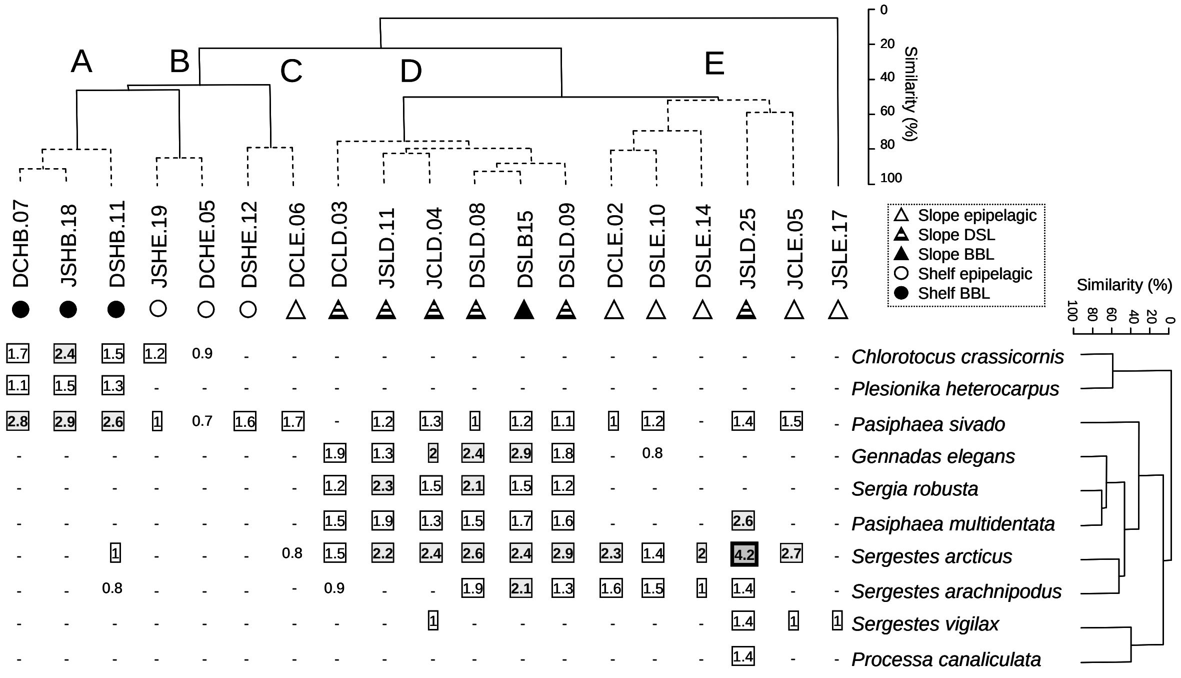


Figure 5: Two-way clustering analysis of Pelagic Trawl samples and species. The central matrix shows the density values (fourth root transformed) of species in each sample; white squares show values greater or equal to 1, in light grey squares values greater than or equal to 2 and the dark grey square values higher than 3.. The sample codes show the combination of season, area, strata, position in the water column and sample number within each survey (December/July + Cabrera/Sóller +shelf(H)/slope(L) + Epipelagic/Deep Scattering Layer/Benthic Boundary Layer +sample number. Solid lines in the sample cluster represent significant branches, as identified by SIMPROF analysis.

particular, all BBL shelf-break samples belonged to cluster A, while the epipelagic samples were located in cluster B. Samples in group A were characterized by two species, *Chlorotocus crassicornis* and *Plesionika heterocarpus*, exclusive of the shelf-break clustering, in addition to *Pasiphaea sivado*, a species widely co-occurring throughout all samples, but that attained its largest densities in the shelf-break BBL. The epipelagic shelf break cluster B was characterized by low densities of only *C. crassicornis* and *P. sivado*. Cluster C was a heterogeneous group of epipelagic July samples located

species widely co-occurring throughout all samples, but that attained its largest densities in the shelf-break BBL. The epipelagic shelf break cluster B was characterized by low densities of only *C. crassicornis* and *P. sivado*. Cluster C was a heterogeneous group of epipelagic July samples located

over both the shelf break and middle slope, with just *P. sivado* occurring in the shelf sample, and *P. sivado* and *S. arcticus* in the slope sample. Samples taken over the deep continental slope clustered in groups D and E. In particular, group D encompassed most of the slope DSL, while group E was formed by most of the epipelagic samples. The species that characterized group D were *Gennadas elegans*, *Sergia robusta* and *Pasiphaea multidentata* which did not occur in any of the epipelagic samples. The most characteristic species of the epipelagic assemblage were different sergestid shrimps, namely *Sergestes vigilax*, *Sergestes arachnipodus*, and *Sergestes arcticus*, together with *P. sivado* which, as stated above, was present throughout all the sampling cells. SIMPER analysis (Table 4) quantifies and further clarifies the role of the different species in the differentiation of the identified assemblages. In the MDS graphic (*Figure* 6) these groups of samples appeared well defined, except for the overlap of the slope epipelagic group (E) with the arrangement of the slope DSL samples. The different sampling areas (Cabrera/Sóller) did not show any significant difference when applying an ANOSIM test to the data, however hints of seasonality, although proved not significant, were present in both the epipelagic and DSL slope assemblages (*Figure* 6).

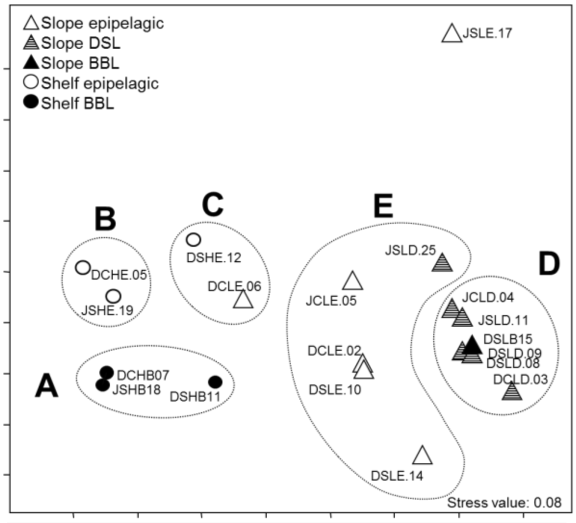


Figure 6: MDS of samples based on species abundances from the Pelagic Trawl. Samples are grouped according to previous results of the cluster analysis. The sample codes show the combination of season, area, strata, position in the water column and sample number within each survey (December/July + Cabrera/Sóller +shelf(H)/slope(L) + Epipelagic/Deep Scattering Layer/Benthic Boundary Layer +sample number.

Table 3: Characteristics of the Pelagic Trawl (PT) and IKMT sample groups identified by cluster analysis.

	Pelagic Trawl Clusters					IKMT clusters	
	A	B	C	D	E	A	B
no. Hauls	3	2	2	6	5	8	19
no. Species	5	2	2	7	7	3	8
Depth range of the samples	180 - 245	35 - 55	33	215 - 635	30 - 430	21 - 414	38 - 600
Mean sample depth	208	45	33	429	143	103.9	289.3
Distance from bottom range	55 - 140	145 - 224	257 - 667	25 - 500	183 - 859	7 - 963	3 - 891
Mean distance from the bottom	85	185	462	364	663	395.6	484.3
Bottom depth range	239 - 340	200 - 259	262 - 700	655 - 939	613 - 906	156 - 988	195 - 995
Bottom mean depth	293	230	481	793	778	499.5	773.5
% Winter	66.7	50.0	100.0	66.7	60.0	0	57.9
% Summer	33.3	50.0	0.0	33.3	40.0	100.0	42.1
% Cabrera	33.3	50.0	50.0	33.3	40.0	25	47.4
% Sóller	66.7	50.0	50.0	66.7	60.0	75	52.6
% Shelf	100.0	100.0	50.0	0.0	0.0	50	21.1
% Slope	0	0	50.0	100.0	100.0	50	78.9
% Epipelagic	0	100.0	100.0	0	80.0	12.5	10.5
% DSL	0	0	0	83.3	20.0	12.5	57.9
% BBL	100.0	0	0	16.7	0	75	31.6
% Day	0	0	0	0	0	37.5	26.3
% Nigth	100.0	100.0	100.0	100.0	100.0	62.5	73.7
Salinity range	38,30 - 38,45	37,96 - 37,98	37,99 - 38,04	38,45 - 38,52	37,88 - 38,52	37.83 - 38.51	37.98 - 38.52
Mean Salinity	38.39	37.97	38.02	38.51	38.12	38.07	38.35
Bottom mean Salinity	38.48	38.35	38.48	38.49	38.49	38.41	38.45
Temperature range	13,09 - 13,38	16,76 - 17,07	17,18 - 17,53	13,12 - 13,27	13,22 - 19,05	13.16 - 22.18	13.12 - 16.61
Mean Temperature	13.25	16.92	17.36	13.23	16.47	17.05	14.00
Bottom mean Temperature	13.23	13.27	13.21	13.10	13.10	13.12	13.15
Oxygen range	4,38 - 4,63	5,18 - 6,04	4,92 - 5,17	4,05 - 4,38	4,08 - 5,79	4.11 - 6.06	4.05 - 6.05
Mean Oxygen	4.53	5.61	5.05	4.18	5.04	5.38	4.58
Fluorescence range	0,01 - 0,022	0,075 - 0,11	0,069 - 0,085	0,009 - 0,026	0,026 - 0,113	0.024 - 0.417	0.008 - 0.162
Mean Fluorescence	0.018	0.093	0.077	0.014	0.073	0.103	0.045
Time range	12,3 - 21,5	2,8 - 19,6	21,6 - 23,2	2 - 23,4	1,7 - 23,4	1.8 - 22.7	2.0 - 22.8
Mean time	15.4	11.2	22.4	14.7	17.8	12.8	12.1

The main diversity indexes of the Pelagic Trawl samples within each significant group of samples identified by cluster analysis are shown in Table 5, namely number of species (S), Shannon-Wiener $\log_2$ diversity index (H) and Equitability (J). Concerning the number of species present, the most diverse assemblages were those sampled above the middle slope bottoms, both in epipelagic (D) and DSL (E) samples, with a total of eight species within each group; the lowest number of species was found in the epipelagic shelf assemblages, B and C, with just two species in each. The Shannon-Wiener diversity index ranged between 0.09 in assemblage C, which was dominated by *P. sivado*, to 1.24 in the DSL slope (D). Equitability was high in assemblage B (shelf epipelagic) with just two co-occurring species with similar relative densities, as well as in the most diverse slope DSL (group D) in which no high dominance of any particular species was found.

Table 4: Species accounting for the main differentiation between groups of Pelagic Trawls and IKMT samples (percentage contribution to dissimilarity), according to SIMPER analysis. The three most relevant species within each pair of groups are shown in bold.

	Pelagic Trawls										IKMT
Species	A x B	A x C	A x D	A x E	B x C	B x D	B x E	C x D	C x E	D x E	AxB
Acanthephyra pelagica	-	-	-	-	-	-	-	-	-	-	0.9
Chlorotocus crassicornis	**17.8**	**37.5**	14.1	**20.7**	**47.2**	10.4	**17.9**	0	0	0	-
Gennadas elegans	0	0	**15.2**	1.9	0.0	**19.9**	2.8	**22.4**	3.6	**24.0**	**18.7**
Pasiphaea multidentata	0	0	11.9	3.6	0.0	**15.7**	4.4	17.6	5.6	**19.5**	0.8
Pasiphaea sivado	**42.0**	**22.6**	**13.9**	**20.3**	**36.7**	4.3	9.1	8.2	**16.2**	6.9	15.7
Plesionika heterocarpus	**28.1**	**25.9**	9.8	14.3	0	0	0	0	0	0	1.6
Processa canaliculata	0	0	0	2.0	0	0	2.4	0	3.0	2.5	-
Sergestes arachnipodus	5.4	4.9	6.8	10.3	0	9.6	**18.3**	10.8	**23.0**	10.7	15.0
Sergestes arcticus	6.7	9.1	**14.9**	**22.7**	**16.1**	**22.6**	**39.3**	**21.2**	**41.4**	9.5	**20.1**
Sergestes vigilax	0	0	1.3	4.2	0	1.7	5.8	1.9	7.3	5.8	**24.1**
Sergia robusta	0	0	12.2	0	0	**16.0**	0	**18.0**	0	**21.0**	3.2

Table 5: Diversity indexes of the Pelagic Trawl and IKMT samples within each significant group of samples identified by cluster analysis: Number of species (S), Shannon diversity (H) and Equitability (J).

		Pelagic Trawls					IKMT	
		A	B	C	D	E	A	B
		SH Epi.	SH BBL	SH	SL DSL	SL Epi.	SH Epi.	SL DSL
No. species	Mín S	3	2	1	5	2	1	1
	Máx S	5	2	2	6	6	2	6
	Median S	3.7	2	1.5	5.7	3.6	1.3	2.6
	Total S	5	2	2	8	8	3	8
Diversity	Mín H	0.43	0.64	0	0.9	0.24	0	0
	Máx H	0.77	0.64	0.2	1.44	1.17	0.69	1.72
	Median H	0.62	0.64	0.1	1.24	0.6	0.17	0.81
Equitability	Mín J	0.39	0.92	0	0.5	0.32	0	0
	Máx J	0.7	0.92	0.3	0.81	0.85	1	1
	Median J	0.5	0.92	0.1	0.72	0.48	0.25	0.77
Number of Samples		7	6	5	11	1	8	19

4.3.4. IKMT samples

Overall, a total of nine decapod crustacean species were present in the IKMT samples (Table 2). The species showing the highest densities were *G. elegans* and *Sergestes arcticus*, followed by *S. vigilax* and *S. arachnipodus*.

The relatively higher number of samples taken with the IKMT gear allowed the analysis of day and night samples separately. The species rank plots show that the dominance pattern of pelagic decapod species varied between sampling cells (*Figure* 4). Most day samples showed a higher dominance of a single species which accounted for over 75% of the abundance, with respect to the rest of species, while night samples were more evenly distributed.

Cluster and MDS analyses (*Figure* 7 and 8) showed the occurrence of two significant assemblages, one encompassing most shelf-break samples (A), and the other (B) most slope samples. Assemblage A was characterized by the occurrence of exclusively *P. sivado* in most samples, although

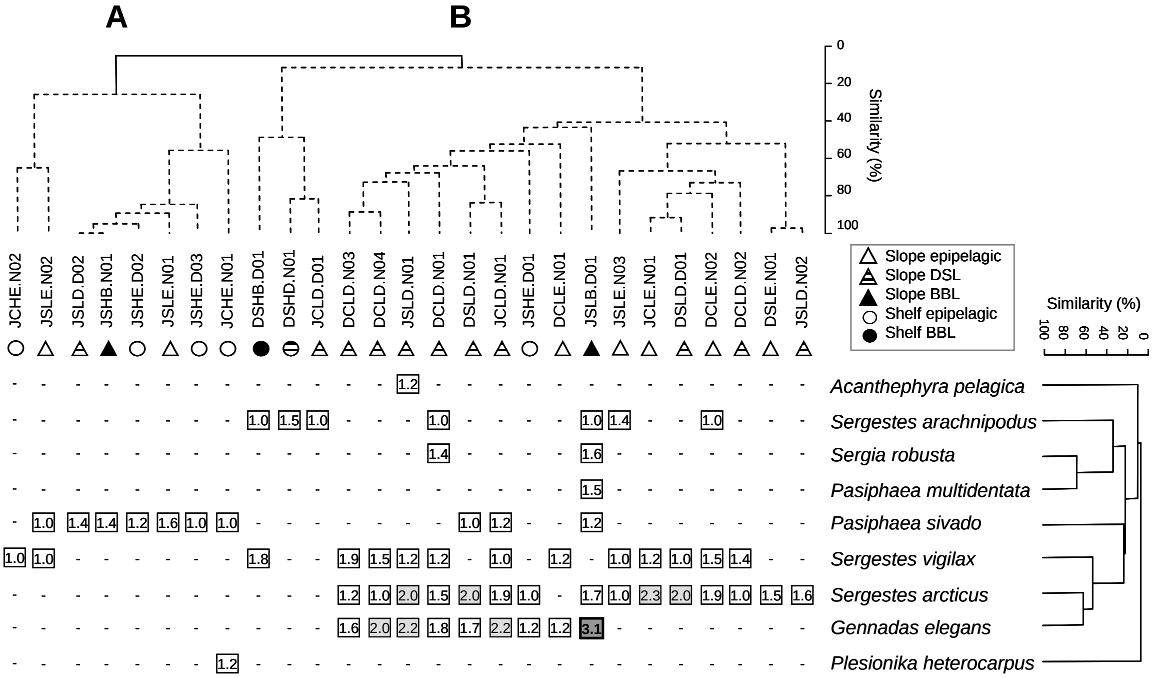


Figure 7: Two-way clustering analysis of IKMT samples and species. The central matrix shows the density values (fouth root transformed) of species in each sample; white squares show values greater or equal to 1, in light grey squares values greater than or equal to 2 and the dark grey square values higher than 3. The sample codes show the combination of season, area, strata, position in the water column and sample number within each survey (December/July + Cabrera/Sóller +shelf(H)/slope(L) + Epipelagic/Deep Scattering Layer/Benthic Boundary Layer +sample number. Solid lines in the sample cluster represent significant branches, as identified by SIMPROF analysis.

S. vigilax was also present in some of them, and one single occurrence of *P. heterocarpus*; all samples of this group were taken in July. Within assemblage B, a subcluster, although identified as non-significant by the SIMPROF subroutine, included most of the DSL slope samples, while another subgroup contained most of the epipelagic slope samples. The DSL subcluster was characterized by the co-occurrence of *S. vigilax*, *S. arcticus* and *G. elegans*, while in the epipelagic group *G. elegans* was absent. *P. sivado* showed very few occurrences in assemblage B and *S. robusta* was only present in the DSL subgroup. No clearer differential distribution patterns could be assessed concerning the MDS results.

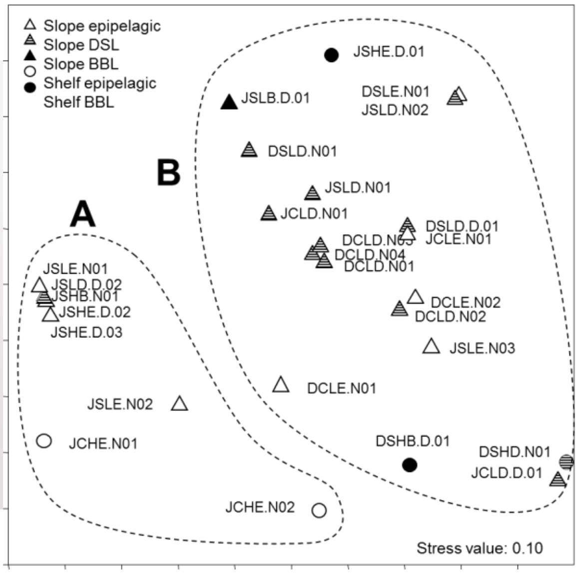


Figure 8: MDS of samples based on species abundances from the IKMT net. Samples are grouped according to previous results of the cluster analysis. The sample codes show the combination of season, area, strata, position in the water column and sample number within each survey (December/July + Cabrera/Sóller +shelf(H)/slope(L) + Epipelagic/Deep Scattering Layer/Benthic Boundary Layer +sample number.

4.3.5. Population size structures

For the most abundant species size frequency distributions were generated by pelagic layers using both Pelagic Trawl and IKMT samples (Figure 9). Additionally, samples from the demersal and epibenthic surveys were also used to generate SFD for the population fraction present in the epibenthic environment during the day. Species-specific patterns were clearly shown for most species. Although the codend mesh size of each sampling gear was different (according to the gear size, structure and organisms size spectra targets), all of them were able to sample both the juvenile and adult fraction of the populations of most species, except perhaps the GOC demersal trawl for the smallest species, such as *Gennadas elegans* and *Sergestes vigilax*.

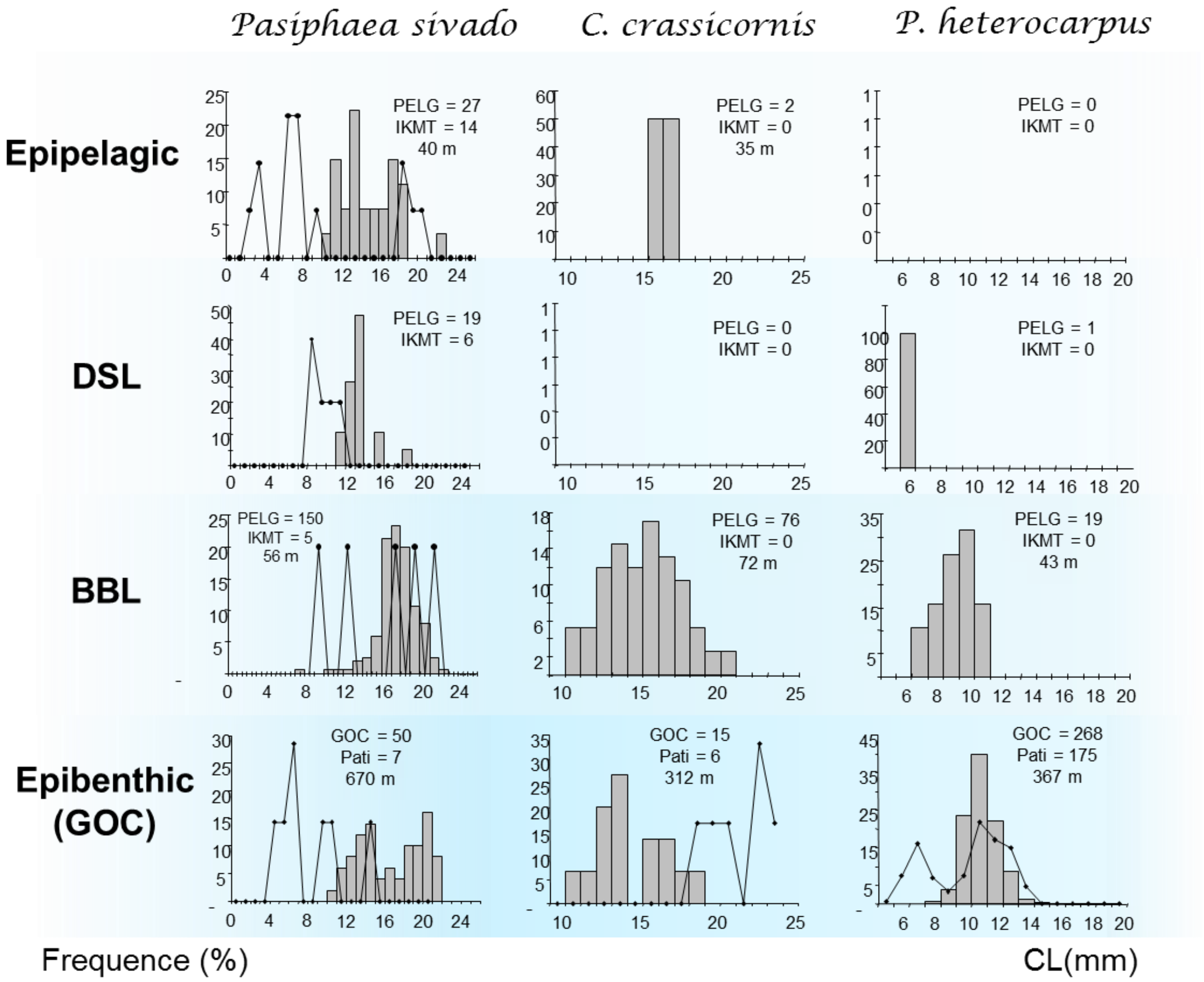


Figure 9: Size frequency distribution (in percentage of the total number of individuals sampled within each cell) of the main decapod crustacean pelagic species according to the strata sampled: Epipelagic, DSL, BBL and Epibenthic. Bars represent Pelagic and Demersal Trawl samples, while lines represent IKMT and epibenthic samples. The number of individuals sampled within each cell as well as the mean sampled depth are also shown. The depth of the epipelagic stratum was presented as mean water column depth sampled, the DSL depth was fixed around 400 m, the depth of BBL stratum is presented as distance from the bottom and the epibenthic stratum as bottom depth.PELG: pelagic samples; IKMT: IKMT samples; DEM: demersal samples; EPI: epibenthic samples.

In the case of the species occurring only over the shelf-break, *Chlorotocus crassicornis* showed a similar size structure in both the epibenthic and BBL layers. In *Plesionika heterocarpus* two cohorts were sampled in the epibenthos, while most of the adult fraction did not appear to show pelagic habits; it is also worth mentioning that *P. heterocarpus* was not found in any epipelagic sample. In the case of *P. sivado,* who was present throughout the pelagic and demersal environments, both adult and juvenile individuals were present in the water column.

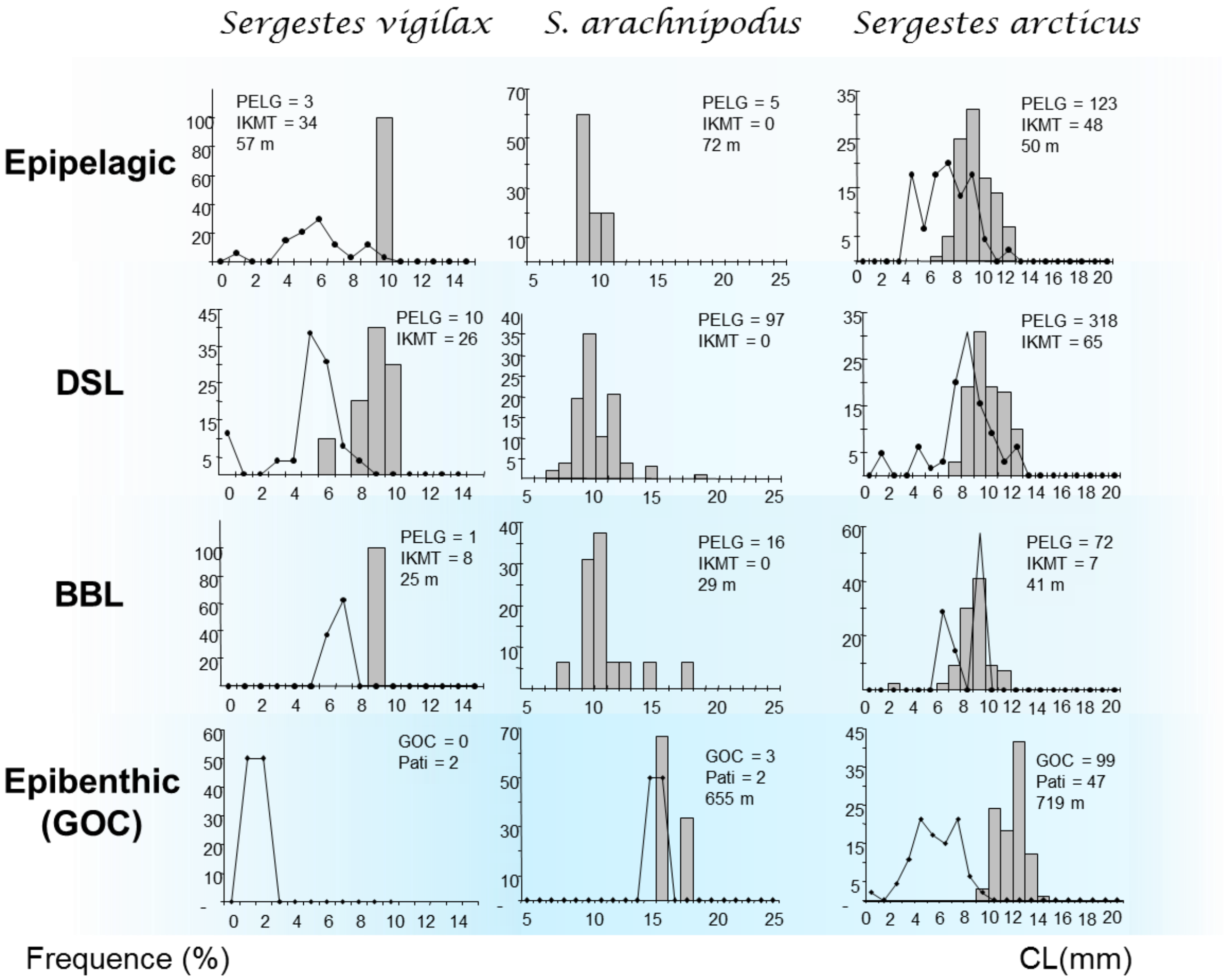


Figure 9: Continuation.

Concerning the bathyal benthopelagic species, practically all size classes of *Genadas elegans* were present in the water column, although just a few adults were collected with the larger mesh-size demersal gear, a fact most probably due to the small size of the species coupled to the codend mesh size of the gear. The case of *Pasiphaea multidentata* is interesting because both the juvenile and adult fractions of the population showed pelagic habits; it must also be remarked that the vertical movements of the species did not reach the epipelagic layer, but rather reached the deper DSL.

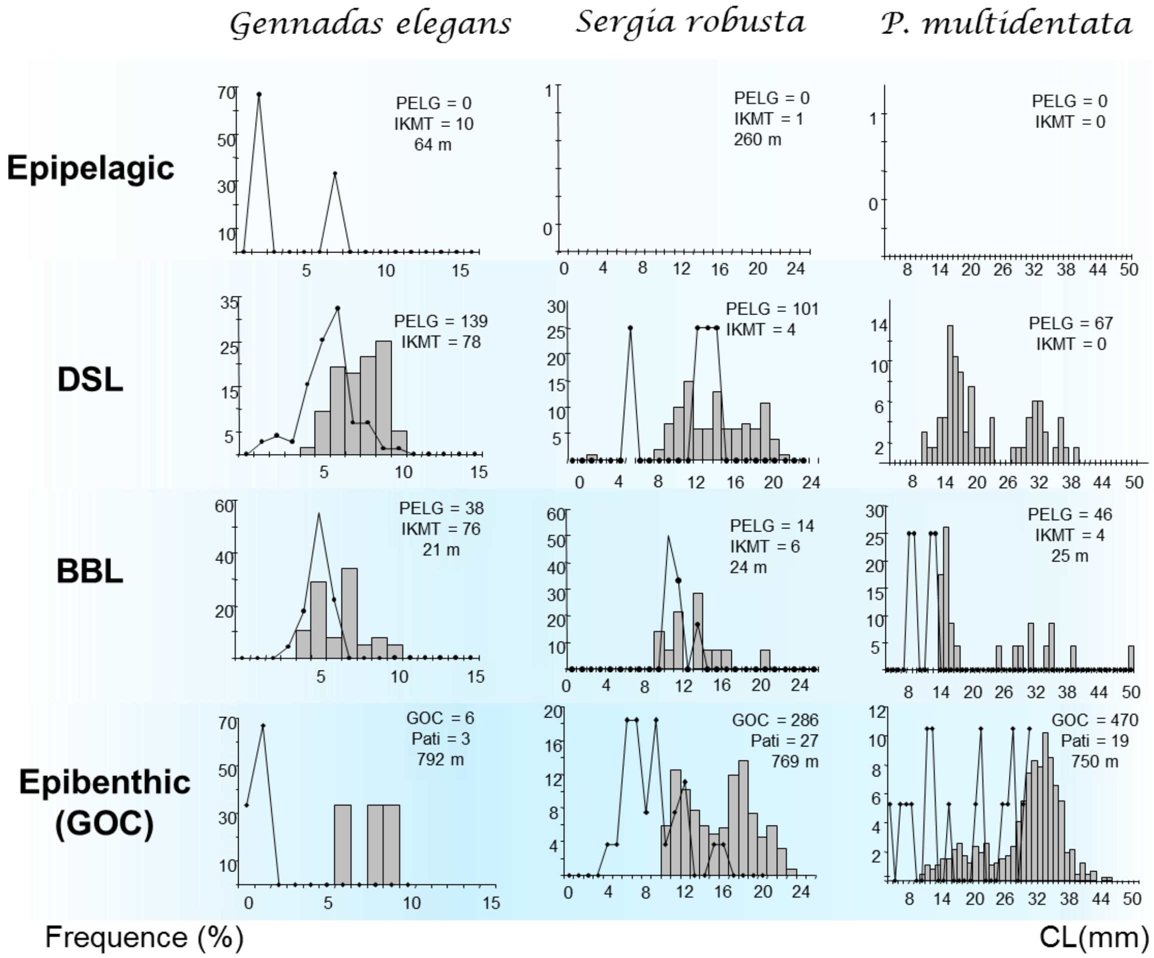


Figure 9: Continuation.

4.4. Discussion

4.4.1. Study area

The pelagic decapod crustacean fauna in the Mediterranean Sea has not been as thoroughly studied as the epibenthic/demersal one (Mauchline 1972, Casanova 1977, Orsi-Relini and Relini 1990, Koukouras *et al.* 2000). Although specific approaches have focused on some life history aspects and on the ecological role of benthopelagic species (Cartes 2001, Company *et al.* 2001, Papiol *et al.* 2012), information on the distribution, dynamics and role of pelagic shrimps within the Mediterranean pelagic environment is scarce (Macquart-Moulin and Patriti 1993, Koukouras 2000, Froglia and Giannini 1982). There is certainly information on patterns of occurrence, vertical range, etc, especially so concerning their association with the bottom layers on the continental slope, where most of the species with pelagic habits occur during the day and can be sampled using demersal trawl gears used in bottom fisheries and research cruises (Abelló *et al.* 2002, Fanelli *et al.* 2007, Cartes *et al.* 2009). Present results contribute to assess the role of pelagic and benthopelagic species over the continental edges of the western Mediterranean Sea. Most pelagic decapod shrimps have been reported to be preyed upon by many pelagic and epibenthic predators, including fish, crabs, and shrimps (Abelló 1989, Mauchline and Gordon 1991, Cartes 1993a,b, Valls *et al.* 2011).

The sampling schedule allowed the differential identification of the relevant species occurring on the Balearic shelf edge and slope, their night/day vertical distribution, and provided hints on their seasonality characteristics. The two geographic areas studied, off Sóller and Cabrera in the Balearic Islands, were selected based on previous information on their different oligotrophy degrees and hydrographic dynamics (Massutí *et al.* 2008, Moranta *et al.* 2008, Balbín *et al.* 2012). Overall they did not show significant differences concerning their decapod crustacean faunistic composition. Neither was seasonality in faunistic composition and structure found to vary significantly. In the present study most species showed species-specific patterns of distribution occurrence, especially so concerning the two geomorphologic areas sampled (over the shelf break and over the middle slope) as well as concerning depth in the water column. On the shelf break, species diversity was low, with just two to five decapod crustacean species occurring in the samples; a high dominance of just one or two species was evident in that area. The slope community was more diverse, with up to six species per sample and a total of eight species in the area, followed by a higher overall equitability. In the colder December samples the variation of the dominance pattern between shelf break and slope was more evident than in summer. The species composition, diversity and dominance between the geographic regions, Cabrera and Sóller, was similar in both seasons, suggesting that the dynamics of the pelagic environment (López-Jurado *et al.* 2008, Torres *et al.* 2011, Balbín *et al.* 2012) would tend to uniformize the ecological dynamics of the system, witch is in agreement with Omori (1974).

Differences in pelagic species communities have been reported at different oceanographic scales in many oceanic regions, such as in the Gulf of Mexico, off southern California, western North Pacific, central-eastern Atlantic, Norwegian Sea, etc, and have been mainly attributed to large scale oceanographic patterns and differences between coastal/neritic and oceanic waters, especially so concerning island environments (e.g. Macquart-Moulin and Patriti 1993, Gibbons *et al.* 1994, Dalpadado *et al.* 1998, Burghart *et al.* 2007, Suntsov and Domokos 2013). Pelagic decapod crustaceans include individuals belonging to several families, but most importantly to Sergestidae, Penaeidae and Aristeidae, among the Penaeoidea, and Pasiphaeidae, Oplophoridae and Pandalidae among the Caridea (Hopkins *et al.* 1994, Karuppasamy *et al.* 2006). Seasonal differences in the pelagic community composition have also been reported and attributed to seasonal differences in hydrological features, such as occurrence and disruption of thermoclines, upwelling strength, etc. (e.g. Franqueville 1971, Williams and Koslow 1997).

In the Balearic Sea no pelagic decapod species were detected in epipelagic waters shallower than 400 m depth during the day light hours, in a similar way as that reported by Fasham and Foxton (1979) in the Atlantic Ocean. Differences in depth distribution limits between seas can be attributed to the oligotrophic characteristics of the study area in the Mediterranean Sea which allows light spectra to penetrate deeper than in more productive areas (Aguzzi *et al.* 2004, 2007). The avoidance of displacement to illuminated waters by small pelagic species is suggested as a way to avoid the pressure by visual predators (De Robertis 2002, Kronfeld-Schor and Dayan 2003).

At the shelf break, species present in the pelagic samples occur also on the bottom during the day (Abelló *et al.* 1988, Moranta *et al.* 2008). Concerning the species sampled over the middle slope, most of them were also sampled during the day in the DSL and BBL layers, and all of them can also be sampled in close contact with the bottom with both demersal gears (Abelló *et al.* 2002, Moranta *et al.* 2008) and epibenthic sledges (Ramón *et al.* 2013), becoming in this way important actors in their role as prey of epibenthic and demersal predators (Orsi-Relini and Relini 1990, Cartes *et al.* 2009).

Present results also showed the occurrence, in all pelagic decapods, of a rapid vertical displacement during the crepuscular hours (Franqueville 1971). This remarkable cyclic migration pattern has been well studied in many works (Omori 1974, Mauchline 1972) and interpreted as of high adaptive value to reduce predation in this period (Reid *et al.* 1991, Naylor 2010, Olivar *et al.* 2012).

4.4.2. Species and faunistic assemblages

The distribution of the pandalid shrimps *Chlorotocus crassicornis* and *Plesionika heterocarpus* in the present samples was restricted to the continental shelf-break. These species, which typically inhabit the deep continental shelf and the upper slope (Company and Sardá, 1997, Carbonell and Abelló, 1998) have been usually considered to be epibenthic/demersal (Colloca *et al.* 2004, Muñoz *et al.* 2012), but this study has clearly shown their ability to perform pelagic daily movements into the water column. The presence of both species in the diet of *Merluccius merluccius*, by Cartes *et al.* (2004) can corroborate the pelagic character of these species. The presence of *Chlorotocus crassicornis* in the stomach content of fishes sampled in depths above the upper limit of the bottom distribution of this prey together with their absence in the diet of fishes captured on their preferential depth suggests that *M. merluccius* is preying specially on the pelagic fraction of *C. crassicornis*. Other pandalid species widely occurring in the western Mediterranean Sea, particularly in the study area, such as *Plesionika antigai, P. edwardsii, P. narval, P. martia, or P. acanthonotus* (Abelló *et al.* 1988, 2002, Moranta *et al.* 2008), were not found to show incursions into the pelagic environment. *Plesionika narval* and an unidentified juvenile *Plesionika* specimen were only identified in one pelagic trawl sample. The processid shrimp *Processa canaliculata* was also only found once.

In the present study the pasiphaeid shrimp *Pasiphaea sivado* was found to be the commonest species in the study area, occurring both over the shelf break in neritic waters, and over the middle slope, as well as through all sampled depth strata, from epipelagic waters through DSL and BBL to the epibenthic layers. During the day time the species is known to occur near the bottom, preferentially between 350-500 m depth where it can be sampled by bottom gears along the continental slope (Abelló *et al.* 2002, Aguzzi *et al.* 2007, Simão *et al.* submitted). In the Mediterranean Sea, *P. sivado* shows a preferential occurrence depth shallower than its congeneric species *Pasiphaea multidentata* (Simão *et al.* submitted). On the contrary, the distribution of *P. multidentata* was restricted to the middle slope, and never reached epipelagic waters.

The family Sergestidae was that with the highest taxonomic richness in the samples, with up to four different species occurring commonly in the samples. The most abundant species was *Sergestes arcticus* and was clearly a dominant species in many samples. Its distribution was however confined to the middle slope, although it reached epipelagic waters during the night (Franqueville 1971, Froglia and Giannini 1982). The patterns of occurrence and abundance of *S. arcticus* establish it as a key species that characterizes the epipelagic samples for both shelf-break and middle slope areas. The characterization of *S. arcticus* as the dominant species in the epipelagic community is well documented in the Mediterranean Sea and Northeast Atlantic (Froglia and Giannini 1982, Vestheim and Kaartvedt 2009), often being the second most abundant crustacean after the euphausid *Meganyctiphanes norvegica* (Franqueville 1971). Larvae of *S. arcticus* are commonly and abundantly found in the study area in both summer and late autumn, being included among the three more abundant decapod crustacean larvae (Torres *et al.* 2013). *S. arcticus* co-occurred with the larger *Sergestes arachnipodus* in many samples, but the former was dominant in abundance in all sampling cells. *S. arachnipodus* was also restricted to the middle slope and had a wide distribution along the water column from epipelagic waters to the epibenthic environment. *Sergestes vigilax* has a smaller mean size than the before mentioned species and occurred more often in IKMT trawls, probably due the smaller mesh size of this gear. In these samples the species was more common than *S. arachnipodus* but never as abundant as *S. arcticus*. The preferential distribution of *S. vigilax* was similar to that of S*. arcticus* but showed a much shallower median depth of occurrence, located at around 200 m. This shallower mean distribution depth, compared with the 400 m depth for *S. arcticus*, could have adaptive value to minimise competition by other sergestids. Also, food targets and feeding behaviour are probably different given the large differences occurring in feeding structures, such as the third maxillipeds, much larger in *S. vigilax* than in *S. arcticus* or *S. arachnipodus*.

Sergia robusta is the largest sergestid shrimp found in the area, and its distribution was clearly restricted to the middle slope, never reaching epipelagic waters. Its number of occurrences was not very high but clearly characterized the middle slope environment (Froglia and Giannini 1982).

The small aristeid shrimp *Gennadas elegans* was practically only sampled in the DSL, BBL and epibenthic layers of the middle slope, not on the shelf break, and rarely in epipelagic waters (Omori 1974, Andersen *et al.* 1998).

4.4.3. Size structure

The size structure of pelagic decapods in the water column is poorly studied in the literature (Omori and Gluck 1979, Orsi Relini and Relini 1990, Vestheim and Kaartvedt 2009, Suntsov and Domokos 2013). The Sergestidae, except *Sergestes vigilax*, in which no large sized individuals were captured with the epibenthic samplers, showed a quite homogeneous size structure in the water column, with both juveniles and adults occurring in the pelagic environment, in a similar pattern as that shown by Vestheim and Kaartvedt (2009). Size structure was however slightly displaced towards the largest size classes in the epibenthic environment and/or deeper strata (Mauchline 1972), although small sized individuals were also present in the epibenthic samples.

Pasiphaea sivado individuals of all size classes were present in the epibenthic environment during the daytime, but in the water column the population showed a size structure of increasing predominance of large individuals towards the deeper strata, with the smallest sized individuals occurring predominantly in the epipelagic environment at night.

The huge size range of *Pasiphaea multidentata* sampled in the water column showed that all size classes do perform vertical movements in the water column, although the predominance of small size classes in the DSL can suggest a higher independence of the seabed in juvenile individuals. In the present study adult *P. multidentata* in both the DSL and BBL revealed that adults are able to usually perform vertical migrations. The occurrence of pelagic habits in the adult population had only been clearly documented once in this species (Koukouras *et al.* 2000). The adults of the species had been previously thought to perform daily bathymetric movements along the middle slope (Cartes *et al.* 1993, Aguzzi *et al.* 2007). Present results clearly showed that adults of this species may also perform vertical migrations into the water column, however restricted to non-epipelagic layers (up to around

300-400 m depth), in agreement with the low ratio between size and wet weight characteristic of pelagic species (Cartes *et al.* 1993).

4.4.4. Colouration

Pelagic species body colour has been related to depth distribution (Herring and Roe 1988). Transparency is common in pelagic species that dwell in both the euphotic and aphotic zones primarily because it is the only camouflage that is successful from all points of view and at all depths (Johnsen 2001). At the aphotic environment (i.e. below 1000 m) transparent species are rare, being generally replaced by species red or black pigmented. At these depths the visual predation is based on bioluminescent light. The bioluminescent wavelength is almost totally absorbed by red or black pigmentation conferring a more effective cripsis in species with all red body pigmentation than transparency, due the characteristic surface reflections of transparent objects. Despite the red is the first spectral colour that becomes extinct in the first few meters of the water column, the red pigmentation confers opacity to the body at low light intensity waters in a way that the animal will present the same colour of the background (Herring and Roe 1988, Aguzzi *et al.* 2007).

In the study area, the DSL environment was mainly characterized by species showing intense red pigmentation such as *Gennadas elegans, Sergia robusta* and *Pasiphaea multidentata*. The depth distribution of these species did not, or rarely did, reach the epipelagic environment. The transparent shrimps of the Sergestidae family presented a huge vertical distribution encompassing all the depths sampled. *Sergestes arcticus* concentrates intense red pigments due the presence of numerous large chromatophores in a restricted area of the cephalothorax, the gut and the extremities of maxillary appendixes, the rest of the body being transparent/translucent. This coloration pattern probably allows *S. arcticus* to be visually identified by other organisms as a much smaller sized mesozooplankton species, which could be of adaptive value. The same mimicry can also be suggested for *Sergestes arachnipodus*, which additionally presents a dorsal dispersion of small red spots and a larger size than *S. arcticus*. Indubitably, to assume these speculations more studies about translucence and light reflection of these species as well as related to the visual sensibility of small pelagic species are needed (Herring and Roe 1988, Johnson *et al.* 2000).

The ontogeny of *P. multidentata* is a remarkable evidence of the influence of preferential depth distribution in body pigmentation pattern of pelagic decapods, since juveniles, which have a much shallower habitat than adults, present an all transparent body (which often leads to identification confusions with *P. sivado*), while along growth the species accumulates red pigments, becoming redder with age, with larger individuals inhabiting deeper waters (Herring and Roe 1988, Company *et al.* 2001). Differential distribution between juvenile and adult components of a population may be of adaptive value to decrease competition between different age groups.

Conclusions

Conclusions

- In both *Pasiphaea sivado* and *Pasiphaea multidentata* the bathymetric distribution, density, maturity size, and population size structure found in the Alboran Sea were clearly different from those in the Balearic basin. The populations in the intermediate Alacant sector showed more affinities with the Alboran Sea populations, especially so in *P. multidentata*, than with the northern populations, in agreement with their common location in the Algerian basin.

- Densities of *P. sivado* showed a heterogeneous distribution with two main nuclei, one in the Alboran Sea (especially so in the western sector) and another along the North Catalan coast. Additionally the bathymetric distribution in the western Alboran sector reached shallower depths than in the rest of sectors, in agreement with the occurrence of coastal upwellings along the northwesternmost region of the Alboran Sea, around Malaga.

- The relationship between occurrence and density patterns by depth allowed delimitating an optimal depth range of 300-500 m in *P. sivado*, and of 500-800 m (the deepest sampled depth) in *P. multidentata*.

- The size structure analysis showed that these species have a marked size increase with depth. *P. sivado* juveniles were present in the upper slope strata of all sectors. However, *P. multidentata* juveniles were restricted to the Balearic basin, implying that seasonality of reproduction significantly differs between basins.

- Juveniles of both species showed a wider occurrence thermohaline window, suggesting their ability to cope with a wider variability in temperature and salinity conditions.

- The occurrence of sexual dimorphism in the shape of the pleopod protopod of *P. sivado* was evidenced by both the traditional method of biometric ratios and by applying geometric morphometrics methodologies: Overall, in all five pleopod pairs, adult female protopods were clearly much thinner than those in adult males. Additionally, the anterior edge of the male protopods was clearly much more convex than in females, while the posterior edge was straight in both males and females.

- The studied populations of Mediterranean Sea *P. sivado* showed the occurrence of sexual dimorphism in size, with females being slightly smaller than males in all geographic areas and depth strata, suggesting that this pattern is intrinsic to the population biology of the species.

- In all geographic sectors females were dominant at sizes smaller than around 18-19 mm CL; after that size, males were dominant. The precise size at which males became dominant decreased progressively from the Alboran Sea (21 mm CL) to the Catalan Sea (19 mm CL) suggesting the occurrence of environmental and ecological processes affecting growth rates.

- The genetic chapter of this thesis is a first attempt to investigate the influence of the Gibraltar Strait on the population genetic structure of pasiphaeid shrimps, namely *P. sivado* and *P. multidentata*. Additionally, the chapter also presents a first approximation to the phylogenetic tree of the *Pasiphaeidae* using the sequences obtained during this study as well as all the COI sequences of pasiphaeid species available in the Genbank database.

- The Gibraltar Strait was clearly identified as a barrier to the genetic flux in both *P. sivado* and *P. multidentata* due the marked genetic structure characterized by the presence of two different main haplotypes in each species, one Atlantic and another Mediterranean. The absence of a genotype gradient and also the absence of a shared haplotype between samples from the different seas indicated that the Gibraltar Strait is a strong and unique geographical barrier to the genetic flux of both pasiphaeid species at the geographical scale analysed.

- The phylogenetic reconstruction obtained showed that *P. sivado* formed a well-differentiated group from the rest of *Pasiphaea* species studied, in agreement with morphological studies performed within the family. Within the second group, all haplotypes significantly grouped by species, except in the case of *P. tarda* in which, as already shown by other researchers, two well differentiated subgroups clustered according to their Atlantic or Pacific origin. *P. multidentata* has shown its close affinities with *P. tarda* complex.

- The study *of the pe*lagic decapod crustacean fauna of two different zones *around M*allorca (Sóller and Cabrera), characterized by different hydrographic dynamics and oligotrophy levels, evidenced the occurrence of several distinct faunistic assemblages related to the bathymetry of the area sampled (shelf-break and middle slope), as well as to the sampling depth (near-surface epipelagic waters and Deep Scattering *Layers).*

- The two studied zones showed a similar faunistic diversity, with the DSL showing the highest species diversity. No significant differences c*oncerning sampl*ing area or seasonality were found. S*ome spec*ies were r*e*stricted to the continental shelf-break: *Chlorotocus crassicornis* and *Plesionika heterocarpus*. Others were exclusive of deeper waters over the middle slope region, such as *Gennadas elegans*, *Pasiphaea multidentata*, and *Sergia robusta*. *Pasiphaea sivado* was common in all pelagic strata. The virtually absence of differences in the species composition, diversity and dominance between the geographic regions (Cabrera and Sóller) and seasons (early winter and summer), suggested that the dynamics of the pelagic environment would tend to uniformize the ecological dynamics of the system.

- Size analysis showed the occurrence of species-specific patterns concerning the size/age movements into the water column throughout the day-night cycle.

- The present study clearly confirms the presence of adult *P. multidentata* in the water column, evidencing that the adult fraction of the population has also the ability to perform vertical migrations.

- The importance of several species, such as *Sergestes arcticus*, *Pasiphaea sivado*, *Gennadas elegans* or *Sergia robusta,* due to their abundance and vertical migration patterns, must be taken into account when trying to explain transfers of matter and energy in the pelagic environment coupled to the epibenthic ecology.

- Overall, the size structure of both pasiphaeids and sergestids was displaced towards the largest size classes in the epibenthic environment. This pattern was more marked in *P. sivado*.

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