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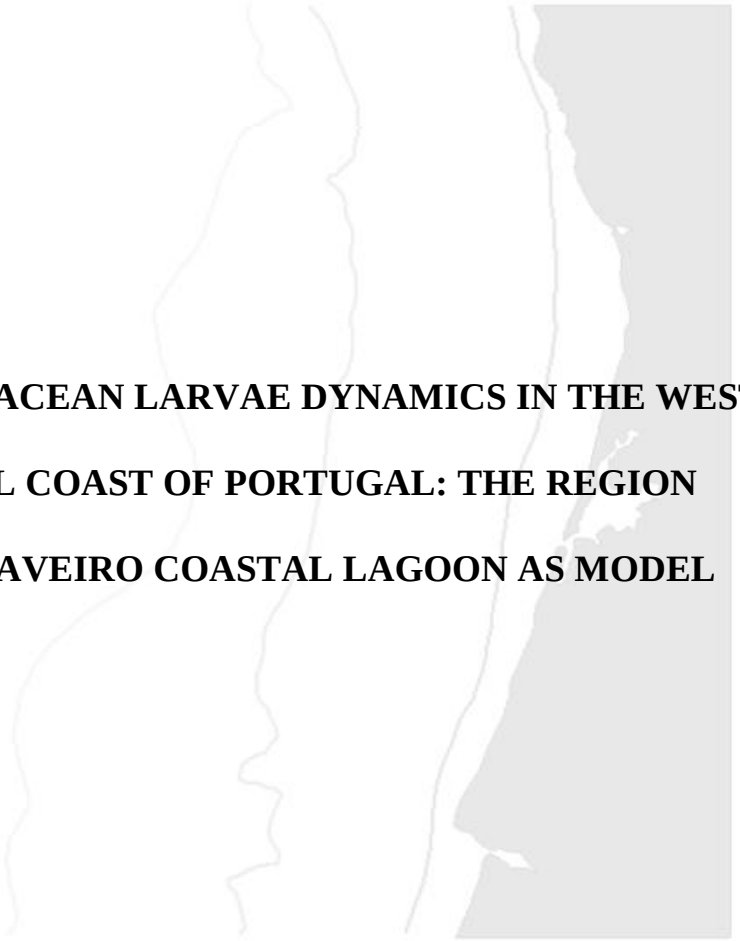
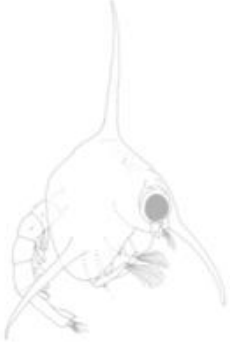


DECAPOD CRUSTACEAN LARVAE DYNAMICS IN THE WEST CONTINENTAL COAST OF PORTUGAL: THE REGION ADJACENT TO AVEIRO COASTAL LAGOON AS MODEL

CÁTIA ALEXANDRA VIEIRA BARTILOTTI

Doutoramento em Ciências do Mar, especialidade de Ecologia Marinha



FARO

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Tese orientada por:

Investigadora Doutora Antonina dos Santos

Professora Doutora Margarida Castro

FARO

(2010)

"Porque sou do tamanho do que vejo

E não do tamanho da minha altura"

Bernardo Soares, in: *O Livro do Desassossego*

ACKNOWLEDGEMENTS

I would like to express my gratitude to all of those that gave me support through these years of work, even knowing that the words are not enough to recognize all the received assistance. First of all I must thank to FCT for the financial support (SFRH/ BD/ 16695/ 2004), to IPIMAR that gave me the conditions to fulfill my PhD working plan, and to my supervisors Dr. Margarida Castro and Dr. Antonina dos Santos for all the guidance and support. To Dr. Margarida Castro, thank you for all the help at the University, for the statistical analysis and for all the nice talks about such varied themes; to Dr. Antonina dos Santos, thank you for these eight years of lessons, friendship and confidence… As I always say, Gurney is a visionary, so if he had a time machine (as you always say), I am sure that he would like to discuss everything about decapod larvae with a generalist like you!

To Dr Miguel Santos for the constant care. To the "ProRecruit- Shelf processes controlling recruitment to littoral populations in an eastern oceanic boundary: using barnacles and crabs as models" (POCTI/1999/BSE/36663) staff, in particular, Dr. Henrique Queiroga, Dr. Álvaro Peliz and Dr. Patrícia Lourenço.

To the Crustacea friends: Dr. Andrew Rhyne, thank you for all the *Lysmata* larvae that you gave me (I believe that they will keep me far from Alzheimer); Dr. Juan Ignacio González-Gordillo for the *Ilia nucleus* larval series and all the confidence deposited in my work; and last but not least, to Dr. Ricardo Calado that is an excellent person but also an excellent scientist always with a new idea to discuss and develop. I also express thanks to all the co-authors of my papers for all the suggestions made that greatly improved the quality of my work and taught me so much.

To those that shared IPIMAR with me: Fátima Quintela, my friend, always looking for me and taking care of me, thank you for your good mood and thank you for your nice smile; Dr. Sofia Palma and Dr. Alexandra Silva, friends of “marmita” lunch time, living at the next door lab, were the perfect companionship for the larval description pauses; Dr. Susana Garrido, the “perfumed” friend, always looking for digested phyto- and zooplankton in sardines stomachs, shared the smell but also her friendship; Dr. Juan Zwolinski, the tea time friend, who shared the activities of the weekend and also the GAM’s and other statistical methodologies.

To my friends, that always believed: Carla, Daniela, Eugénio, Irina, Joana, Marta, Miguel, Sara, Rui, Vanessa, thank you for your love, your understanding, your support. You make my life happier, simpler and funnier. To all those that I don’t refer but in some way were important to me, thank you!

To my Super-heroes family! Thank you! Aqueles que são meus e que estão comigo sempre, que me amam e protegem incondicionalmente, a eles dedicam o meu trabalho! À Avó, Mãe, Luis e Nino, obrigada por serem os meus anjos! Ao Pai que considero um super-homem a cada dia que passa. Aos tios, primos e bebés, por me mimarem sempre. A todos estes que me ajudaram a percorrer o caminho, muito obrigada! Foi um fim difícil… Aos que não me acompanharam, também agradeço, porque me mostraram que os nossos limites são sempre ultrapassáveis…

NOME: Cátia Alexandra Vieira Bartilotti

FACULDADE: Ciências do Mar e do Ambiente

DATA: 12 de Fevereiro de 2010

TÍTULO DA TESE: Dinâmica larvar de crustáceos decápodes na costa ocidental de Portugal Continental: a região adjacente à Ria de Aveiro como modelo.

RESUMO

O Filo Crustacea é um dos maiores taxa do Reino Animal, e a Ordem Decapoda é a maior com cerca de 50000 espécies, um número que continua a aumentar. A maioria dos decápodes distribui-se no oceano e nas zonas estuarinas adjacentes, no entanto algumas espécies invadiram a água doce, e um pequeno número de espécies ocupou os habitats terrestres. A diversidade de espécies de decápodes reflecte a diversidade de estilos de vida, e sendo na sua maioria bentónicos, estes animais podem viver nos habitats tropicais, mangais, recifes de coral, regiões polares, zonas do oceano profundo ou fontes hidrotermais. Os crustáceos decápodes são bastante importantes pois constituem um dos grupos biológicos com pesca dirigida e são muitas vezes elos da cadeia alimentar de inúmeros recursos pesqueiros da nossa costa. Uma estratégia adequada de gestão pesqueira para uma espécie com valor económico requer o conhecimento do seu ciclo de vida. Assim, para os crustáceos decápodes os estudos de plâncton dão informação acerca da distribuição e abundância das espécies durante a fase larvar planctónica. A fase de recrutamento é particularmente crítica nestes organismos, uma vez que implica a articulação de duas fases do ciclo de vida separadas espacialmente: a fase larvar planctónica e a fase adulta bentónica, ou seja, o "stock" explorável depende da sobrevivência dos estádios larvares. A fase larvar constitui assim um período vital no ciclo de vida das várias espécies de decápodes e tem o papel fundamental nos processos de intercâmbio genético, de recrutamento e consequente renovação de populações e "stocks".

As larvas de crustáceos decápodes são nadadoras activas, capazes de regular a sua posição vertical na coluna de água, controlando a extensão e direcção da dispersão horizontal, mantendo a posição favorável ao transporte necessário e adequado à fase do ciclo de vida em que se encontram. Os processos de transporte são decisivos para o mecanismo de fornecimento larvar aos habitats onde o assentamento e o desenvolvimento juvenil irão ocorrer, separando a ontogenia no tempo e no espaço, expondo as larvas a diferentes factores de mortalidade. A compreensão dos padrões dos movimentos temporais e espaciais larvares é fundamental para estudar a ecologia dos decápodes, permitindo o design de estratégias efectivas de conservação e gestão dos recursos. As migrações verticais são a estratégia adoptada pelas larvas de crustáceos decápodes para o transporte adequado e necessário à fase do ciclo de vida em que se encontram, e normalmente são separadas em dois grandes grupos: as migrações verticais diárias (DVM) e as migrações ontogénicas.

A fase larvar pelágica apresenta uma morfologia diferente da do adulto. O desenvolvimento larvar dos crustáceos decápodes é descrito como uma sequência de estádios larvares

distintos. Existem três fases de desenvolvimento, separadas por uma metamorfose: nauplius, zoé e megalopa (formas com natação cefálica, torácica e abdominal, respectivamente). As larvas de crustáceos decápodes podem ter diferentes formas mas usualmente apresentam padrões de desenvolvimento estáveis. Na análise dos processos bio-ecológicos envolvidos nesta fase do ciclo de vida, deverão considerar-se além dos factores hidrológicos, a duração do desenvolvimento larvar, e o número de estádios larvares que cada ciclo larvar planctónico comporta, bem como as suas características morfológicas como estratégias adaptativas aos ecossistemas costeiros, sendo que a forma hidrodinâmica das diferentes larvas poderá corresponder a diferentes padrões de dinâmica larvar, ou seja, as características morfológicas podem ser encaradas como estratégias adaptativas aos ecossistemas. Tendo em conta o universo considerado (larvas de crustáceos decápodes), no que diz respeito à forma, podemos agrupá-las nas seguintes formas tipo: tipo A (carapaça e abdómen achatados lateralmente; telson de forma triangular com invaginação central; camarões *sensu lato*); tipo B (corpo fortemente achatado dorso-ventralmente; apêndices muito longos, projectados lateralmente; infraordem Palinura); tipo C (carapaça achatada lateralmente mas ligeiramente arredondada; abdómen de forma cilíndrica; telson de forma triangular ou bifurcado; infraordens Astacidea e Anomura); tipo D (carapaça quase esférica; usualmente apresenta espinhos laterais, dorsais e um rostro ventralmente direccionado; abdómen de forma cilíndrica e telson em forma de furca; caranguejos). Destas vamos estudar apenas as formas tipo camarão carídeo (tipo A), caranguejo eremita (tipo C) e caranguejo (tipo D).

Esta tese pretende descrever os padrões de distribuição horizontal e vertical (ontogénica) das larvas de crustáceos decápodes no sistema de afloramento costeiro português. Pretende também fazer a descrição morfológica externa do desenvolvimento larvar de espécies de cada uma das formas tipo escolhidas: os camarões carídeos, os caranguejos eremita e os caranguejos. Para completar os objectivos ecológicos e morfológicos deste trabalho, desenvolveram-se 5 capítulos, os dois primeiros relativos à ecologia e os três últimos relativos à taxonomia e morfologia dos estádios larvares de decápodes.

Nos capítulos 2 e 3 descrevem-se os padrões de distribuição horizontal e vertical (ontogénica) das larvas de crustáceos decápodes no sistema de afloramento português adjacente à Ria de Aveiro.

O capítulo 2 pretende testar a hipótese de que a distribuição horizontal larvar de todos os crustáceos decápodes na área estudada reflecte um padrão de retenção. As larvas deverão estar distribuídas em bandas, paralelas à costa, concordantes com os intervalos das distribuições dos adultos. Demonstra-se que as migrações verticais diárias (DVM) anteriormente descritas por dos Santos et al. (2008) são consistentes ao longo do desenvolvimento, de quase todos os taxa estudados, e a estratégia de retenção larvar suposta pelos autores existe. As larvas de decápodes estiveram distribuídas ao longo de bandas meridionais alongadas, paralelas à costa, concordantes com as respectivas origens: as espécies da plataforma interna e Ria de Aveiro distribuíram-se junto à costa, as espécies da plataforma distribuíram-se ao longo da zona média da plataforma, e as espécies da vertente distribuíram-se junto ao bordo da plataforma. Assim, um dos resultados mais importantes do presente trabalho é que a distribuição larvar reflecte a distribuição dos adultos. Definem-se três grupos: espécies da "Inner shelf" distribuídas junto à costa com os máximos de abundância a 8 km de distância à costa (ex: *Diogenes pugilator*); espécies da "Shelf": distribuídas na plataforma média com os máximos de abundância entre os 20 e os 60 km de distância à costa (ex: *Polybius henslowii*); e espécies da "Slope": distribuídas

sobre a vertente da plataforma com os máximos de abundância a 60 km de distância à costa (ex: *Solenocera membranacea*). O "Self recruitment" parece existir para as espécies costeiras e da plataforma. As espécies da vertente deverão ter vantagens em ter parte do seu ciclo de vida na plataforma. No que diz respeito ás distribuições em relação aos factores ambientais verificou-se que as espécies da Ria de Aveiro e da plataforma interna estiveram associadas a temperaturas mais elevadas reflexo do relaxamento do afloramento ocorrido durante a amostragem, e por contrário as espécies da vertente estiveram associadas a temperaturas mais baixas reflexo do afloramento ocorrido antes da amostragem. Os zoés de camarões estiveram associados à lente de água menos salina WIBP que estava a ser advectada para o largo, e os megalopas de caranguejo parecem evitar esta massa de água.

O capítulo 3 pretende descrever as migrações verticais ontogénicas num ponto fixo, e também pretende descrever as respostas comportamentais dos estádios larvares das espécies seleccionadas aos factores ambientais. Em geral o primeiro estádio de zoé tem uma posição mais superficial que o segundo estádio, e o último zoé teve sempre uma distribuição mais profunda. O megalopa teve uma profundidade média de distribuição semelhante à calculada para o ultimo estádio de zoé, mas teve em geral muitas vezes concentrado na camada de neuston durante a noite, reflectindo provavelmente um mecanismo de transporte. Durante a amostragem a distribuição dos campos hidrológicos foi complexa.

Nos capítulos 4 a 6 apresentam-se as descrições morfológicas externas dos desenvolvimentos larvares de crustáceos decápodes obtidos no laboratório das três formas tipo seleccionadas: os camarões carídeos de duas espécies próximas de *Lysmata*, *L. galapagensis* e *L. moorei*, os caranguejos eremitas *Clibanarius aequabilis* e *C. erythropus*, e o desenvolvimento larvar do caranguejo *Ilia nucleus*.

O capítulo 4 teve como objectivos descrever os estádios larvares disponíveis de duas espécies próximas *L. galapagensis* e *L. moorei*, comparar estas descrições com as da única espécie descrita no "Cosmopolitan Clade" a *L. seticaudata*, e com o "género larvar" *Eretmocaris* dadas as semelhanças existentes entre *L. galapagensis* e *E. corniger*, e finalmente rever e discutir os caracteres do género. Ambas as espécies apresentaram um desenvolvimento larvar homogéneo: no primeiro zoé os olhos estão fundidos, a carapaça tem um espinho pterigostomiano seguido de 4-5 dentículos marginais, o escafocerito tem 5 segmentos, o 5º segmento abdominal tem um par de espinhos dorso-laterais, os rudimentos dos pereiópodes 1 e 5 estão presentes; no segundo zoé os olhos são pedunculados, os espinhos antenar e supraorbital estão presentes, um espinho pós-rostral, o pereiópodes 1 e 5 estão funcionais; no terceiro zoé o pedúnculo antenar tem dois segmentos e os dois flagelos estão já presentes, o sexto segmento abdominal está separado do telson, o rudimento do pereiópode 2 está presente, os urópodes estão presentes com um endópode rudimentar; no zoé IV o escafocerito não apresenta qualquer segmento, o segundo pereiópode está funcional, e os urópodes são tão longos quanto o telson; o zoé V tem os rudimentos birramosos dos pereiópodes 3 e 4, e os urópodes são tão longos quanto o telson; zoé VI tem os pereiópodes 3 e 4 funcionais e apresenta já uns pequenos rudimentos dos pleópodes no abdómen; no penúltimo estádio (ZVII ou ZVIII) o flagelo da antena é tão longo quanto o escafocerito e tem 8 segmentos, os pleópodes são já birramosos e os endópodes e exópodes têm pequenas sedas apicais; último zoe (ZVIII ou ZIX) tem já o flagelo da antena mais longo que a escama e com mais de 8 segmentos, os pleópodes apresentam o apêndice interno, o telson muito semelhante ao de um adulto. Ambas as espécies eclodem com uma forma semelhante à de *L. seticaudata* com os rudimentos dos pleópodes 1 e 5. Como o presente trabalho demonstra o estudo das larvas de *Lysmata* será certamente uma ajuda

preciosa para os investigadores que estudam a filogenia deste género, uma vez que ao analisar as descrições larvares disponíveis para o género podemos supor pelo menos dois padrões de desenvolvimento larvar para estas espécies: o primeiro semelhante a *L. galapagensis*, *L. moorei* e *L. seticaudata* em que as larvas eclodem com os primeiro e quinto pereiópodes como rudimentos que estarão funcionais no estádio seguinte e que completarão o seu desenvolvimento com 8 ou 9 estádios de zoe, e um segundo mais longo com as larvas a eclodirem sem qualquer rudimento dos pereiópodes. O primeiro grupo de espécies pertence ao "Cosmopolitan Clade"/ "*Lysmata* Clade" (Baeza et al. 2009 e Fiedler et al. submetido), o que nos leva a supor que a presença dos rudimentos do 1º e 5º pereiópodes nas larvas recém eclodidas é uma característica partilhada por todas as espécies deste clade. A presença de um espinho anteriormente curvo na região dorsal do 3º segmento abdominal nos estádios mais velhos de *L. galapagensis* torna esta espécie muito característica. Esta espécie é muito semelhante aos exemplares descritos por Gopalakrishnan & Laurs (1971), portanto demonstramos que as larvas descritas pelos autores como o *E. corniger* do Pacífico Este são de facto *L. galapagensis*. Gurney (1937) descreve Sp. A. V, *Eretmocaris corniger*, do Oceano Atlântico, e esta larva apresenta a mesma forma geral que *L. galapagensis*. Tendo em conta a proximidade filogenética sugerida pelos estudos moleculares de Baeza et al. (2009) e Fiedler et al. (submetido) entre a *L. galapagensis* do Pacífico Este e a *L. moorei* do Atlântico Oeste, o carácter larvar mais evidente que ambas partilham é a presença de um rostro semelhante, razão que nos leva a concluir que provavelmente existe uma espécie de *Lysmata* desconhecida provavelmente filogeneticamente mais próxima de *L. galapagensis* que de *L. moorei* no Atlântico Este. Tendo em conta que *L. galapagensis* ocorre no Pacífico Este e a larva mais semelhante conhecida até à data é o *Eretmocaris corniger* colhido no Atlântico Este, questiona-se a biogeografia deste complexo de espécies trans-istmo do Panamá.

O capítulo 5 apresenta o estudo do desenvolvimento larvar dos caranguejos eremita do género *Clibanarius*. Descreve-se *C. aequabilis* e re-descreve-se *C. erythropus*. Os estádios larvares das duas espécies de *Clibanarius* do nordeste Atlântico são muito semelhantes, e não são fáceis de distinguir. Uma comparação exaustiva das características morfológicas larvares das espécies do género descritas até à presente data e das descritas no presente trabalho demonstram que este género é muito homogéneo. Apesar da homogeneidade no género *Clibanarius* verificam-se pequenas variações entre espécies, particularmente no que respeita à formula do telson e à morfologia dos seus processos depois do segundo estádio de zoé. Também o número de estádios larvares pode variar. Sugere-se que se considere como característica geral dos estádios de zoé do género *Clibanarius* a transformação do 4º processo plumoso do telson num espinho fundido, reduzido ou desenvolvido, do Segundo para o terceiro estádio de desenvolvimento.

Finalmente o capítulo 6 pretende descrever com detalhe os quatro estádios de zoé e o megalopa do caranguejo *Ilia nucleus*. As características gerais dos 4 zoés e do megalopa destaespécie correspondem às propostas anteriormente por Rice (1980) para os estádios de zoé e por Quintana (1986) para o megalopa da família Leucosiidae. Os estádios larvares descritos no presente estudo são muito semelhantes aos anteriormente descritos. Ng et al. (2008) propuseram recentemente uma nova classificação da família Leucosiidae, com apenas 3 subfamílias: Cryptocneminae, Ebaliinae e Leucosiinae. Os autores afirmaram que a actual subfamília Ebaliinae é um grupo muito heterogéneo. Considerando as características larvares estudadas no presente trabalho, verifica-se que a subfamília Ebaliinae inclui as espécies mais ancestrais e também as mais derivadas nos Leucosiidae, o

que leva à conclusão que esta subfamília é um grupo heterogéneo. Verificou-se também que para a correcta identificação do estádio de zoé além do número de sedas dos exopoditos dos maxilípedes deve também utilizar-se o desenvolvimento da antenula e dos pereiópodes. Os objectives morfológicos desta tese demonstram a importância da taxonomia no estudo das larvas dos crustáceos decápodes. As espécies descritas e as questões levantadas nos três capítulos podem servir de base a novas hipóteses de trabalho.

Palavras-chave: larvas de crustáceos decápodes, retenção larvar, distribuição vertical ontogénica, desenvolvimento larvar de *Lysmata*, desenvolvimento larvar de *Clibanarius*, desenvolvimento larvar de *Ilia*.

DECAPOD CRUSTACEAN LARVAL DYNAMICS IN THE WEST CONTINENTAL COAST OF PORTUGAL: THE ADJACENT REGION TO AVEIRO COASTAL LAGOON AS MODEL

ABSTRACT

Present thesis pretends to describe the horizontal and vertical distribution patterns of decapod crustacean larvae in the Portuguese upwelling ecosystem. The studied taxa were retained close to their parental populations; so, species from the inner shelf had their larvae concentrated close to the shore, species from the shelf had their larvae over the shelf, and the slope species had their larvae along the shelf break. The ontogenetic vertical distribution was also analysed, and the average depth of distribution of the larval stages varied through the larval development. The majority of the studied species had their early zoeal stages more close to the surface and the last zoeal stages in a more deep position.
The morphological larval descriptions from laboratory cultured material of the three selected larval forms of decapod larvae are presented: the larval development of two closely related species of *Lysmata*, *L. galapagensis* e *L. moorei* (caridean shrimps), the complete larval developments of *Clibanarius aequabilis* and *C. erythropus* (hermit crabs), and the complete larval development of the crab *Ilia nucleus*.

Key-words: decapod crustacean larvae, larval retention, ontogenetic vertical migration, *Lysmata* larval development, *Clibanarius* larval development, *Ilia* larval development.

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LIST OF ABBREVIATIONS

Bir.- biramous

Bottom- depth of the station

CL- carapace length

CTD- conductivity-temperature-depth recorders

CW- carapace width

CWls- carapace width with lateral spines

D- dactylus

Decr. post.- decreasing in size posteriorly

DEPTH_CAT – upper limit of the depth stratum

DIST_COAST - distance to the coast

DL- dactylus length

DVM- Diel vertical migration

GAM- Generalized Addictive Model

Hour1- night and day

Hour2- night, dawn, day, dusk

HOUR_WAVE - hour of the day

ISWM – Intermediate Salinity Water Mass

IPC – Iberian Poleward Current

LHPR- Longhurst Hardy plankton recorder

LM- Logistic Model

M- megalopa or decapodid

MAX_TOW_DEPTH – Maximum depth towed in the station

MID_DEPTH – Midpoint of depth stratum

NA- not available

ND- not developed

n.s.- not significant

P- propodus

p.-page

Part.- partially

pc- pseudochaetae

PL- propodus length

RDL- rostro-dorsal length

REAL_TIME – Hour of the day

RL (or R)- rostrum length

SAL – salinity

Seg.- segmented

Sub-ac.pr- sub-acute processes

T- transect

TEMP – temperature

TL- total length

Unir.- uniramous

Unseg.- unsegmented

WIBP - Western Iberia Buoyant Plume

WMD- Weighted Mean Depth

Z- zoea

ZN- early zoeal stages

ZO- old zoeal stages

CHAPTER 1

General Introduction

1.1- The importance of decapod crustaceans

Tropical and temperate coastal zones are inhabited by 110000 species of benthic invertebrates, of which approximately 80% have complex life cycles with a pelagic larval phase, a vital period for gene-flow, recruitment and consequent renovation of populations (Thorson 1964, McConaugha 1992). The Phylum Crustacea is one of the largest taxa in the animal kingdom only exceeded by insects and gastropods, and the Decapoda Order is the largest one with around 50000 species (Tudge 2000), a number that stills increasing (e.g. dos Santos et al. 2008a). Most of the decapods are found in the sea or adjacent brackish waters, some species invaded the freshwater, and a small number of species occupied the terrestrial habitats (e.g. Anger 2001). The diversity of decapod species reflects the diversity in their life styles, even knowing that most decapods are benthic they can live in the floors of the oceans from the high latitudes to the tropical environments, in mangroves, in coral reefs, polar and deep seas or hydrothermal vents. They are also a relevant part of the food web in the marine ecosystems.

A high number of species of decapods are commercially exploited, and in Portugal they constitute important fisheries resources (e.g. dos Santos 1999). An adequate fisheries management strategy for a species with economical value requires the knowledge of the species life cycle, so in the case of decapods the plankton studies give information about the distribution and abundance during their pelagic larval phase (e.g. dos Santos 1998). The exploited stock depends mainly on the larval stages survival (e.g. Roughgarden et al. 1988, Queiroga et al. 1994, dos Santos 1999), so the larval phase is a vital period in the life cycle of most decapod species and it is crucial for genetic flux, recruitment and consequent populations and stocks preservation (Paula 1993).

1.2- Decapod crustaceans life cycle: ecology

The decapod crustacean larvae are active swimmers, that can actively regulate their vertical position in the water column, controlling the extent range and direction of their horizontal dispersal (Queiroga & Blanton 2005), maintaining a favourable position to the adequate and necessary transport (e.g. Forward et al. 1997, Christy & Morgan 1998). The transport processes are the decisive components of the supply mechanism of larvae to habitats where settlement and juvenile development will occur (e.g. Botsford 1986), separating the ontogeny in time and in space (different environments), exposing the larvae to different mortality factors (Queiroga & Blanton 2005). The understanding of temporal and spatial larval movement patterns is fundamental to study decapods ecology leading to the design of effective conservation and resource management strategies (e.g. Pittman & McAlpine 2001, Mace & Morgan 2006). The larval vertical migrations are the strategy adopted by decapod larvae for the adequate and necessary transport at a life cycle phase (e.g. Oishi & Saigusa 1997, Christy & Morgan 1998, Paula 1998, Queiroga 1998).

The Diel Vertical Migration (DVM) behaviour seems to be the rule for decapod larvae, but it is rarely described in detail (see Queiroga & Blanton 2005 for examples), being most of the studies obtained by the neuston and/or discrete depth levels sampling (e.g. Shanks 1985, Jamieson & Phillips 1988, Abelló & Guerao 1999). Dos Santos et al. (2007, 2008b) showed that decapod zoeae and megalopae, as well as *Chthamalus stellatus* cyprids, displayed the DVM in the upwelling ecosystem adjacent to the Ria de Aveiro lagoon system. Pineda et al. (2007) concluded that the vertical swimming behaviour, changes in buoyancy, and ontogenetic changes in vertical position in the water column influenced the horizontal larval movements, and recently, dos Santos et al. (2008b) verified the diel vertical migration in

decapod crustacean larvae over the Portuguese upwelling ecosystem, corroborating the hypothesis presented by the models developed for the study area (Marta-Almeida et al. 2006, Peliz et al. 2007).

The ontogenetic migrations defined as the change in the average depth of distribution during the larval life period, is obligatory for benthic crustacean that hatch close to the bottom, feed in the surface, and must return to the adult habitat (Queiroga & Blanton 2005). Most of the times, the studies describing the ontogenetic migrations (see Queiroga & Blanton 2005 for details and references), considered brachyuran species, and in the study area, Queiroga (1996) described the ontogenetic variations in the vertical distribution of *Carcinus maenas*.

For the complete understanding of the larval transport and settlement locations is essential to describe the local hidrography and the three-dimensional current fields, as well as the growth, survival and behavioural responses of larvae to their environment (e.g. Botsford et al. 1994). The northwest coast of Portugal is characterised by complex mesoscale variability and a strong seasonality (Peliz et al. 2002, Relvas et al. 2007). Dos Santos et al. (2008b) studied the decapod larvae and discussed their results with the modelling studies for the area (Marta-Almeida et al. 2006, Peliz et al. 2007), confirming that the larval dispersal in the inner and middle shelf is made alongshore and the observed retention greatly depends on the diel vertical migration behaviour. Moreover, the authors supposed that the horizontal distribution patterns of several species retained over the shelf were associated with their presumable settlement areas. The apparent retention of decapod larvae close to parental populations was recently discussed by Morgan et al. (2009) and seems to be more frequent than considered until present date.

1.3- Decapod crustaceans life cycle: morphology

As referred before, the majority of decapods have complex life cycles, with an indirect development, and after the embryonic development within the egg the larvae are release, floating in the water column (e.g. Anger 2001). These pelagic larvae usually differ entirely in their morphology and habits from juvenile and adults, showing their own evolutionary adaptations. The larval development of crustaceans is described as the sequence of morphologically distinct stages.

To avoid misunderstandings and due to the particular importance of the concepts, according to Anger (2001):

- phase: is defined as the sequence of morphologically equal developmental stages, e.g. all naupliar, zoeal or megalopal stages combined as the “larval phase”;
- instar: is defined as a numerical stage, e.g. the appearance of a new instar may not be associated with morphological changes;
- stage: is defined as a distinguishable morphologically different instar, e.g. the roman numbers to identify successive stages in a given phase zoea I, zoea II, etc.;
- metamorphosis: is defined as the sudden and dramatic changes in the morphology of two subsequent stages, e.g. the transition from nauplius to zoea, from zoea to the megalopa, from the megalopa to juvenile.

During the larval development of decapod crustacean there are three possible phases separated by one metamorphosis: the nauplius, the zoea and the decapodid or megalopa (Williamson1969), depending on the appendages used by the larva for swimming (cephalic, thoracic and abdominal, respectively).

The nauplius is considered the most ancestral type of larva in the Crustacea, and in decapods, it is only present in the Suborder Dendrobranchiata. All the other decapods have this phase in the egg, during the embryonic development. The nauplius swims with the three pairs of cephalic appendages: antennules, antennae and mandibules. In a subsequent stage of the naupliar phase, the metanauplius, can develop other appendages however those are nonfunctional; the nauplius has a rudimentary small median eye.

The Suborder Pleocyemata has an embryonic development longer than the Dendrobranchiata because the nauplius develops within the egg, that when hatches releases a zoea. This phase is the most common in the plankton collections, and as a consequence it is the most studied (e.g. Paula 1993). The zoea presents as locomotion appendages the biramous thoracic appendages. It has compound eyes, in general sessile in the first zoea and mobile in subsequent stages. The number of stages in the zoeal phase is greatly variable, varying between groups (from two zoeal stages in Majidae crabs, to 15 or more in the Palinura lobsters) but also within a species (e.g. Anger 2001).

The megalopa or decapodid is characterized by the presence of functional pleopods as swimming appendages. Generally this phase has only one stage, which is the last larval stage that will metamorphose to a juvenile. It is a stage more similar to the adult (e.g. Paula 1993, dos Santos 1999), and presents a more or less uniform shape among the various taxonomical groups.

The decapod crustacean larvae may have different forms, but usually present stable developmental patterns (Paula 1993). The analysis of bio-ecological processes involved in the larval phase of the life cycle, the hydrological factors, the duration of the larval development,

and the number of larval stages in each larval series, as well as their morphological characters should be assigned as adaptive strategies to the coastal ecosystems. It can be supposed that the larval hydrodynamic form may correspond to different dynamic patterns, so having in mind the decapod crustacean larvae we grouped them in four different types:

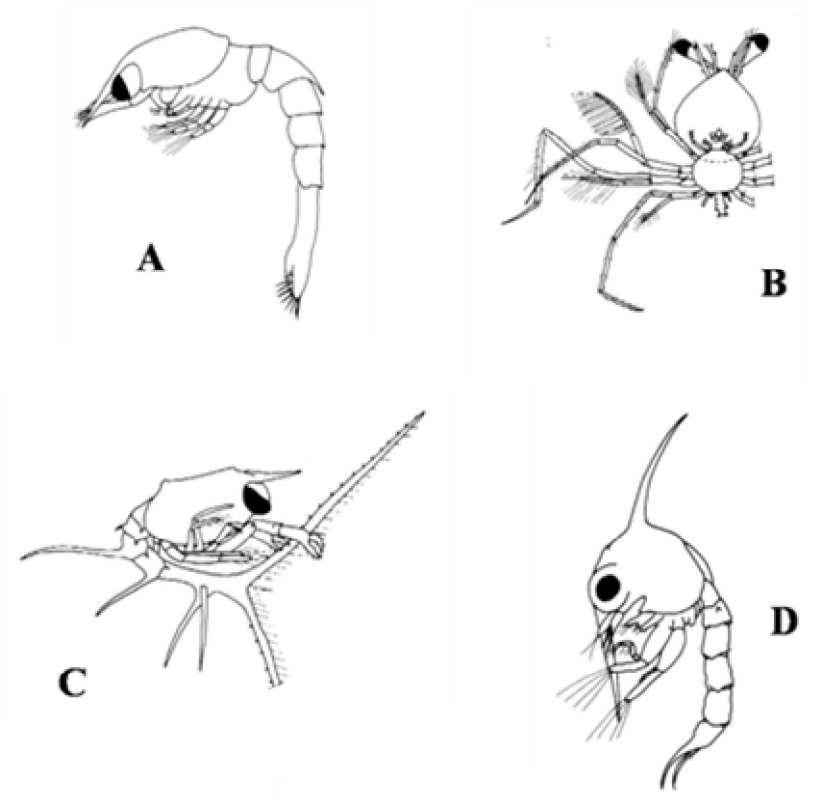

Figure 1. A: Zoea I of *Callianassa tyrrhena*; **B:** Zoea II of *Palinurus elephas*; **C:** Zoea I of *Nephrops norvegicus*; **D:** Zoea I of *Carcinus maenas*. *In*: dos Santos (1999).

Type A (Figure 1A): carapace and pleon laterally flattened; triangular telson. Suborder Dendrobranchiata, Infraorders Caridea, Stenopodidea and Thalassinidea, all shrimps sensu lato.

Type B (Figure 1B): the body strongly dorso-ventrally flattened; with very long appendices, laterally projected. Infraorder Palinura, the lobsters.

Type C (Figure 1C): with a laterally flattened but slightly rounded carapace; a cylindrical pleon (or abdomen); a bifurcated or triangular telson. Infraorders Astacidea and Anomura, the Norway lobster and the hermit crabs.

Type D (Figure 1D): a carapace almost spherical; usually with dorsal and lateral spines, and a rostrum ventrally projected; cylindrical telson or as a furca. Infraorder Brachyura, the crabs.

1.4- Objectives

The aim of present thesis is to describe the horizontal and vertical distribution patterns of decapod crustacean larvae in the Portuguese upwelling ecosystem, as well as to present the morphological larval descriptions from laboratory cultured material of the three selected larval forms of decapod larvae: the caridean shrimps, the hermit crabs, and the crabs. In the working plan of present thesis five specific objectives related with the larval ecology and morphology of decapod crustacean larvae were delineated: (1) to verify if the larval vertical distribution in the water column is related with the day cycle variation and (2) to describe the vertical migration rhythms of the considered larval forms (larval form and stage), (3) to establish an average depth of distribution through the larval development and (4) to verify the existence of ontogenetic vertical migration in the water column. The last objective is to make the morphological description of the larval development of three selected taxa of the three selected larval forms (a caridean shrimp, a hermit crab and a crab). To fulfil the ecological and morphological aims of present dissertation, five different studies were conducted:

- dos Santos et al. (2008b) studied the decapod larvae and hypothesised that the horizontal distribution patterns of several decapod species retained over the shelf were

related to their presumable settlement areas. So, in Chapter 2 our objective was to test the hypothesis that the decapod crustacean larval distribution, in the studied coastal upwelling ecosystem, clearly shows a retention strategy being coincident with the adults' distributional ranges. As a result, we also pretend to demonstrate that the larvae concentrated very close to the shore will be the inner shelf species larvae; those concentrated somewhere in the middle shelf, will include the shelf species larval stages; and those that will appear over the continental shelf break, will concentrate the shelf slope species. To verify our hypothesis we analyse the relationships between the larval distributions of 15 selected taxa with the physical environment in the Western Iberia upwelling ecosystem.

- dos Santos et al. (2008b) described the diel vertical migration of decapod larvae. Knowing this, in Chapter 3 we pretend to analyse in detail the vertical distribution patterns of the larval stages of 9 species, in order to determine their ontogenetic vertical migration and also to describe the behavioural responses of each stage to the environment.

- present work in Chapter 4 pretends to describe the available zoeal characters of two closely related trans-isthmian caridean shrimp species, *Lysmata moorei* (Rathbun, 1901) and *L. galapagensis* Schmitt 1924, using laboratory cultured material. Both descriptions are compared with the larval features previously described for the only species in the "Cosmopolitan Clade" with their larvae known, *L. seticaudata* (Calado et al. 2004), and with the *Eretmocaris* larval genus (Bate 1888) given the evident resemblances between *L. galapagensis* larvae and *E. corniger* from the Tropical Eastern Pacific.

- the hermit crabs *Clibanarius aequabilis* and *C. erythropus* are both present in the eastern Atlantic, and present work pretends to describe and compare the complete larval series of *Clibanarius aequabilis* with the previously described larval stages of other species of the genus, in particularly with those of *C. erythropus*. These two species do not co-occur in the same area, but a careful comparison was required, so Chapter 5 also presents a redescription of *C. erythropus* larval morphology according to modern standards.

- finally, the Chapter 6 describes in detail the four zoeal stages and the megalopa of the crab *I. nucleus* from laboratory reared material.

1.5- Structure of this Dissertation

As the objectives list indicates, this dissertation is organized in seven chapters where the ecological and morphological aspects of decapod crustacean larvae are studied. Chapters two and three present the ecological results, while chapters four to six present the morphological results. Present chapter, the "Introduction", includes a general introduction to the study of decapod crustacean larvae, with the state of the art, the presentation of the objectives of this thesis, and a brief description of the study area were the research cruise was carried.

The second chapter, the "Circulation patterns and decapod larvae distribution in a coastal upwelling ecosystem", analyses the spatial distribution of decapod larvae off the northwest Portuguese shelf. This chapter is in preparation for publication.

The third chapter, the "Ontogenetic vertical migration behaviour of decapod larvae in the Portuguese upwelling ecosystem", describes the ontogenetic vertical migration behaviour of decapod larvae on the western Iberia upwelling ecosystem. This chapter is also in preparation for publication.

The fourth chapter, "Shedding light over the larval genus *Eretmocaris* – morphological larval features of two closely related trans-isthmian *Lysmata* species using laboratory cultured material", the morphological larval description of the zoeal stages of the caridean shrimps of the genus *Lysmata* was made. This chapter was submitted to "Systematics and Biodiversity" for publication.

The fifth chapter, "Complete larval development of the hermit crabs *Clibanarius aequabilis* and *Clibanarius erythropus* (Decapoda: Anomura: Diogenidae), under laboratory conditions, with a revision of the larval features of genus *Clibanarius*", presents the complete morphological larval description of the hermit crabs of the genus *Clibanarius*. This chapter was published in *Helgoland Marine Research*.

The sixth chapter, "Complete larval development of the crab *Ilia nucleus* (Linnaeus, 1758) (Decapoda, Brachyura, Leucosiidae) reared under laboratory conditions", describes the complete morphological larval development of the brachyuran crab of the species *Ilia nucleus*. This chapter was published in *Scientia Marina*.

Finally in the seventh and last chapter the general discussion and conclusions are listed.

1.6- Study area

In the Portuguese coast, until the beginning of the 21st century, Queiroga (1996) and dos Santos (1999) were the only authors studying the decapods larval distribution over the continental shelf, so most of the studies considering the spatio-temporal distribution of planktonic larval stages of decapod crustaceans were carried in estuaries and nearshore zones (e.g. Flores et al. 2002, Almeida & Queiroga 2003) and generally had as objective the brachyuran larval stages being the exceptions Paula's works with Mira estuarine species (Paula 1987, 1989, 1993, 1998), dos Santos description of decapods occurring in the Portuguese coastal area (dos Santos 1999) and Pereira et al. (2000) description of the decapods larval fluxes at Aveiro coastal lagoon.

Queiroga (1996) studied the distribution and drift of the crab larvae of *Carcinus maenas* over the continental shelf off northern Portugal, demonstrating that the first zoea was clearly associated with the estuarine inlets, the older stages were dispersed progressively offshore, and the megalopal stage experienced an onshore transport. Sustained on these results a survey was conducted in May 2002 in the adjacent area to the Aveiro coastal lagoon, and several studies addressing the planktonic larval stages distribution resulted from the collected data (the most recent: dos Santos et al. 2008b, Moreno et al. 2009, Garrido et al. 2009).

The area adjacent to Aveiro coastal lagoon, the northwest coast of Portugal, is characterised by complex mesoscale variability and a strong seasonality (Peliz et al. 2002, Relvas et al. 2007). The Western Iberia Shelf is a seasonal upwelling system where the northerlies (upwelling favourable) are observed in summer (June to September), developing the typical upwelling phenomena like coastal jets and long filaments (e.g. Peliz et al., 2002). the presence

of a poleward current along the coast of northwestern Iberia, generally described as a slender poleward flow along the upper slope/shelf break zone, carrying warm and salty waters in the upper 200- 300 m during autumn and winter was reported (Frouin et al. 1990, Haynes & Barton 1990). Peliz et al. (2003, 2005) showed that the Iberian Poleward Current (IPC) circulates along mid-latitude western and northern Iberia continental margins, driven by density forcing associated with larger-scale meridional thermal gradients, and is intensified during the winter. A large portion of land runoff from Iberian Peninsula is directed to the north of Lisbon shelf zone contributing to a fresh water input to the shelf, and generating a low salinity lens termed the Western Iberia Buoyant Plume (WIBP, Peliz et al. 2002, Santos et al. 2004). The joint effect of both these features, the Western Iberia Buoyant Plume (WIBP) and the Iberian Poleward Current (IPC) together with the alternating downwelling and upwelling episodes, produces extremely variable circulation patterns at short time scales in which the plankton distributes.

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CHAPTER 2

Circulation patterns and decapod larvae distribution in a coastal upwelling ecosystem*

*****Bartilotti C., Castro M., Santos A. M. P., Peliz Á., Queiroga H., dos Santos A. (*in prep.*)

Circulation patterns and decapod larvae distribution in a coastal upwelling ecosystem.

2.1- Abstract

Spatial distribution of decapod larvae off the northwest Portuguese shelf was analysed. Two grid samplings were performed: a first one to characterise the hydrology and circulation of the area and a second one addressing physical and biological parameters. Decapod larvae were associated with upwelled waters and with a deeper intrusion of warmer offshore waters and were separated in three groups. The "Inner Shelf Species" larvae, *Diogenes pugilator* and *Necora puber*, were concentrated near the shore along a band 8 km off the coastline. The "Shelf Species" larvae, *Eualus occultus*, *Processa nouveli*, *Pandalina brevirostris*, *Philocheras bispinosus*, *Callianassa subterranea*, *Upogebia pusilla*, *Anapagurus* spp., *Atelecyclus rotundatus*, *Liocarcinus* spp. and *Polybius henslowii* , were concentrated along meridionally elongated patches parallel to the coast inshore of the 200 m isobath 8- to 40 km far from the coastline. Finally the "Slope Species" larvae, *Solenocera membranacea*, *Parthenope* spp. and *Goneplax rhomboides* were concentrated along the 200 m isobath, in the vicinity of the shelf break, 56 km far from the coastline. These results confirm previous findings, that decapod larvae are retained in the Portuguese continental shelf and the self recruitment for parental populations is the most probable scenario even under upwelling conditions. The decapod larval distribution was modelled in a two steps approach, having 4 explanatory variables. The Inner Shelf species always had their larval distributions positively related with the distance to the coast and with the temperature. The Shelf species due to their wide distribution over the shelf showed more variable responses that were in accordance with the distribution ranges of the adults. The Slope species distributions were negatively related with distance to the coast and with the temperature, and never were affected by the Western Iberia Buoyant Plume (WIBP).

2.2- Introduction

Many marine organisms have complex life cycles with a planktonic larval phase. For most of these the larval phase is the dominant dispersal stage (e.g. Thorson 1950). It has been assumed that during this phase, the larval stages were transported to great distances and were widely dispersed (e.g. Roughgarden et al. 1988). As a consequence, much of the past studies considering larval dispersal supposed that the coastal benthic populations were open and highly "connected" through larval transport (e.g. Swearer et al. 2002). The idea of mechanisms leading to closed populations, in which "self-seeding" occurs at local spatial scales is quite recent (e.g. Swearer et al. 2002, Levin 2006).

The population connectivity is a bio-physical phenomenon since it results of the combination of the physical transport and dispersion, with the biological processes such as the larval behaviour (e.g. Gawarkiewicz et al. 2007, Cowen & Sponaugle 2009). Decapod crustacean larvae are active swimmers that can dynamically modify their vertical position in the water column, controlling the extent range and direction of their dispersal (Queiroga & Blanton 2005). The understanding of temporal and spatial larval movement patterns is fundamental to study decapods ecology leading to the design of effective conservation and resource management strategies (e.g. Pittman & McAlpine 2001, Mace & Morgan 2006).

It is known that the transport processes are the critical components of the supply mechanisms of larvae to habitats for settlement and juvenile development (e.g. Queiroga et al. 2006, 2007), separating the larval development in time (development through stages) and in space (transport in a three-dimensional fluid habitat), exposing the larvae to different mortality factors (Queiroga & Blanton 2005). To completely understand the larval transport and

settlement locations it is fundamental to have a good description of the local hidrography and of the three-dimensional current fields, as well as to describe the growth, survival and behavioural responses of larvae to their environment (e.g. Botsford et al. 1994). The study area, in front of Aveiro coastal lagoon, northwest coast of Portugal, is characterised by complex mesoscale variability and a strong seasonality (Peliz et al. 2002, Relvas et al. 2007). Frouin et al. (1990) and Haynes & Barton (1990) reported the presence of a poleward current along the coast of northwestern Iberia, generally described as a slender poleward flow along the upper slope/shelf break zone, carrying warm and salty waters in the upper 200- 300 m during autumn and winter. Peliz et al. (2003, 2005) showed that the Iberian Poleward Current (IPC) circulates along mid-latitude western and northern Iberia continental margins, driven by density forcing associated with larger-scale meridional thermal gradients, and is intensified during winter. The river runoff is significant to the northern region of Lisbon contributing to a fresh water input to the shelf, generating a low salinity lens named Western Iberia Buoyant Plume (WIBP) (Peliz et al. 2002, Santos et al. 2004).

Dos Santos et al. (2008) studied the decapod larvae three-dimensional distribution and discussed their results with the previous modelling studies for the area (Marta-Almeida et al. 2006, Peliz et al. 2007), confirming the assumptions made by both models that larval dispersal in the inner and middle shelf is mainly alongshore and that their retention greatly depends on their diel vertical migration behaviour. Furthermore, they hypothesised that the horizontal distribution patterns of several decapod species retained over the shelf were related to their presumable settlement areas. The apparent retention of decapod larvae close to parental populations was recently discussed by Morgan et al. (2009) and is now almost an evidence for invertebrate larvae.

We pretend to test the hypothesis that all decapod crustacean larvae distribution, in the studied coastal upwelling ecosystem, clearly shows a retention strategy in accordance with the adults' distributional ranges. We believe that the larvae will be distributed along meridional patches of larvae according to their origins: one, very close to the shore, will include the inner shelf species larvae; another, somewhere in the middle shelf, will concentrate the shelf species larval stages; and a last one, will appear over the continental shelf break, and will concentrate the shelf slope species. To verify our hypothesis we analysed the relationships between the larval distributions of 15 selected taxa with the physical environment in the Western Iberia upwelling ecosystem.

2.3- Material and Methods

2.3.1- Field study

An oceanographic survey was carried out off the northwest coast of Portugal, aboard the RV 'Noruega', from 9-22 May 2002 (Fig. 1).

In the beginning of the survey a mooring array with 3 current meters (Aanderaa RCM 9) and 2 conductivity-temperature-depth (CTD) recorders (Sea-Bird Electronics MicroCats) was deployed on the Portuguese continental shelf (40º 45.9' N 08º 59.0' W), approximately 21 km offshore and at 60 m bottom depth (Fig. 1). In order to obtain a quasi-synoptic view of the mesoscale circulation patterns before the biological sampling, a first grid of CTD stations was also carried out, along 4 transects perpendicular to the coast from 11 to 14 May 2002 (Fig. 1). Temperature, salinity and chlorophyll *a* (chl *a*) concentration were registered in each station with a CTD SBE 9plus (S/N 19860-0530) with a Seapoint fluorometer attached.

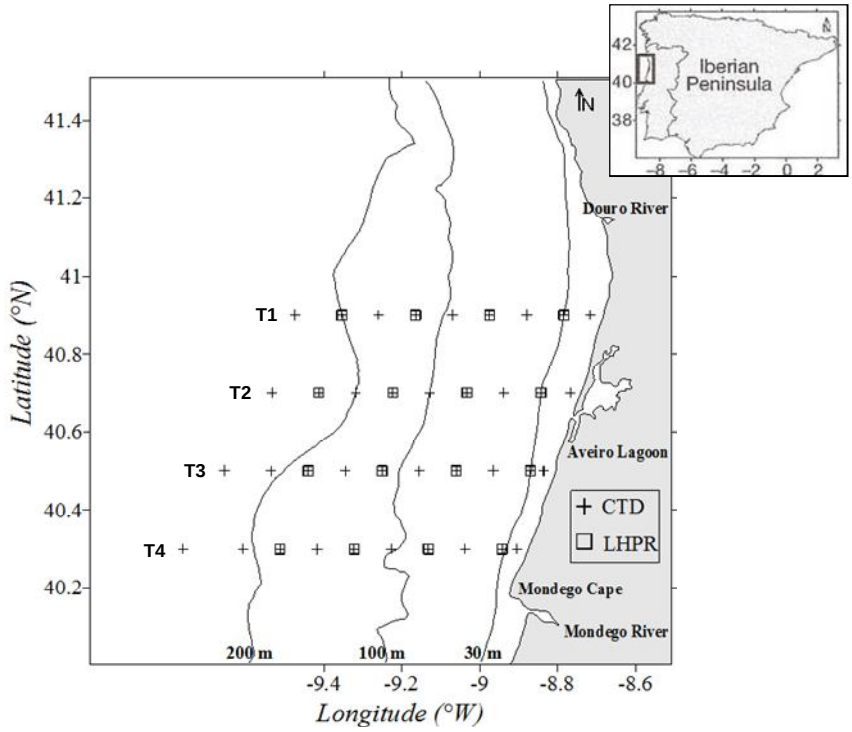

Figure 1- Map of the northwest coast of Portugal showing sampling positions (CTD: conductivity, temperature and depth profiler; and LHPR: Longhurst Hardy plankton recorder) collected aboard the RV *Noruega*, during the ProRecruit project survey. Transects are identified as T1, T2, T3 and T4; the squares represent the stations sampled with the Pro-LHPR. The 30, 100 and 200 m bathymetric lines are also presented.

From 15-17 May, in a total of 60 h, along the same 4 transects perpendicular to the coast (Fig.1), the plankton sampling was carried out in a 16 stations grid, with a Pro-LHPR system, a commercially updated version of the Longhurst Hardy Plankton Recorder (Williams et al. 1983). Each transect (T1 to T4) had 4 stations, separated by approximately 16 km, being the first station in each transect 8 km far from the shore (Fig. 1). Transect T1 was the most northern, and T4 the most southern located one. The Pro-LHPR net had a mouth aperture of 0.42 m, and collected plankton between two rolls of filtering gauze (280 µm mesh size) that are advanced at intervals inside a cod end box to give a series of consecutive samples. It was towed at 3-4 knots on oblique hauls from the surface to a maximum of 200 m depth. Stratified samples of Pro-LHPR were collected at 5 m depth intervals in the first 25 m, at 10 m depth

intervals from 25 m deep until 65 m, and for the deeper stations points, at 20 m depth intervals from 65 m until near the bottom.

2.3.2- Sample processing

Plankton samples were preserved in 4% borax-buffered formaldehyde, prepared with seawater. Samples were sorted for decapod larvae, that were identified to species level and developmental stage whenever possible using dos Santos & Lindley (2001), González-Gordillo et al. (2001), and dos Santos & González-Gordillo (2004). Data were standardized for ind.m^{-3}.

2.3.3- Analysis of decapod larval distribution in relation to the physical environment

The 15 studied taxa were selected having as criterions, the most abundant in the samples and, whenever possible, the presence of the complete larval series; within the most abundant we chose species belonging to different decapod groups, Dendrobranchiata shrimps, caridean shrimps, scavengers, hermit crabs and crabs and the distributional range of the adults, inhabitants of Inner Shelf, Shelf and Slope, according to Zariquiey-Álvarez (1968) and d'Udekem d'Acoz (1999) (Table 1).

Diogenes pugilator and *Necora puber* were considered as Inner Shelf species since both are coastal species inhabiting the intertidal area when adults. According to Zariquiey-Álvarez (1968) and d'Udekem d'Acoz (1999) the first species is distributed up to 35 to 40 m depth and the second has their higher abundances up to 15 m depth. Most of the selected taxa were considered Shelf species. When adults, *Eualus occultus* is distributed to a maximum of 150 m, *Processa nouveli* is distributed between 10 and 330 m, *Pandalina brevirostris* up to 180 m and *Philocheras bispinosus* up to 130 m. *Callianassa subterranea* is usually present between 35 and 500 m, and *Upogebia* spp. is distributed to a maximum of 65 m. Most of the

Anapagurus spp. species in the Portuguese west coast are distributed to a maximum of 200 m depth, *Atelecyclus rotundatus* is distributed between 9 and 100 m depth, *Liocarcinus* spp. is normally distributed in the 50 to 100 m depth band but can reach the upper slope, and finally, *Polybius henslowii* a pelagic crab found from 2 to 518 m (Zariquiey-Álvarez 1968, d'Udekem d'Acoz 1999). The Slope species included *Solenocera membranacea*, *Parthenope* spp. and *Goneplax rhomboides*, species that inhabit the continental shelf but also the continental shelf break or slope, and are common beyond the 400 m depths (Zariquiey-Álvarez 1968, d'Udekem d'Acoz 1999).

Table 1- List of the selected taxa, respective decapod crustacean group, distribution range when adult, and the grouping of the larval stages.

Taxa	**Decapod group**	**Distribution range**	**Larval group**		
			ZN	**ZO**	**M**
Solenocera membranacea	Shrimps	Slope	Z II- III	Z IV- VI	-
Eualus occultus	Caridean shrimps	Shelf	Z I- IV	Z V- IX	M
Processa nouveli	Caridean shrimps	Shelf	Z I- IV	Z V- IX	M
Pandalina brevirostris	Caridean shrimps	Shelf	Z I- III	Z IV- VII	M
Philocheras bispinosus	Caridean shrimps	Shelf	Z I- II	Z III- V	M
Callianassa subterranea	Scavengers	Shelf	Z I- II	Z III- IV	-
Upogebia spp.	Scavengers	Shelf	Z I- II	Z III- IV	-
Diogenes pugilator	Hermit crabs	Inner Shelf	Z I- II	Z III- IV	M
Anapagurus spp.	Hermit crabs	Shelf	Z I- II	Z III- IV	M
Parthenope spp.	Crabs	Slope	Z I- II	Z III- IV	-
Atelecyclus rotundatus	Crabs	Shelf	Z I- II	Z III- V	M
Liocarcinus spp.	Crabs	Shelf	Z I- II	Z III- V	M
Necora puber	Crabs	Inner Shelf	Z I- II	Z III- IV	M
Polybius henslowii	Crabs	Shelf	Z I- II	Z III- V	M
Goneplax rhomboides	Crabs	Slope	Z I- II	Z III- V	M

Zoea (Z) and Megalopa (M); New (N) and Old (O).

The larval stages were grouped in accordance with their ontogeny for each taxa, in order to reduce the zero values, improving the statistics. We considered three groups, the early zoeal stages or zoeae new (ZN), the later zoeal stages or zoeae old (ZO) and the megalopa or decapodid (M). Table 1 presents the chosen taxa, respective decapod crustacean group and distributional range of the adults, as well as the larval stages that were grouped.

Two response variables were considered: abundance (ind.m^{-3}) was log transformed, and presence/ absence (1/ 0) of a given taxa and larval group.

With respect to the explanatory variables, in a first phase of the exploratory data analysis, all environmental variables that were available and potentially useful to explain presence and/or abundance of larvae were investigated. They were:

- BOTTOM – depth of the station, continuous variable, in meters;
- DEPTH_CAT – Upper limit of the depth stratum, discrete variable, in meters;
- MAX_TOW_DEPTH – Maximum depth towed in the station, continuous variable, in meters;
- MID_DEPTH – Midpoint of depth stratum, discrete variable, in meters;
- DIST_COAST - distance to the coast, continuous variable, in km;
- REAL_TIME – Hour of the day, continuous variable, 0 to 24;
- HOUR1 – night and day, categorical variables with two levels, night (18:00-05:59) and day (06:00-17:59);
- HOUR2 – night, dawn, day and dusk, categorical variables with four levels, night (20:00-05:59), dawn (06:00-07:59), day (08:00-17:59) and dusk (18:00-19:59);

- HOUR_WAVE - hour of the day, continuous variable, defined as a circular function of the hour of the day, sunrise=0, noon=1, sunset=0 and midnight=-1, generated by the function:

$$sin\ [(Hour \times \frac{2\pi}{24}) + \frac{3\pi}{2}]$$

  where "Hour" is the hour of the day (0-24);
- WIBP - Western Iberia Buoyant Plume, binomial variable, representing the presence of a less saline continental water mass lens with salinities less than 35.75 (Peliz et al. 2002 for details);
- ISWM – Intermediate Salinity Water Mass, binomial variable, representing the presence of a water mass with salinity within the range [35.75,35.90[;
- IPC – Iberian Poleward Current, representing the presence of a high salinity water mass, with salinity greater than 35.9 (Peliz et al. 2003 for details);
- SAL – Salinity, continuous variable, no units;
- TEMP - temperature, continuous variable, in ºC.

Most of these variables were not significant in any of the studied cases (species and larval group) and were dropped from the models. The variables remaining and used in the construction of a reduced model were: the distance to the coast (DIST_COAST), the hour of the day (HOUR_WAVE), the temperature (TEMP) and the Western Iberia Buoyant Plume (WIBP).

The decapod larval distribution, in relation to environmental parameters, was analysed in two steps, for each species and larval group separately. The two steps approach is used to deal with the problem of zero inflated data (see Wenger & Freeman 2008). In the first step, the presence or absence was predicted using a Logistic Model (LM) with the following

explanatory variables: distance to the coast (DIST_COAST), hour of the day (HOUR_WAVE), temperature (TEMP) and Western Iberia Buoyant Plume (WIBP). The results from this model were used in the second step for situations where at least one of the variables was significant and for data points where "presence" was predicted. For these data points, measured values of abundance were modelled with a Generalized Addictive Model (GAM), using linear predictors for the distance to the coast (DIST_COAST), the hour of the day (HOUR_WAVE), the temperature (TEMP) and the Western Iberia Buoyant Plume (WIBP) and a spline for temperature (TEMP, with 2 degrees of freedom). Statistical analysis was made using SAS 9.2 (SAS Institute, 2008) using PROC LOGIT for fitting the logistic model (stepwise option) and PROC GAM for the generalized additive models.

2.4- Results

2.4.1- Oceanographic conditions

For the complete and detailed description of the oceanographic conditions during the survey see dos Santos et al. (2007). Before the plankton sampling the study area was under coastal upwelling favourable winds (northerlies in this region). As a consequence, the Western Iberia Buoyant Plume (WIBP), present in the first 25-30 m depth as a fresher water lens with salinities inferior to 35.75 (Fig. 2), had a considerably offshore displacement. However during the plankton sampling there was a relaxation of the upwelling due to the reversal of the wind direction. This change leads to coastal convergence conditions and to the appearance of a poleward current over the shelf.

Furthermore, the Iberian Poleward Current (IPC) was represented by the slope waters (Fig. 3) with salinities larger than 35.90 that invaded the shelf (Fig. 2). The appearance of a thermal

front separating the two northern transects (T1 and T2) from the two southern ones (T3 and T4) was also observed during the plankton sampling (see Fig. 2 representing the horizontal distribution of salinity).

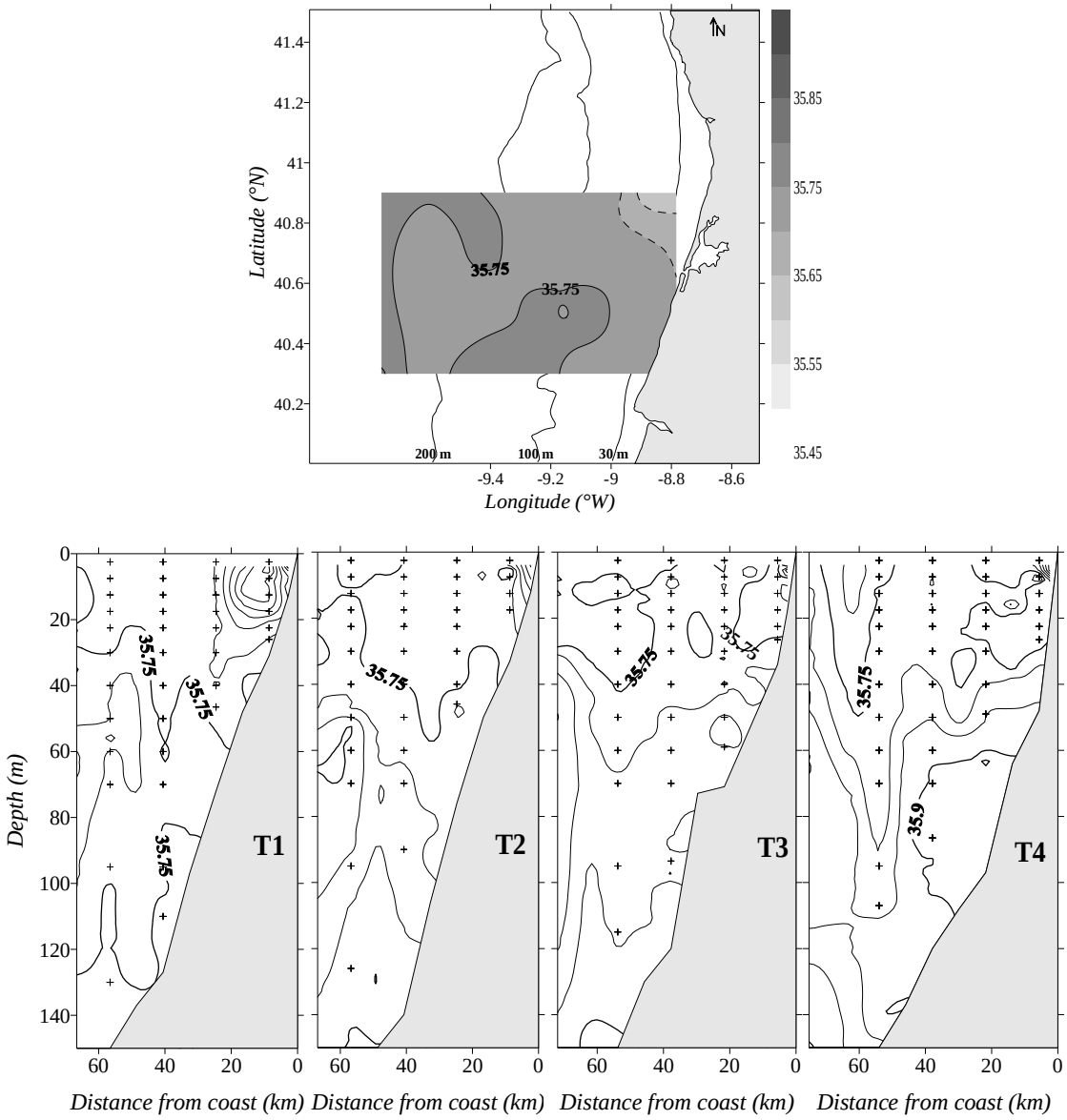

Figure 2- Horizontal (at 20 m depth) and vertical distributions of salinity measured with a CTD. T1, T2, T3 and T4 represent the four sampled transects (respectively 40.9º N, 40.7º N, 40.5º N and 40.3ºN). The 30, 100 and 200 m bathymetric lines are also identified. The horizontal and vertical distributions of salinity are represented by the solid lines with respective labels.

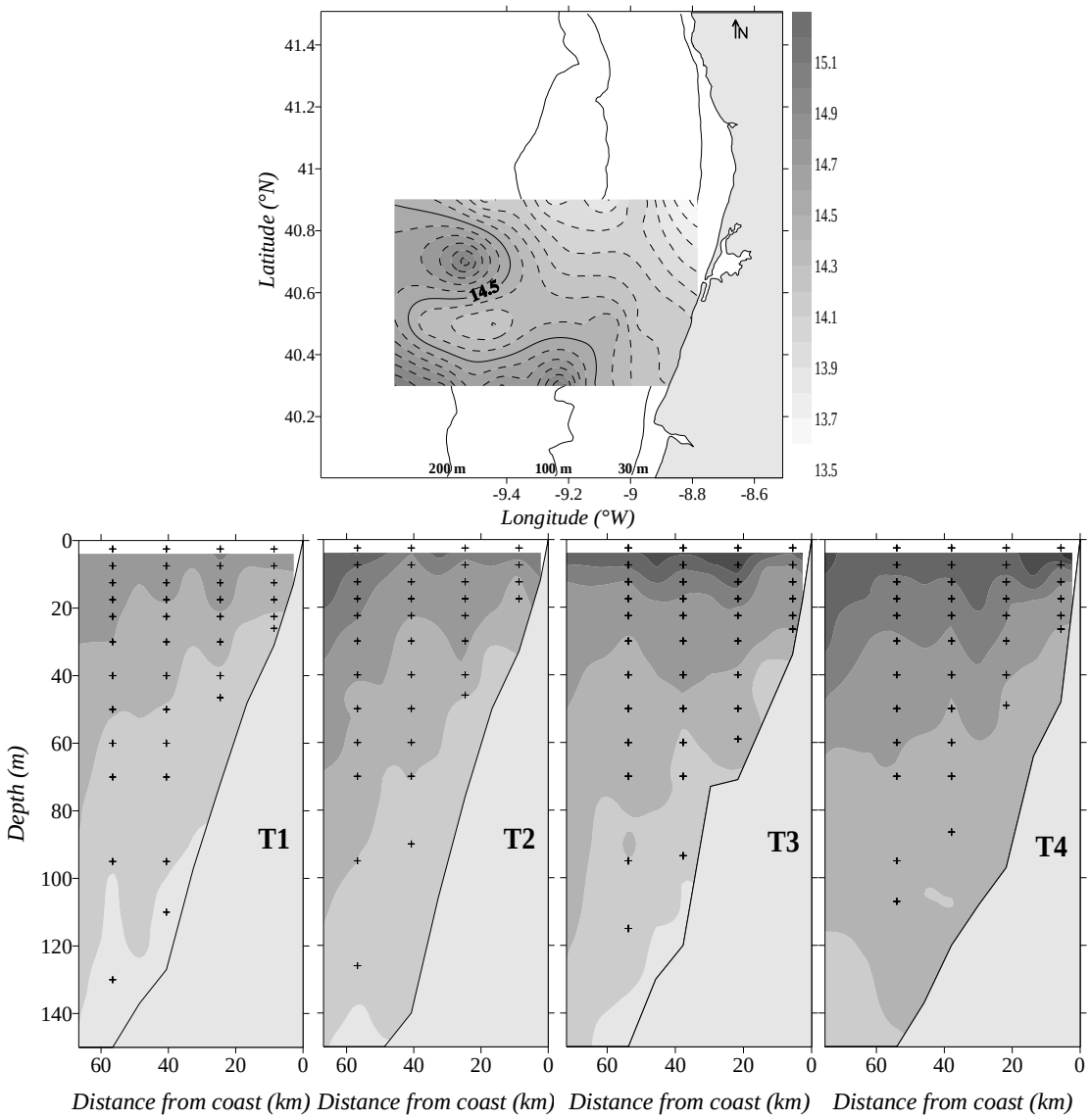

Figure 3- Horizontal (at 20 m depth) and vertical distributions of temperature (º C) measured with a CTD. T1, T2, T3 and T4 represent the four sampled transects (respectively 40.9º N, 40.7º N, 40.5º N and 40.3ºN). The 30, 100 and 200 m bathymetric lines are also identified. The horizontal and vertical distributions of temperature are represented by the grey areas with respective scale.

Surface salinity varied between 35.44 and 35.81 (Fig. 2). In the water column the minimum registered was 35.43 in the first transect (Fig. 2, T1), and the maximum registered was 35.96 in the fourth transect (Fig. 2, T4). The deeper intrusion of the more saline waters of the Iberian Poleward Current (IPC) is clear in the fourth transect (Fig. 2, T4) in the more deep sampled stations. Surface temperature varied between 14.08 and 16.26 ºC (Fig. 3) and

vertically the minimum registered was 12.63 ºC in the first transect (Fig. 3, T1) and the maximum registered was 16.29 ºC in the fourth transect (Fig. 3, T4).

2.4.2- Decapod larvae distribution

The zoeal stages were more abundant than the megalopae, and were more concentrated over the middle shelf (dos Santos et al. 2008). The horizontal and vertical larval distributions of the 15 selected taxa were analysed in detail. The selected planktonic larval stages were concentrated, as expected, in three different horizontal layers of the sampled area. Most of the species had their larvae concentrated along the middle shelf (*Eualus occultus*, *Processa nouveli*, *Pandalina brevirostris*, *Philocheras bispinosus*, *Callianassa subterranea*, *Upogebia* spp., *Anapagurus* spp., *Atelecyclus rotundatus*, *Liocarcinus* spp., *Polybius henslowii*), although there were also species with their higher abundances at the most exterior stations, and at the most coastal stations.

The two steps approach used for the decapod larval distribution modelling in relation to the environmental variables (Table 2) showed that the distance to coast (DIST_COAST) was the variable that explained the larval distribution of the majority of the studied larval stages, being negatively related with most of the zoeal presences of the Slope species, and positively related with the zoeal presences of the Inner Shelf species.

The Western Iberia Buoyant Plume (WIBP) was positively related with the presences of the early zoeal stages (ZN) and negatively related when explaining the presence of the megalopae (M) of some Shelf species.

Table 2- Coefficients of significant explanatory variables of the two steps model (presence or absence predicted using a Logistic Model in the first step, values of abundance modelled with a Generalized Addictive Model in the second step) describing the distribution of the decapod larvae selected taxa collected with the LHPR net in the horizontal grid of stations. Levels of significance are represented as ***p<0.001, **p<0.01, *p<0.05; n.s. not significant.

Species	Larval group	Model phases	n	Cofficients of significant explanatory variables				
				WIBP	HOUR_WAVE	DIST_COAST	TEMP (linear)	TEMP (spline)
Solenocera membranacea	Zoea New	presence	148	n.s.	0.641 *	-0.041 **	-1.601 ***	-
		abundance	34	n.s.	0.130 *	n.s.	n.s.	n.s.
	Zoea Old	presence	148	n.s.	n.s.	-0.051 **	n.s.	-
		abundance	-	-	-	-	-	-
Eualus occultus	Zoea New	presence	148	0.394 *	n.s.	n.s.	-1.290 ***	-
		abundance	20	n.s.	n.s.	n.s.	n.s.	n.s.
	Zoea Old	presence	148	n.s.	n.s.	n.s.	-0.837 **	-
		abundance	34	n.s.	n.s.	n.s.	n.s.	n.s.
	Megalopa	presence	148	n.s.	n.s.	0.102 **	1.717 **	-
		abundance	-	-	-	-	-	-
Processa nouveli	Zoea New	presence	148	0.646 **	0.823 **	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	Zoea Old	presence	148	0.564 **	1.535 ***	n.s.	n.s.	-
		abundance	17	n.s.	0.621 **	0.008 *	-0.025 *	n.s.
	Megalopa	presence	148	n.s.	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
Pandalina brevirostris	Zoea New	presence	148	0.573 *	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	Zoea Old	presence	148	n.s.	n.s.	n.s.	-1.110 ***	-
		abundance	41	0.348 **	n.s.	n.s.	n.s.	n.s.
	Megalopa	presence	148	n.s.	-2.253 **	n.s.	1.379 **	-
		abundance	-	-	-	-	-	-
Philocheras bispinosus	Zoea New	presence	148	n.s.	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	Zoea Old	presence	148	n.s.	n.s.	n.s.	-1.493 ***	-
		abundance	-	-	-	-	-	-
	Megalopa	presence	148	n.s.	-1.931 *	0.110 ***	n.s.	-
		abundance	12	n.s.	4.497 *	n.s.	-0.024 *	n.s.
Callianassa subterranea	Zoea New	presence	148	0.850 ***	n.s.	0.033 *	1.602 ***	-
		abundance	45	n.s.	n.s.	n.s.	n.s.	n.s.
	Zoea Old	presence	148	n.s.	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
***Upogebia* spp.**	Zoea New	presence	148	n.s.	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	Zoea Old	presence	148	-0.740 *	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-

Continued

Table 2 Continued

Species	Larval group	Model phases	n	Cofficients of significant explanatory variables				
				WIBP	HOUR_WAVE	DIST_COAST	TEMP (linear)	TEMP (spline)
Diogenes pugilator	Zoea New	presence	148	n.s.	n.s.	0.289 ***	n.s.	-
		abundance	18	n.s.	n.s.	n.s.	0.044 *	n.s.
	Zoea Old	presence	148	n.s.	n.s.	0.069 **	1.517 **	-
		abundance	-	-	-	-	-	-
	Megalopa	presence	148	n.s.	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
***Anapagurus* spp.**	Zoea New	presence	148	n.s.	-1.466 *	0.057 **	n.s.	-
		abundance	-	-	-	-	-	-
	Zoea Old	presence	148	n.s.	n.s.	n.s.	-0.627 *	-
		abundance	19	n.s.	n.s.	n.s.	n.s.	n.s.
	Megalopa	presence	148	n.s.	-1.466 *	0.057 **	n.s.	-
		abundance	12	n.s.	4.001 **	n.s.	-0.017 **	0.007
***Parthenope* sp.**	Zoea New	presence	148	n.s.	n.s.	-0.137 *	n.s.	-
		abundance	-	-	-	-	-	-
	Zoea Old	presence	148	n.s.	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
Atelecyclus rotundatus	Zoea New	presence	148	n.s.	-1.064 *	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	Zoea Old	presence	148	n.s.	0.829 ***	n.s.	-0.857 **	-
		abundance	85	n.s.	n.s.	-0.0039 *	n.s.	n.s.
	Megalopa	presence	148	-0.781 ***	n.s.	-0.049 ***	-1.557 ***	-
		abundance	36	n.s.	n.s.	n.s.	n.s.	0.044
***Liocarcinus* spp.**	Zoea New	presence	148	n.s.	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	Zoea Old	presence	148	n.s.	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	Megalopa	presence	148	-0.517 **	-0.560 *	n.s.	n.s.	-
		abundance	25	n.s.	0.604 *	n.s.	n.s.	n.s.
Necora puber	Zoea New	presence	148	n.s.	n.s.	0.079 ***	1.287 *	-
		abundance	-	-	-	-	-	-
	Zoea Old	presence	148	n.s.	n.s.	n.s.	1.470 **	-
		abundance	-	-	-	-	-	-
	Megalopa	presence	148	n.s.	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
Polybius henslowii	Zoea New	presence	148	n.s.	-1.017 ***	0.025 *	n.s.	-
		abundance	12	n.s.	n.s.	n.s.	n.s.	n.s.
	Zoea Old	presence	148	n.s.	n.s.	-0.050 ***	n.s.	-
		abundance	126	n.s.	n.s.	0.008 *	n.s.	n.s.
	Megalopa	presence	148	-0.591 **	-1.149 ***	-0.072 ***	n.s.	-
		abundance	68	-0.086 **	-0.070 ***	n.s.	n.s.	n.s.
Goneplax rhomboides	Zoea New	presence	148	n.s.	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	Zoea Old	presence	148	n.s.	n.s.	-0.065 ***	-1.536 ***	-
		abundance	22	n.s.	0.191 *	n.s.	n.s.	n.s.
	Megalopa	presence	148	n.s.	-1.154 *	n.s.	-3.505 ***	-
		abundance	-	-	-	-	-	-

The temperature (TEMP) was also an important factor for the presence of the Shelf species, and it was always negatively related with the larval stages distributions (ZN, ZO and M) except for *P. brevirostris* megalopae (M) and *C. subterranea* early zoeal stages (ZN). The Slope species, except *Parthenope* sp. that did not showed any relation with this predictor (n.s.), also had their presence negatively related with the temperature; the Inner Shelf species zoeal stages presences were positively related with this predictor.

Finally, the effect of the hour of the day (HOUR_WAVE) reflected the vertical migration behaviour previously described by dos Santos et al. (2008). This effect was significantly related with *S. membranacea* (ZN, presence and abundance), *P. nouveli* (ZN presence, ZO presence and abundance), *P. brevirostris* (M, presence), *P. bispinosus* (M, presence and abundance), *Anapagurrus* spp. (ZN, presence and M, presence and abundance), *A. rotundatus* (ZN and ZO, presences), *Liocarcinus* spp. (M, presence and abundance), *P. henslowii* (ZN presence and M, presence and abundance), and *G. rhomboides* (ZO, abundance and M, presence) larval distributions.

2.4.2.1- Horizontal and vertical distributions of the Inner Shelf species

The modelling results (Table 2) showed that both species early zoeal stages presences (ZN) and *D. pugilator* old zoea (ZO) were significantly and positively related with the distance to the coast (DIST_COAST). The temperature (TEMP) was always positively related with both species zoeal stages distribution, the early zoeae (ZN) abundance and old zoeae presence (ZO) of *D. pugilator* and the presence of *Necora puber* zoeal stages (ZN and ZO). The megalopa (M) distribution of both species was not significantly explained by any of the selected predictors.

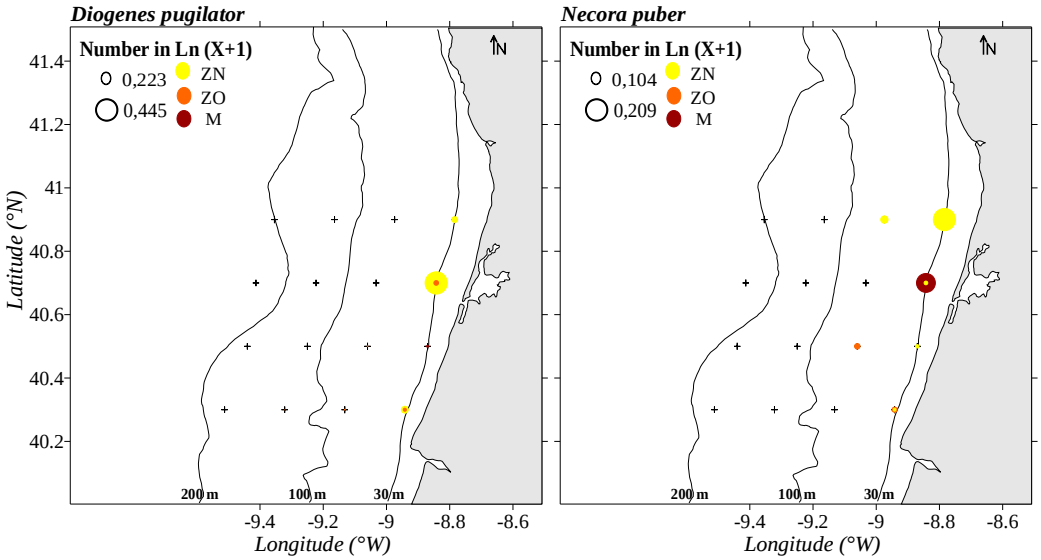

Figure 4- Horizontal distributions of *Diogenes pugilator* and *Necora puber*. The 30, 100 and 200 m bathymetric lines are identified. The abundances are represented in Ln (X+1) with X in ind.10 m^{-3}. ZN corresponds to the early zoeal stages, ZO to later zoeal stages, and M to megalopa.

Horizontally (Fig. 4), all the larval groups (ZN, ZO and M) occurred with higher larval abundances in the northern transects (T1 and T2), above the 30 m isobath, approximately 8 km far from the shore (Fig. 4).

Vertically, both species larval stages were distributed in the first 40 m of the water column (Fig. 5), concentrated in the buoyant plume characterized by a higher temperature and a lower salinity. The vertical migration behaviour was not evident, and none of the species had their distribution related with the hour of the day.

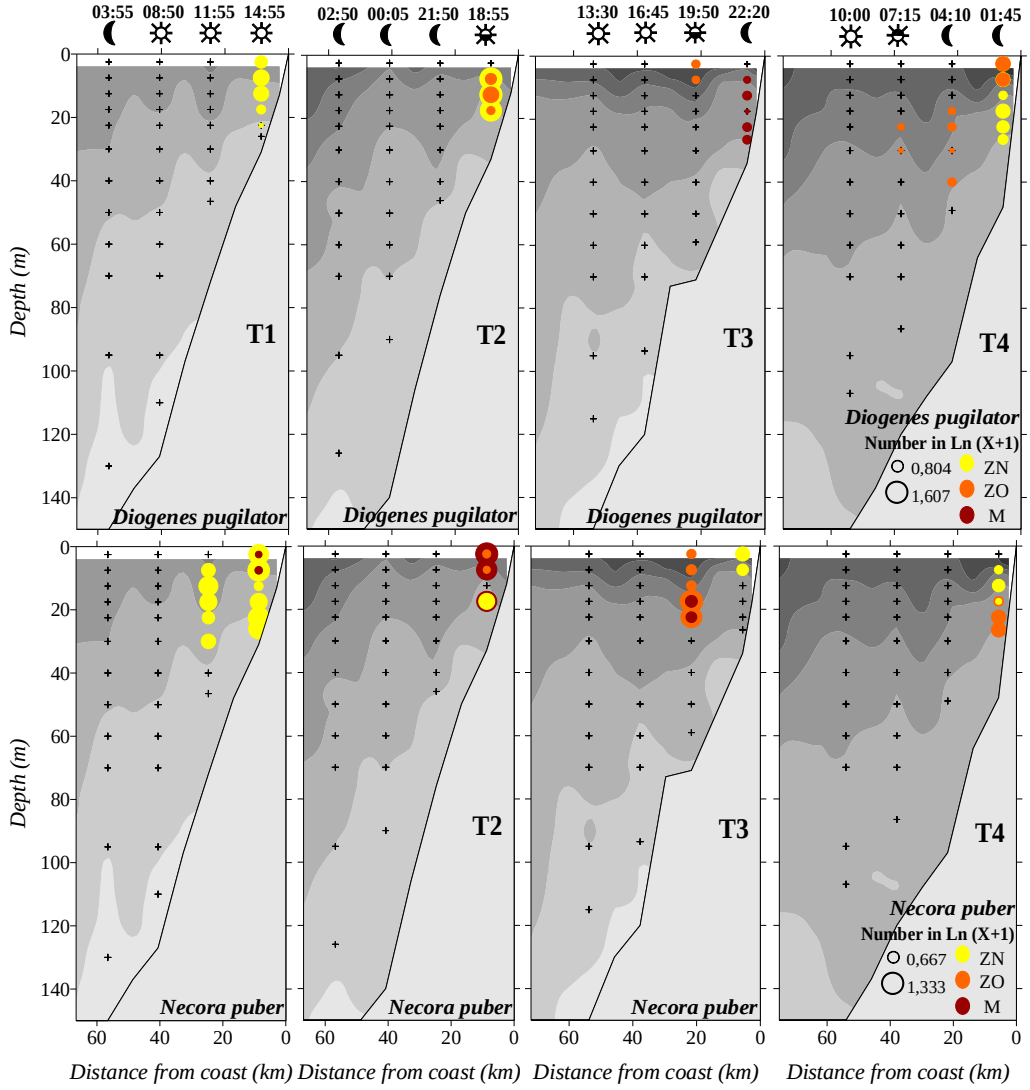

Figure 5- Vertical distributions of *Diogenes pugilator* and *Necora puber*. T1, T2, T3 and T4 represent the four sampled transects (respectively 40.9º N, 40.7º N, 40.5º N and 40.3ºN). The vertical distributions of temperature are represented by the grey areas (scales in Fig. 3). The night and day periods are represented by the moon and sun symbols, and the hours of the day are also represented above the night-day symbols. The abundances are represented in Ln (X+1) with X in ind.10 m^{-3}. ZN corresponds to the early zoeal stages, ZO to later zoeal stages, and M to megalopa.

2.4.2.2- Horizontal and vertical distributions of the Shelf Species

The larval distribution of the Shelf species reflected at least three different patterns.

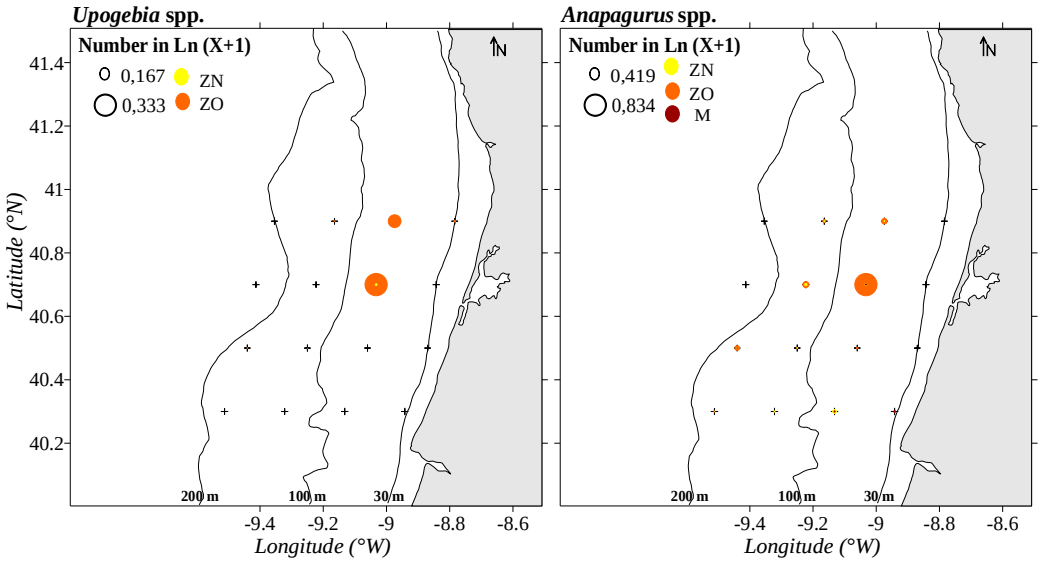

Figure 6- Horizontal distributions of *Upogebia spp. and Anapagurus spp.*. The 30, 100 and 200 m bathymetric lines are identified. The abundances are represented in Ln (X+1) with X in ind.10 m^{-3}. ZN corresponds to the early zoeal stages, ZO to later zoeal stages, and M to megalopa.

Upogebia spp. and *Anapagurus* spp. showed their higher abundances in the northern transects T1 and T2, inshore of the 100 m isobath, in a distance of approximately 20 km far from the shore (Fig. 6). For both species the old zoeal stages (ZO) were the most abundant.

However, the modelling results (Table 2) for the scavenger shrimp *Upogebia* spp. were only significant for the Western Iberia Buoyant Plume (WIBP) effect that was negatively related with the old zoeae (ZO) presence. On the contrary, *Anapagurus* spp. early zoeal stages (ZN) and megalopa (M) presences were significantly and positively related with the distance to the coast (DIST_COAST); the old zoeae (ZO) and the megalopa (M) had their presence and abundance, respectively, significantly and negatively related with the temperature (TEMP and also TEMP Spline for the megalopa).

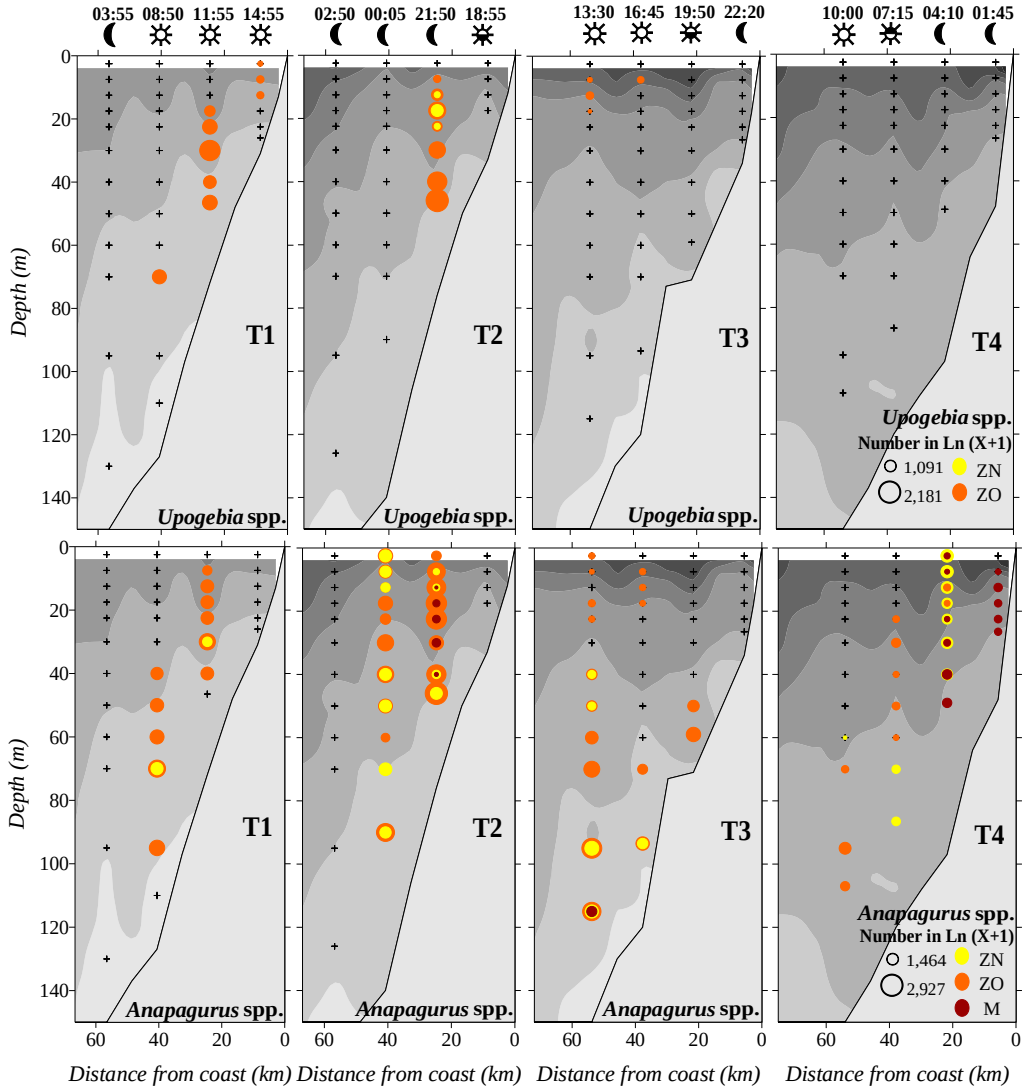

Figure 7- Vertical distributions of *Upogebia spp. and Anapagurus spp.*. T1, T2, T3 and T4 represent the four sampled transects (respectively 40.9º N, 40.7º N, 40.5º N and 40.3ºN). The vertical distributions of temperature are represented by the grey areas (scales in Fig. 3). The night and day periods are represented by the moon and sun symbols, and the hours of the day are also represented above the night-day symbols. The abundances are represented in Ln (X+1) with X in ind.10 m^{-3}. ZN corresponds to the early zoeal stages, ZO to later zoeal stages, and M to megalopa.

Vertically, both species larval stages were distributed from the surface to the 120 m depth (Fig. 7).

The *Processa nouveli* and *Callianassa subterranea* had their higher abundances in the southern transects T3 and T4, inshore of the 100 m isobath, in a distance of approximately 20 km far from the shore (Fig. 8). For both species the early zoeal stages (ZN) were the most abundant.

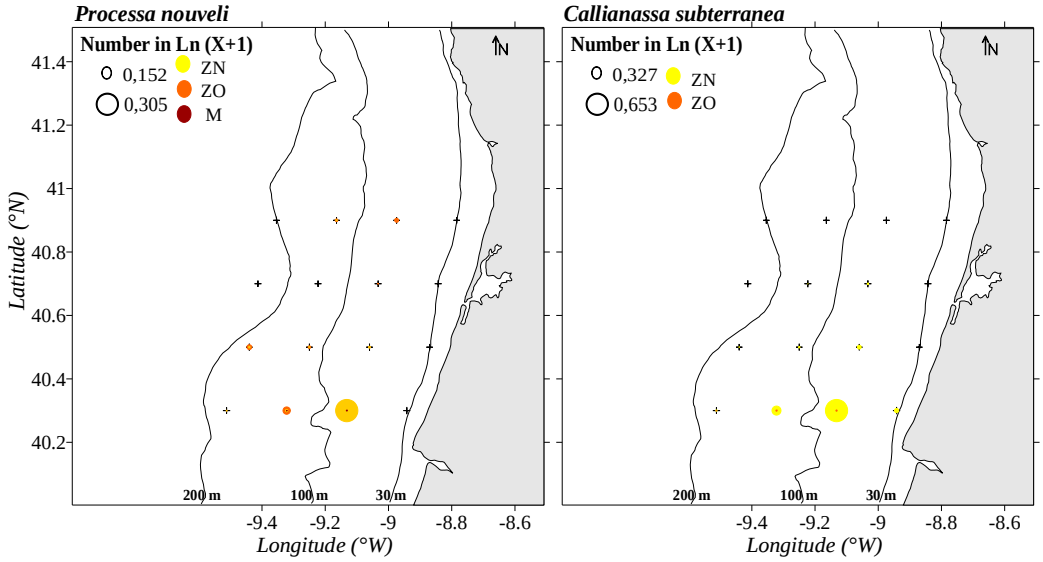

Figure 8- Horizontal distributions of *Processa nouveli and Callianassa subterranea*. The 30, 100 and 200 m bathymetric lines are identified. The abundances are represented in Ln (X+1) with X in ind.10 m^{-3}. ZN corresponds to the early zoeal stages, ZO to later zoeal stages, and M to megalopa.

The modelling results (Table 2) showed that both species zoeal stages distributions were significantly and positively related with the Western Iberia Buoyant Plume (WIBP, *P. nouveli* early and old zoeae and *C. subterranea* early zoeae presences), and with the distance to the coast (DIST_COAST, *P. nouveli* old zoeae abundance and *C. subterranea* early zoeae presence). The temperature (TEMP) influenced negatively the *P. nouveli* old zoeae (ZO) abundance, and positively the *C. subterranea* early zoeae (ZN) presence.

Vertically, both species larval stages were distributed from the surface to the 120 m depth (Fig. 9). *P. nouveli* megalopa (M) was exclusively sampled in the most southern transect (T4),

and during the night (at 04:10) it was more close to the surface than it was at the sunrise (at 07:15). *C. subterranea* was not collected in the northern transect T1, and the old zoeae (ZO) were only sampled in the most southern transect T4.

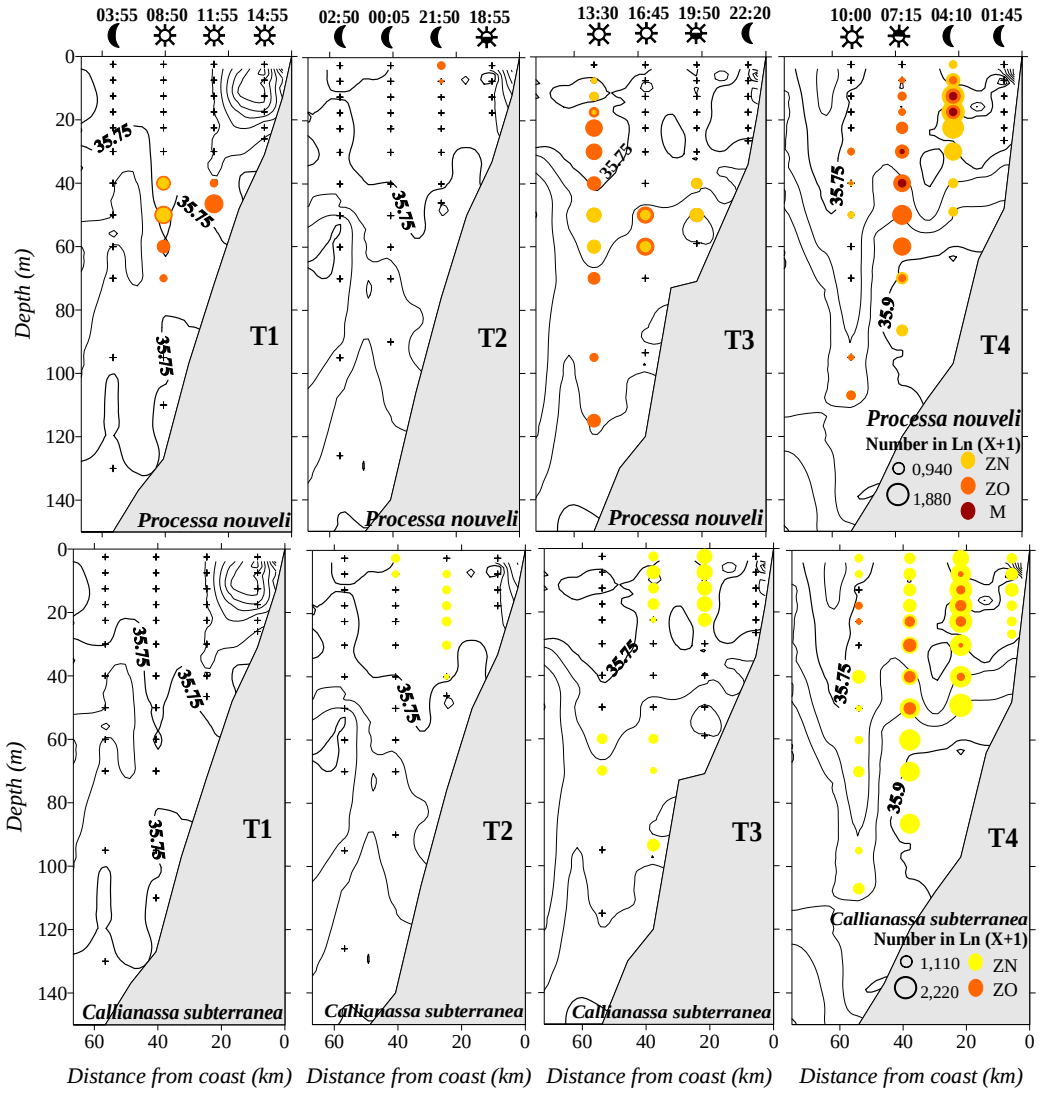

Figure 9- Vertical distributions of *Processa nouveli* and *Callianassa subterranea.* T1, T2, T3 and T4 represent the four sampled transects (respectively 40.9º N, 40.7º N, 40.5º N and 40.3ºN). The vertical distributions of temperature are represented by the grey areas (scales in Fig. 3). The night and day periods are represented by the moon and sun symbols, and the hours of the day are also represented above the night-day symbols. The abundances are represented in Ln (X+1) with X in ind.10 m^{-3}. ZN corresponds to the early zoeal stages, ZO to later zoeal stages, and M to megalopa.

The remaining 6 species (*Eualus occultus, Pandalina brevirostris, Philocheras bispinosus, Atelecyclus rotundatus, Liocarcinus* spp. and *Polybius henslowii*) presented a more or less extended distribution in the shelf (Fig. 10). For the brachyuran taxa the megalopal presences were always negatively related with the WIBP, whenever it explained this stage distribution. All the six taxa had their higher larval abundances along a band, inshore of the 200 m isobath, from 8 to 60 km far from the coast (Fig. 10), and the old zoeal stages (ZO) were the most abundant.

The modelling results for *E. occultus* and *P. brevirostris* (Table 2) were quite similar. Both species had their zoeal stages significantly and positively related with the Western Iberia Buoyant Plume (WIBP, *E. occultus* and *P. brevirostris* early zoeae presence, *P. brevirostris* old zoeae abundance). The temperature was significantly and negatively related with both species zoeal stages presence (*E. occultus* early and old zoeae, *P. brevirostris* old zoeae), and was significantly and positively related with both species megalopae presence. The distance to the coast (DIST_COAST) only explained the *E. occultus* megalopal presence. Vertically, both species larval stages were distributed from the surface to the 120 m depth (Fig. 11).

For *P. bispinosus* and *A. rotundatus* the temperature (TEMP) was significantly and negatively related with the old zoeae (ZO) presences and with the megalopal presence (*A. rotundatus*) and abundance (*P. bispinosus*, Table 2). The distance to the coast (DIST_COAST) and the hour of the day (HOUR_WAVE) were also explaining both species distribution. Vertically, both species larval stages were distributed from the surface to the 120 m depth (Fig. 12). The bathymetrical range distribution of the adults goes to a maximum of 100 m depth.

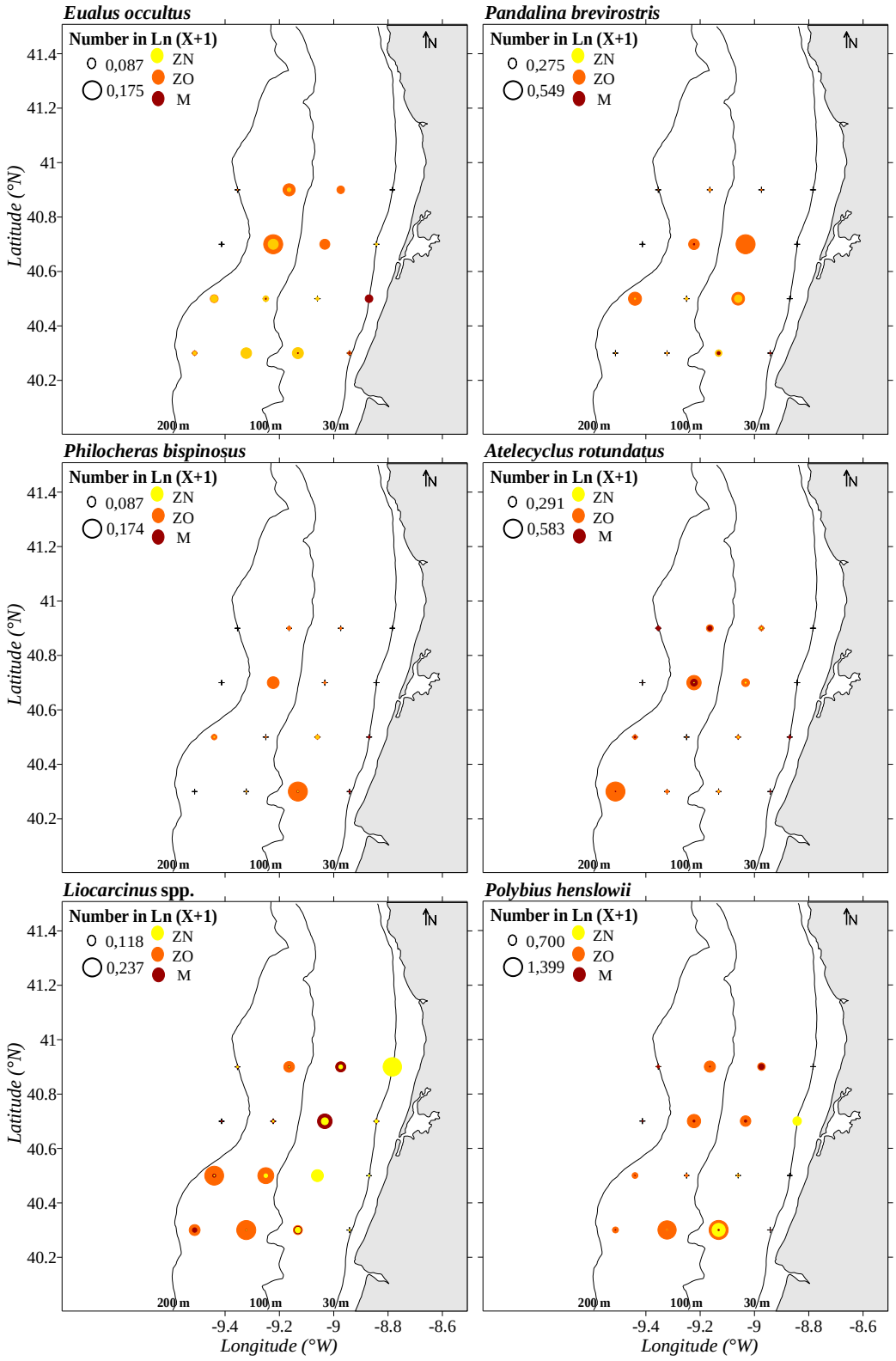

Figure 10- Horizontal distributions of *Eualus occultus*, *Pandalina brevirostris*, *Philocheras bispinosus*, *Atelecyclus rotundatus*, *Liocarcinus* spp. and *Polybius henslowii*. The 30, 100 and 200 m bathymetric lines are identified. The abundances are represented in Ln (X+1) with X in ind.10 m^{-3}. ZN corresponds to the early zoeal stages, ZO to later zoeal stages, and M to megalopa.

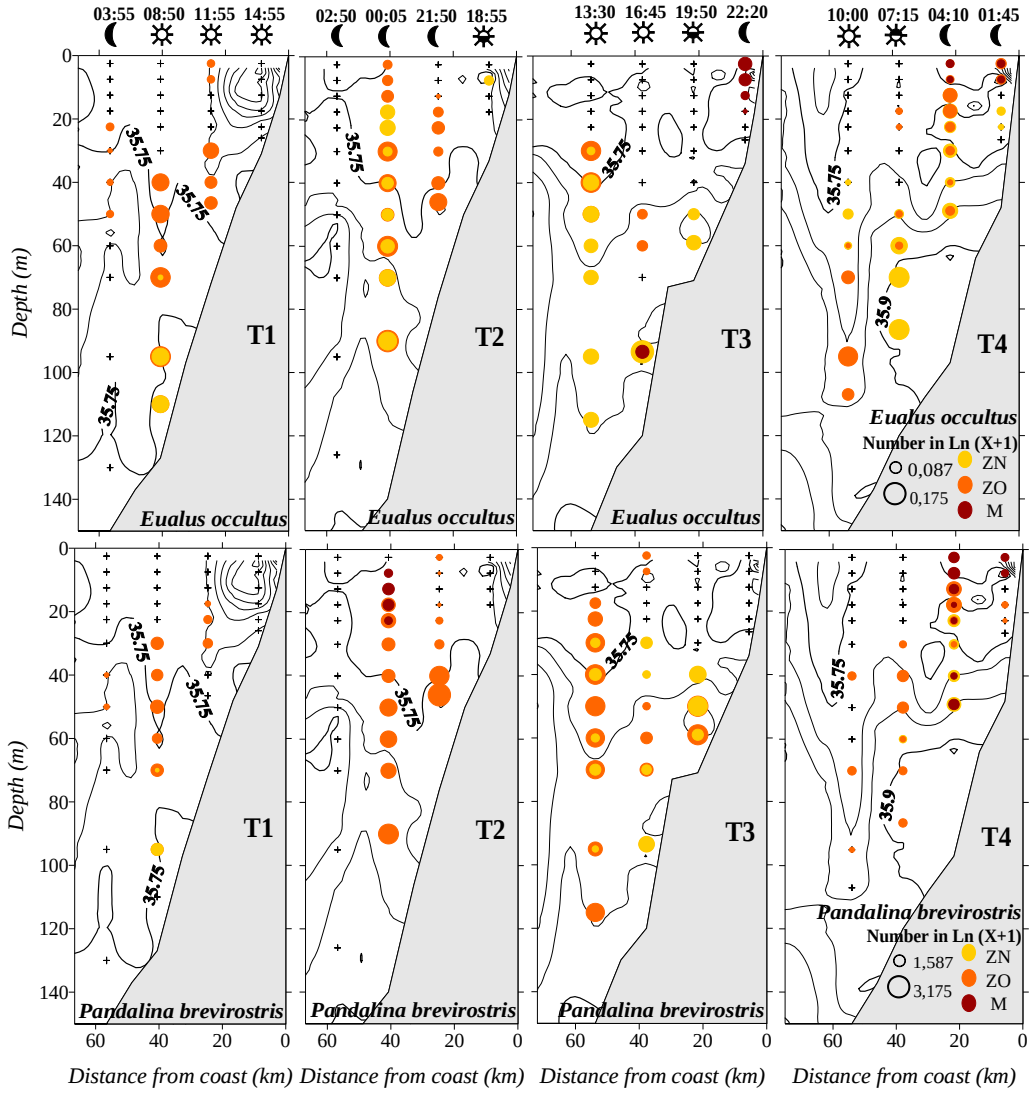

Figure 11- Vertical distributions of *Eualus occultus* and *Pandalina brevirostris*. T1, T2, T3 and T4 represent the four sampled transects (respectively 40.9º N, 40.7º N, 40.5º N and 40.3ºN). The vertical distributions of temperature are represented by the grey areas (scales in Fig. 3). The night and day periods are represented by the moon and sun symbols, and the hours of the day are also represented above the night-day symbols. The abundances are represented in Ln (X+1) with X in ind.10 m^{-3}. ZN corresponds to the early zoeal stages, ZO to later zoeal stages, and M to megalopa.

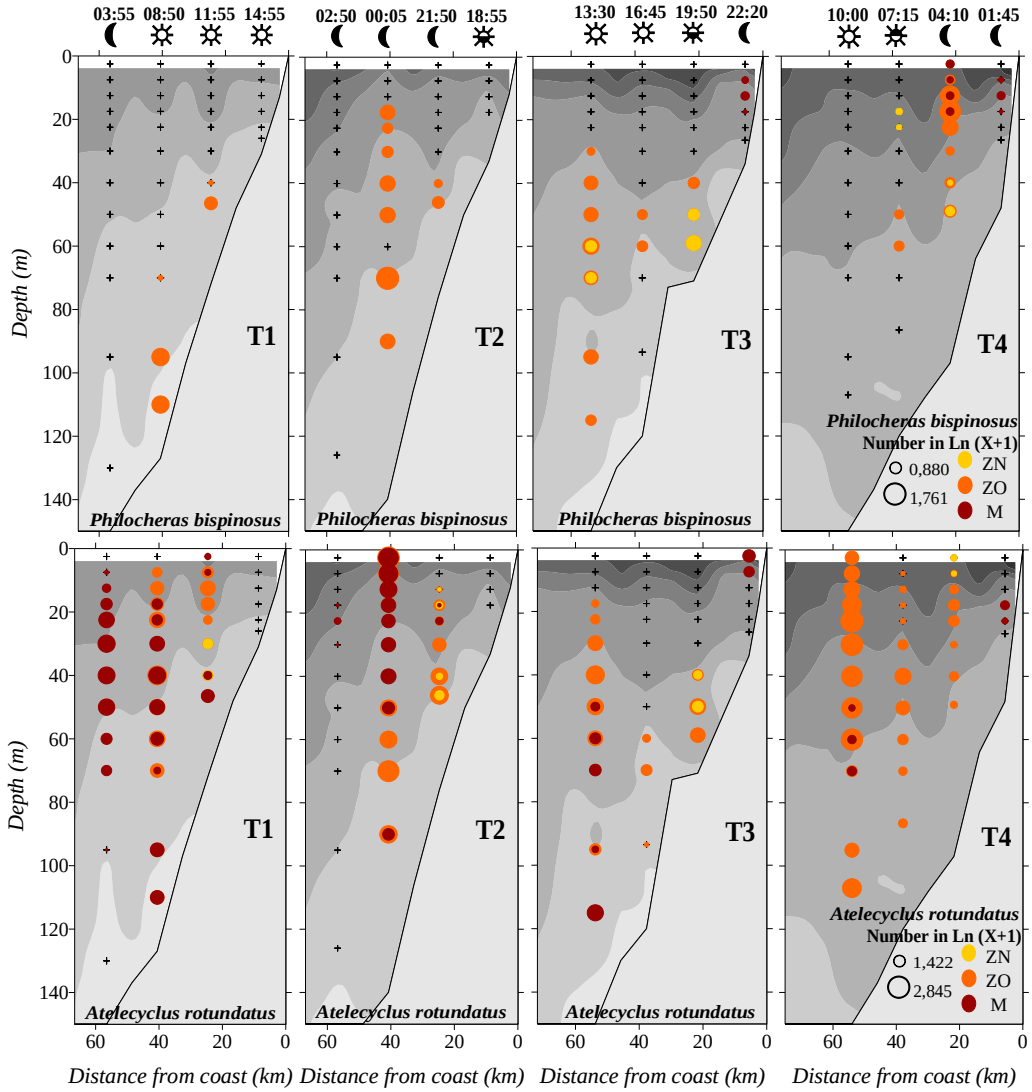

Figure 12- Vertical distributions of *Philocheras bispinosus* and *Atelecyclus rotundatus*. T1, T2, T3 and T4 represent the four sampled transects (respectively 40.9º N, 40.7º N, 40.5º N and 40.3ºN). The vertical distributions of temperature are represented by the grey areas (scales in Fig. 3). The night and day periods are represented by the moon and sun symbols, and the hours of the day are also represented above the night-day symbols. The abundances are represented in Ln (X+1) with X in ind.10 m^{-3}. ZN corresponds to the early zoeal stages, ZO to later zoeal stages, and M to megalopa.

For *Liocarcinus* spp. and *Polybius henslowii* the temperature (TEMP) was non significant probably because these larval stages were ubiquitous. *P. henslowii* zoeal and megalopal distributions were significantly related with the distance to the coast (DIST_COAST). This environmental predictor was not significant for *Liocarcinus* spp.. Finally the hour of the day

(HOUR_WAVE) was significantly related with the megalopal presence and abundance of both species (see Table 2 with the coefficients of significant explanatory variables values and signals). Both taxa larval stages were sampled in the more deep stations of the survey (Fig. 13).

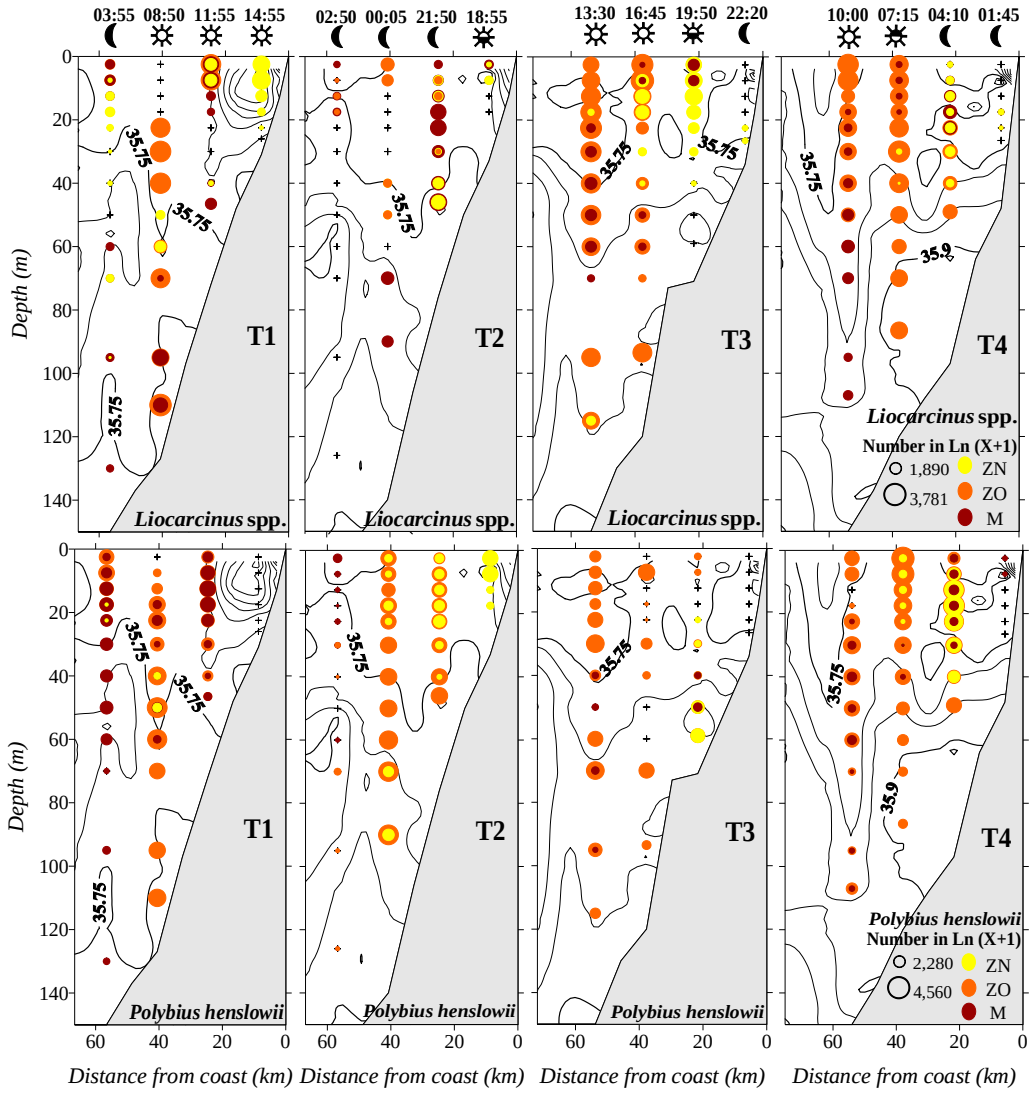

Figure 13- Vertical distributions of *Liocarcinus* spp. and *Polybius henslowii*. T1, T2, T3 and T4 represent the four sampled transects (respectively 40.9º N, 40.7º N, 40.5º N and 40.3ºN). The vertical distributions of temperature are represented by the grey areas (scales in Fig. 3). The night and day periods are represented by the moon and sun symbols, and the hours of the day are also represented above the night-day symbols. The abundances are represented in Ln (X+1) with X in ind.10 m^{-3}. ZN corresponds to the early zoeal stages, ZO to later zoeal stages, and M to megalopa.

2.4.2.3- Horizontal and vertical distributions of the Slope species

Horizontally (Fig. 14), *S. membranacea, Parthenope* spp. and *G. rhomboides* had their higher larval abundances registered in the southern transects (T3 and T4), offshore of the 100 m isobath, 40- 56 km far from the shore.

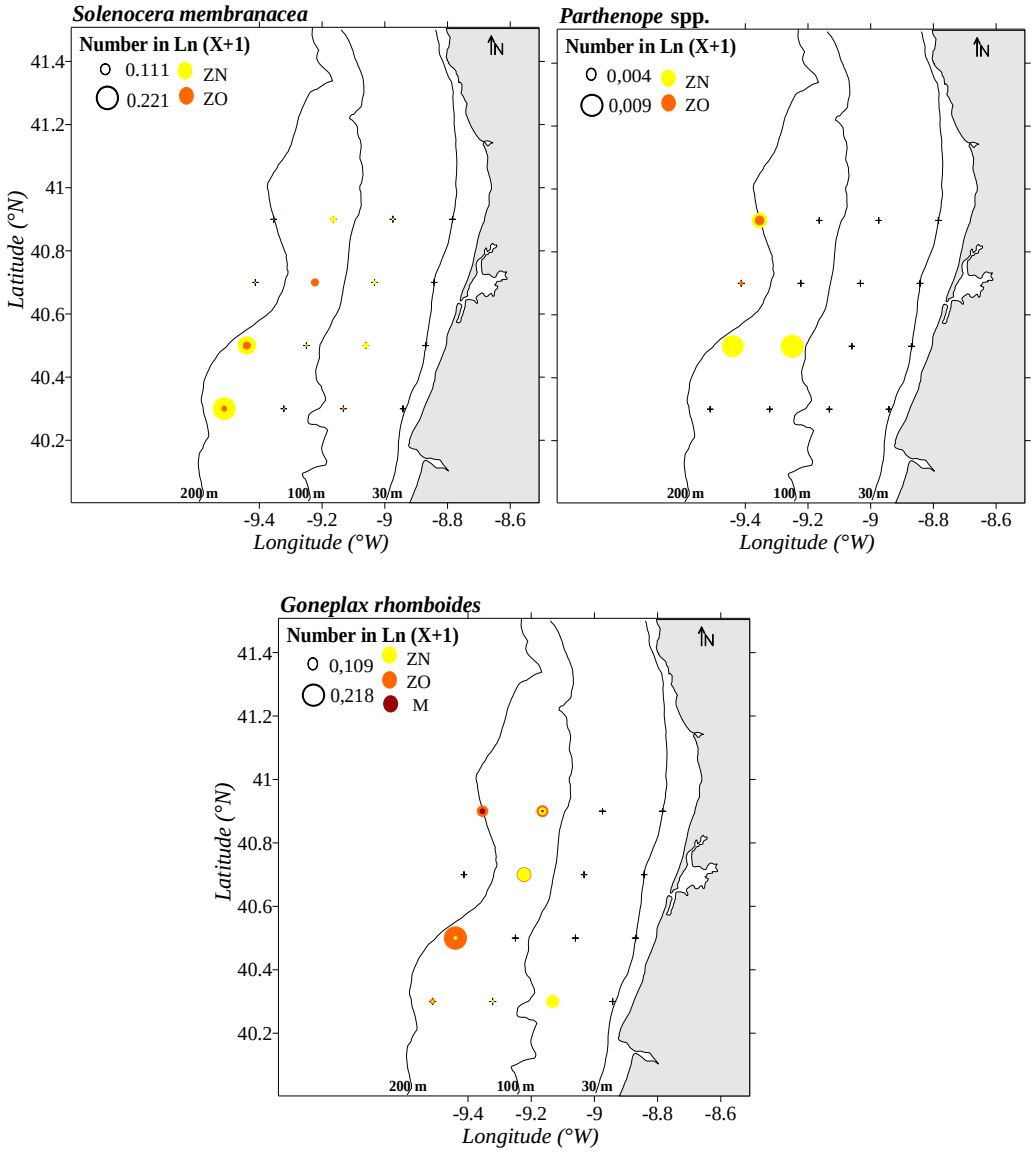

Figure 14- Horizontal distributions of *Solenocera membranacea, Parthenope spp. and Goneplax rhomboides.* The 30, 100 and 200 m bathymetric lines are identified. The abundances are represented in Ln (X+1) with X in ind.10 m^{-3}. ZN corresponds to the early zoeal stages, ZO to later zoeal stages, and M to megalopa.

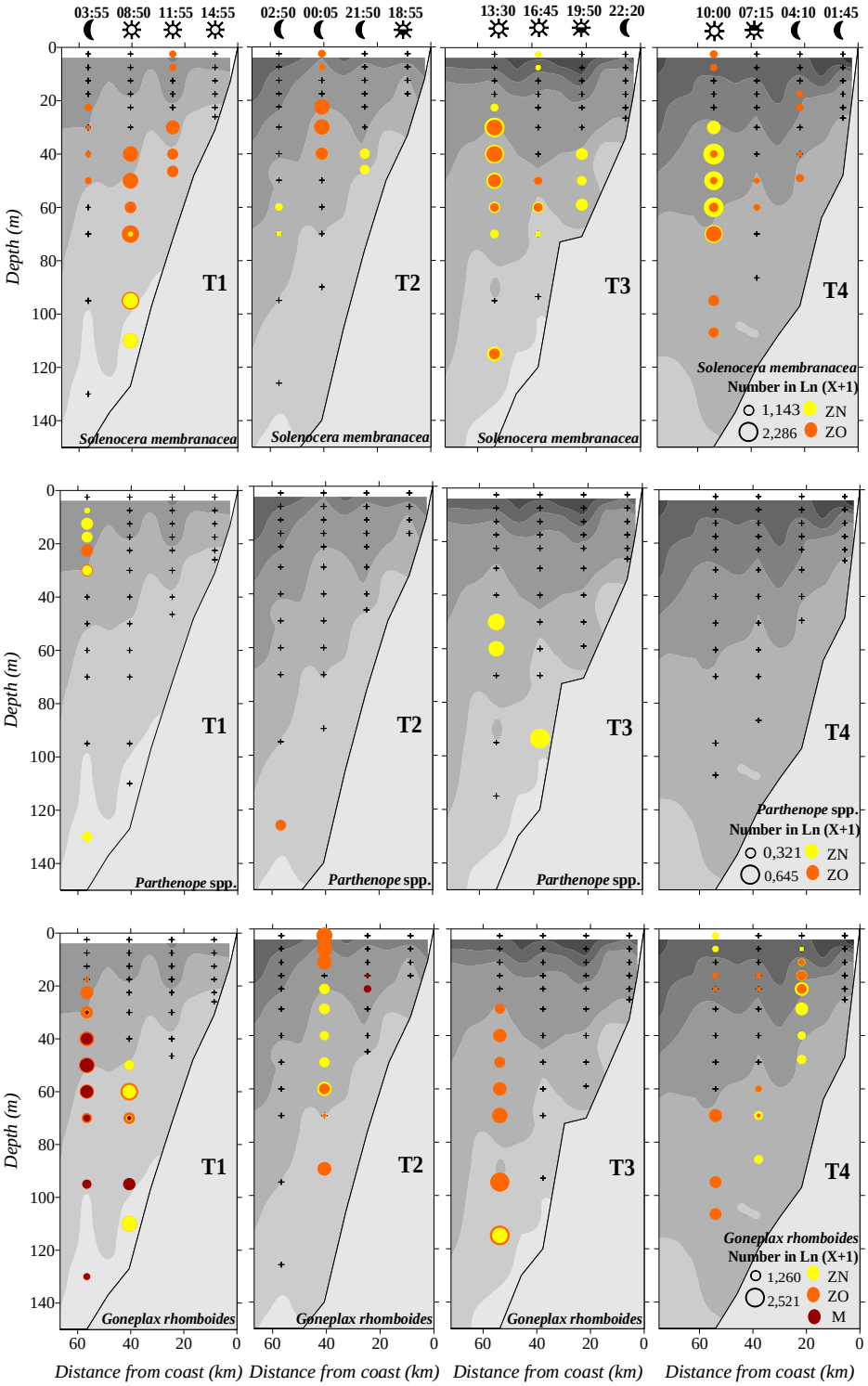

Figure 15- Vertical distributions of *S. membranacea*, *Parthenope* spp. and *G. rhomboides*. T1, T2, T3 and T4 represent the four sampled transects (respectively 40.9º N, 40.7º N, 40.5º N and 40.3ºN). The vertical distributions of temperature are represented by the grey areas (scales in Fig. 3). The night and day periods are represented by the moon and sun symbols, and the hours of the day are also represented above the night-day symbols. The abundances are represented in Ln (X+1) with X in ind.10 m^{-3}. ZN corresponds to the early zoeal stages, ZO to later zoeal stages, and M to megalopa.

The early zoeal stages (ZN) had their higher abundances in the southern transect T4 where an intrusion of slope waters was happening. The *S. membranacea* older zoeal stages (ZO) were also sampled in the northern transect, probably because of the larval advection in the upwelled water mass to northeast.

The modelling results (Table 2) showed that the three species zoeal stages presences (early zoeae for *S. membranacea* and *Parthenope* spp., and old zoeae for *S. membranacea* and *G. rhomboides*) were significantly and negatively related with the distance to the coast (DIST_COAST). As expected these species never had their distributions explained by the Western Iberia Buoyant Plume, once that they were distributed along the shelf break where the WIBP is not present. The three species zoeal stages were negatively related with the distance to the coast and with the temperature.

The Slope species larval stages presence was always negatively related with the temperature (TEMP). This environmental variable explained the early zoeae (ZN) of *S. membranacea* and *Parthenope* spp. and the old zoea (ZO) and megalopal stage (M) of *G. rhomboides* (the only species in the group with this stage). The Western Iberia Buoyant Plume never explained these species larval distributions.

Vertically, all the three species larval stages were distributed from the surface to close to the bottom (Fig. 15). The vertical migration behaviour is once again clear, although the hour of the day was only significantly related with *S. membranacea* early zoeal stages presence and abundance (both positive) and with *G. rhomboides* old zoeal stages abundance (positive) and megalopa presence (negative).

2.5- Discussion

Present results demonstrate that the decapod crustacean larvae distribution in the Portuguese continental shelf, clearly showed a retention strategy in accordance with the adults' distributional ranges as already hypothesized by dos Santos et al. (2008). During the survey, the larvae were distributed along meridional patches in accordance to their origins: the inner shelf species were distributed very close to the shore, the shelf species were distributed in the middle shelf, and the slope species appeared over the continental shelf break, thus, we can suppose that the self recruitment for parental populations is a probable scenario for these larvae even under upwelling conditions. The first evidence to support our hypothesis is the occurrence of the complete larval series for most of the studied species: when the early zoeal stages were found, the older zoeal stages and the megalopa were also sampled in the same area, many times in the same station. Present results disagree with Queiroga (1996) that described a different scenario for the distribution of *Carcinus maenas* larval stages. According to the author, the horizontal distribution of *C. maenas* reflected an ontogenetic pattern: the first zoea was associated with the estuarine inlets while the older zoeal stages were dispersed progressively offshore. As dos Santos et al. (2008) affirmed present results as well as archive data analysed by the authors positioned the *C. maenas* larvae inshore to the middle shelf position described before, and show the mixture of all larval stages in the same sampling stations. We agree with dos Santos et al. (2008) that pointed as justification for such differences a particular oceanographic situation that lead to an exceptional sampling period. Another evidence to support our theory is that the several larval forms studied (the shrimps sensu lato, the scavenger shrimps, the hermit crabs and the crabs) and their respective developmental stages (early and old zoeae, and megalopa) had similar behavioural responses to the selected environmental variables, and their distribution was along meridionally

elongated patches parallel to the coast inshore of the 200 m isobath as previously described in the study area for decapod larvae (dos Santos et al. 2008) and for cirripede cyprids (dos Santos et al. 2007). Present work agree well with Peliz et al. (2007) model results, therefore the larval transport in the study area is mainly alongshore and there is an aggregation along band-like meridional patches parallel to the coast usually inshore of the 100 m of the larvae.

We decided to model the distribution of the selected taxa in relation to four environmental predictors: the Western Iberia Buoyant Plume, the hour of the day, the distance to the coast and the temperature. The distance to the coast predictor was the one that explained most times the decapods larval distribution in the studied area. It was always positively related with the Inner Shelf species, and on the contrary, was always negatively related with the Slope species distribution. We can conclude that this predictor confirmed that the larvae are distributed in accordance to the adults: the species that were close to the coast were positively related with the distance to the coast and those that were far from the coastline were negatively related with it.

It is known that the temperature effect is important in decapod larval development, abundance and diversity (e.g. Anger, 2001). It was always positively related with the Inner Shelf species zoeal distribution, and when explaining the Slope species distributions was negatively related, probably because the Inner Shelf species were more coastal and were distributed in higher temperatures than the Slope species.

The effect of salinity, in particular the Western Iberia Buoyant Plume (WIBP) presence, contrary to what we were expecting never explained the Inner Shelf species distribution, and was only related with the Shelf species distribution, probably because during the survey the

WIBP was considerably displaced to offshore. The WIBP is a different water mass in the study area that brings stratification and stability to the surface layers, and as a consequence can constitute a retention mechanism that leads to a higher survival of fish larvae (Santos et al. 2006). We can assume that *E. occultus, P. nouveli, P. brevirostris* and *C. subterranea* zoeal distributions were always positively related with this less saline water lens because this is a more productive area and also a less turbulent water mass (Santos et al. 2006). Contrasting to the zoeal stages distributions, the crab megalopae (*A. rotundatus, Liocarcinus* spp. and *P. henslowii*) were negatively related with the WIBP, reflecting the avoidance of this less saline water lens by this stage.

Finally, the hour of the day gave information about the existence of diel vertical migrations. It was shown that in the absence of diel vertical migration the *Carcinus maenas* larval stages would be highly dispersed off the Western Iberia (Marta-Almeida et al. 2006), and that the larvae in the study area were retained over the shelf due to the presence of this mechanism (dos Santos et al. 2008). Present study also demonstrates that the diel vertical migration behaviour is clear for most of the sampled species: *P. nouveli, P. brevirostris, P. bispinosus, Anapagurus* spp., *Liocarcinus* spp., and *P. henslowii* megalopal stage, *Parthenope* spp. and *G. rhomboides* early zoeae, and *A. rotundatus* early zoeae and megalopa. Therefore, the larval retention mechanism in meridionally elongated bands parallel to the shore, more or less coincident with the adults distribution is in fact the result of the synchronized diel vertical migration behaviour of the larvae. This will maintain the larval patch close to their parental population, minimizing the horizontal larval excursion, enhancing the species recruitment (DiBacco et al. 2001).

As referred before, the very recent study of dos Santos et al. (2008) discussed the larval retention possibility for decapod larvae off the northwest Portuguese coast, affirming that larvae belonging to coastal species, as *Carcinus maenas* were mainly restricted to a 10 km-wide band along the coast, over bottom depths less than 30 m. Present study confirms that the Inner Shelf species were mainly restricted to a 10 km wide band along the coast, over bottom depths less than 30 m, even under upwelling events. These results also corroborate Shanks et al. (2002), Wing et al. (2003), Shanks & Eckert (2005). *D. pugilator* and *N. puber* zoeal distributions were positively related with the temperature predictor, probably because they were distributed close to the shore, above the depth of the thermocline, where the higher temperatures were registered.

The retention mechanisms in the coastal environments have been well described for estuarine species (e.g. Epifanio & Garvine 2001), although for shelf species it is almost unknown. Marta-Almeida et al. (2006), Peliz et al. (2007) and dos Santos et al. (2008) demonstrated that the retention strategies for the species more widely distributed in the continental shelf resulted of the diel vertical migration between water masses moving in different directions at different depths. Present study demonstrates that the Shelf Species are retained close to their parental populations, over the shelf near their release sites. Crossing the larval distribution results with the adults' spatial distribution (Zariquiey-Álvarez 1968, d'Udekem d'Acoz 1999), the larval presences reflected the place where the adults' populations were. During this study the Shelf species larvae showed three different distributional patterns: some species were more abundant in the north, others were more abundant in the south, and the third group includes those species that were widely distributed over the shelf.

The first two groups reflect the larval distribution response to the environmental variables, in particular to the thermal front and to the WIBP displacement. *Upogebia* spp. and *Anapagurus* spp. were concentrated in the northern transects, approximately 25 km off the shore, inshore of the 100 m isobaths and never had their distributions explained by the WIBP, while *P. nouveli* and *C. subterranea* the two more abundant in the southern transects, approximately 25 km off the shore, inshore of the 100 m isobath, were positively related with the WIBP. Probably this less saline water mass is the responsible for the larval retention over the shelf. In the WIBP is well-developed, the transport in the surface layers would be mainly offshore during upwelling, but the presence of the the IPC in the deeper stations of the southern transects creates a blocking effect to the offshore transport, generating a mechanism of larval retention (Santos et al. 2004).

All the other six species were widely distributed over the shelf and concentrated their higher abundances along the middle shelf. *E. occultus* and *P. brevirostris* abundances were mainly registered in a band 20 to 60 km off the coastline. Both species had their zoeal stages positively related with the WIBP and negatively related with the temperature that was positively related with both species megalopae. This difference in the temperature significance signal for the zoeal and megalopal stages may be reflecting different ontogenetic responses to the environment. Most likely, the distribution of the adults to a maximum of 180 m depth may be the reason why these species larval stages have such similar responses to the environmental predictors. Probably both species hatched in the same environment and migrate vertically in the shelf water column with similar buoyancy and stratification andas a consequence, the surface Ekman layer transport is similar for both species.

Also, *P. bispinosus* and *A. rotundatus* when adults are distributed to 100 m depth, had their higher larval abundances along the 20 to 60 km off band, and the temperature affected them in a similar way. Finally, *Liocarcinus* spp. and *Polybius henslowii* have higher distribution bathymetrical ranges when adults, and had their larvae widely distributed in the area from 8 to 60 km to the shore, with the megalopae similarly affected by the WIBP. The temperature did not affected these species distribution, probably when adults these species are almost everywhere (Zariquiey-Álvarez 1968, d'Udekem d'Acoz 1999), and as a consequence their larvae are ubiquitous and can hatch or settle in any place of the continental shelf.

We suppose that *S. membranacea*, *Parthenope* spp. and *G. rhomboides* were advected to the shelf, probably because here their survival will be enhanced due to higher food availability and certainly to favour their retention (e.g. Levin 2006). The absence of the megalopal stage may be because they were not present in the plankton for this time of year, or they were not in the shelf anymore once that they already started the return to the parental population where they will metamorphose and settle.

The larval horizontal transport will be determined by the interactive effect of the position of the larvae in the water column (vertical distribution) and the hydrodynamics (e.g. Sameoto & Metaxas 2008). The particular oceanographic conditions during the survey, lead to the appearance of a frontal structure. This frontal structure was perpendicular to the coastline and separated the two northern transects (T1 and T2) from the two southern ones (T3 and T4), concentrating the decapod larvae along the shelf (dos Santos et al. 2008). Analysing the general distribution of the considered taxa, it is possible to state that the Shelf species larval distribution reflects the transport in the upwelled water mass, which was advected for the northern transects (a higher presence of old zoeae in transects T1 and T2). Probably, during

the south-north dislocation of this water mass, the early zoeal stages were transported continuing to developed, and completing their ontogenetic cycle. As a result, these larvae reached a northern position older. The examples of species that might be experiencing this transport are: *Solenocera membranacea, Eualus occultus, Pandalina brevirostris* and *Anapagurus* spp.. Based on dos Santos et al. (2008) data, as well as on the behavioural reactions described in the literature and described in present study, we agree with Queiroga et al. (2007) proving that in general decapod zoeae and megalopae are not trapped at frontal convergence zones as described before (e.g. Shanks et al. 2000). Decapod larval diel vertical migration behaviour conjunct with the local wind-driven circulation and the coastline orientation, are capable of keep their distribution along meridionally elongated bands coincident with the adults bathymetric distribution.

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CHAPTER 3

Ontogenetic vertical migration behaviour of decapod larvae in the Portuguese upwelling ecosystem*

*Bartilotti C., Castro M., Santos A. M. P., Queiroga H., dos Santos A. (*in prep.*) **Ontogenetic vertical migration behaviour of decapod larvae in the Portuguese upwelling ecosystem**.

3.1- Abstract

The ontogenetic vertical migration behaviour of decapod larvae was analysed on the western Iberia upwelling ecosystem. An oceanographic cruise conducted in May 2002 sampled vertically a fixed station point during 69 h, approximately 21 km off the coast. Plankton samples were collected every 2 hours at the surface with a Neuston net and through the water column with a Longhurst Hardy Plankton Recorder (Pro-LHPR), in order to obtain a detailed resolution of larval vertical distribution. Temperature and salinity were registered hourly. The vertical migration behaviour of nine different species of decapod crustacean, three caridean shrimps, three hermit crabs, and three crabs were analysed in detail, by stage. The results showed that all the studied species larval stages had diel vertical migration (DVM) behaviour, and the average depth of distribution of the studied larval stages varied through the ontogenetic development. The majority of the studied species had their early zoeal stages more close to the surface and the last zoeal stages in a more deep position. We also analysed the larval distributions in relation with the hour of the day which indicated the DVM and as expected was the predictor that explained most times the larval distributions; the midpoint of depth stratum reflected the positioning of the larvae in a certain favourable depth giving information about the average depth of distribution. Finally the temperature and the salinity gave information about the larval distributions and the probable transport mechanisms in the water column in the sampling area. During sampling, a less saline water lens appeared in the second day, and, we suppose that it transported newly hatched larvae to the fixed station point.

3.2- Introduction

Decapod crustacean larvae can dynamically regulate their vertical position in the water column, controlling the range and direction of their horizontal dispersal (Queiroga & Blanton 2005), maintaining a favourable position to the adequate and necessary transport (e.g. Forward et al. 1997, Christy & Morgan 1998). The Diel Vertical Migration (DVM) behaviour seems to be the rule for decapod larvae, although, it is a pattern rarely described in detail (see Queiroga & Blanton 2005 for examples). Most of the studies present results obtained by neuston and/or discrete depth levels sampling (e.g. Shanks 1985, Jamieson & Phillips 1988, Abelló & Guerao 1999), being the studies with a high vertical discrimination rare (Lindley 1986, Lindley et al. 1994, dos Santos et al. 2008). The recent results for the upwelling ecosystem adjacent to the Ria de Aveiro lagoon system showed that decapod zoeae and megalopae, as well as *Chthamalus stellatus* cyprids, displayed the DVM (dos Santos et al. 2007, 2008).

Most decapod crustaceans present DVM ascending to the surface water layer during the night (Queiroga & Blanton 2005). Observing inert larvae Pineda et al. (2007) stated that vertical swimming behaviour, changes in buoyancy, and ontogenetic changes in vertical position in the water column were influencing the horizontal larval movements. Recently, dos Santos et al. (2008) concluded that the diel vertical migration is the mechanism responsible by the larval retention in the Portuguese upwelling ecosystem, corroborating the hypothesis presented by the models developed for the study area (Marta-Almeida et al. 2006, Peliz et al. 2007). The authors concluded that the horizontal larval distribution of the studied species in the inner and middle shelf was mainly alongshore clearly related to the presumed settlement

areas, and the capacity to perform diel vertical migrations was the crucial factor for the established distributional larval patterns (dos Santos et al. 2008).

The larval vertical distribution behaviour may reflect environmental responses to light (e.g. Sulkin 1975), salinity (e.g. Latz & Forward 1977) and temperature (e.g. Dawirs 1985). In the laboratory, small variations of salinity or temperature lead to changes in the larval behaviour (e.g. Forward 1989), although in the study area, these small variations reflected the water mixing, which did not modified the larval behaviour (dos Santos et al. 2008). Salinity is one of the environmental factors with a higher selective pressure for decapod crustacean larvae (Anger 2003), and the freshwater input can enhance vertical mixing affecting the distribution and transport of planktonic larvae (e.g. Garrison 1999). Also the temperature takes to various metabolic responses by crustacean larvae reflecting their tolerance (Agard 1999), and it is known that a shallow thermocline can create vertically sheared environments that can limit the larval transport for species with DVM (Pineda et al. 2007). Other important factors for the establishment of the larval vertical position are the predators' avoidance (e.g. Hobbs & Botsford 1992, Forward & Rittschof 2000) or the food availability (e.g. Lindley et al. 1994, Pearre 2003).

Another category of vertical migrations is the ontogenetic migration, defined as a change in the average depth of distribution during the larval life period, a process obligatory for benthic crustacean that hatch close to the bottom, feed in the surface, and when reaching the end of their larval life must return to the adult habitat (Queiroga & Blanton 2005). Several studies described these ontogenetic migrations (see Queiroga & Blanton 2005 for details and references), and most of them considered brachyuran species. In the study area, Queiroga (1996) demonstrated these ontogenetic differences in the vertical distribution of *Carcinus*

maenas: the first and second zoeal stages were closer to the surface and a gradual displacement to deeper waters was observed from then on, for the older stages; the megalopa was equally distributed at the surface and at a higher depth of the water column.

Knowing that the decapod crustacean larvae in the study area perform vertical migrations (dos Santos et al. 2008), present work pretends to analyse in detail the vertical distribution patterns of the larval stages of the 9 selected taxa, in order to determine their ontogenetic vertical migration in the Portuguese continental shelf, and also to describe the behavioural responses of each larval stage to the environment (depth, salinity, temperature and hour of the day).

3.3- Material and Methods

3.3.1- Field study

An oceanographic survey was carried out off the northwest coast of Portugal (project ProRecruit'2002-02050502), aboard the RV 'Noruega', from 9-22 May 2002 (Fig. 1).

From 11 to 14 May a mooring with 3 current meters (Aandera RCM 9) and 2 conductivity-temperature-depth (CTD) profilers (Sea-Bird Electronics MicroCats) was carried out on the Portuguese continental shelf (40º45.9' N 08º59.0' W), approximately 21 km offshore, positioned at a 60 m bottom depth, in an area adjacent to Aveiro coastal lagoon (Fig. 1).

From 18 to 21 May 2002, the fixed station was sampled continuously in two hours intervals for 69 hours. Environmental sampling was carried (see dos Santos et al. 2007 for details). Temperature and salinity were measured every hour with a Seabird SBE 9 plus CTD (conductivity/ temperature/ depth). The depth stratified samples were collected with the Pro-

LHPR system (a commercially updated version of the Longhurst Hardy Plankton Recorder, Williams et al. 1983). The Pro-LHPR had a mouth aperture of 0.42 m and collects plankton between two rolls of filtering gauze with 280 µm mesh size that are advanced at intervals inside a cod end box to give a series of consecutive samples. It was towed at 3-4 knots on oblique hauls from the surface to near the seabed for 30 min intervals. Stratified samples of Pro-LHPR were collected at 5 m depth intervals in the first 25 m, and 10 m depth intervals from 25 m deep until near the bottom. The first 20 centimetres of the water column were also sampled every 2 hours with a Neuston net. It had a rectangular mouth aperture of 0.2× 1.0 m with a flowmeter mounted and a 335 µm mesh size. It was towed horizontally, for a 3 minutes period, at a velocity of 1.5 knots.

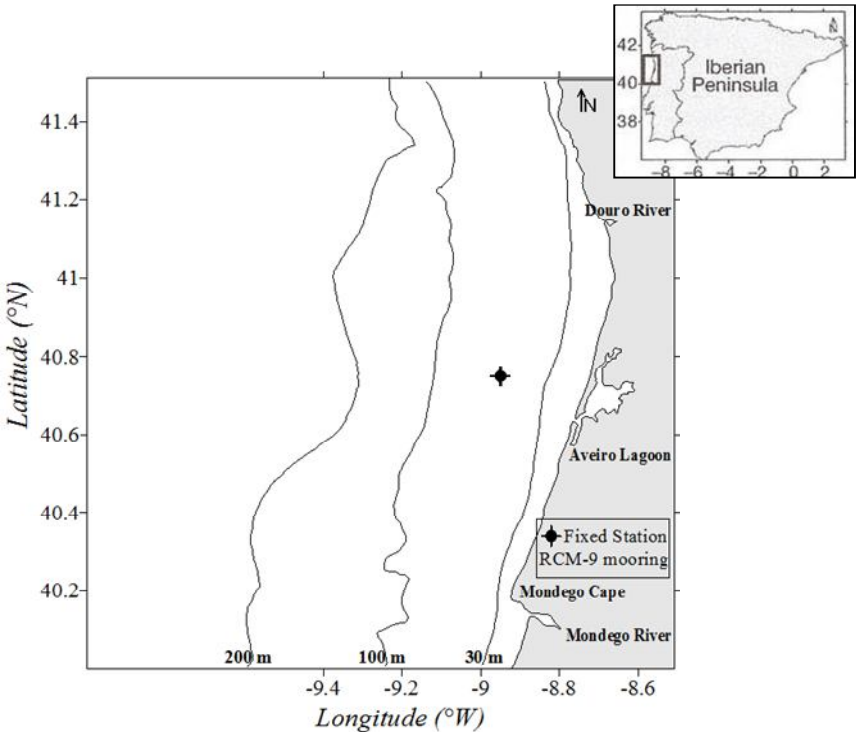

Figure 1- Map of the northwest coast of Portugal: sampling position of the fixed station collected aboard the RV *Noruega*, from 15-17 May 2002, during ProRecruit project. The 30, 100 and 200 m bathymetric lines are also identified.

3.3.2- Sample processing

Plankton samples were preserved in 4% borax-buffered formaldehyde, prepared with seawater. Samples were sorted for decapod larvae, that were identified to species level and developmental stage whenever possible using dos Santos & Lindley (2001), González-Gordillo et al. (2001), and dos Santos & González-Gordillo (2004). Data were standardized for ind.m^{-3} using the flow information.

3.3.3- Analysis of decapod vertical distribution in relation to the physical environment

The average depth was calculated according to Pearre (2003) for each species, by stage, as the Weighted Mean Depth (WMD, in m). It consisted of the weighted abundance per species, of each stage collected in each sampling stratum *z*, at each sampling time *t*:

$$WMDt = \frac{\sum_{z=1}^{9}(Azt \times Dzt)}{\sum_{z=1}^{9} Azt}$$

where *A* is the abundance (ind. m^{-3}) and *D* is the midpoint of depth stratum (m).

Present study considered nine of the more abundant species of the three most represented decapod crustacean groups, the caridean shrimps, hermit crabs and crabs, previously studied by dos Santos et al. (2008). Each species larval series was analysed stage by stage. Table 1 presents the list of chosen taxa, the respective decapod crustacean group and the constitution of the larval series with the number of zoeal stages (Z) and the megalopa or decapodid stage (M).

Table 1- List of the selected taxa, respective decapod crustacean group, and larval series.

Taxa	Decapod group	Larval series
Processa nouveli	Caridean shrimps	Z I to IX and M
Pandalina brevirostris	Caridean shrimps	Z I to VII and M
Philocheras bispinosus	Caridean shrimps	Z I to V and M
Anapagurus spp.	Hermit crabs	Z I to IV and M
Pagurus bernhardus	Hermit crabs	Z Ito IV and M
Pisidia longicornis	Porcelain crabs	Z Ito II and M
Atelecyclus rotundatus	Crabs	Z I to V and M
Liocarcinus spp.	Crabs	Z I to V and M
Necora puber	Crabs	Z I to V and M

The statistical procedure was similar to the one used in Chapter 2. Two response variables were considered, the abundance expressed as number of individuals per cubic meter of filtered water (ind.m^{-3}) that was log transformed to homogenise the variance, and the presence/absence of a given taxa and larval stage, binomial variable equal to one if abundance greater than zero, and zero otherwise.

Having in mind that our objective was to determine the ontogenetic larval vertical migration behaviour in a fixed point, four explanatory variables were established and used in the construction of the model. These were:

- MID_DEPTH – Midpoint of depth stratum, discrete variable, in meters;
- HOUR_WAVE - hour of the day, continuous variable, defined as a circular function of the hour of the day, sunrise=0, noon=1, sunset=0 and midnight=-1, generated by the function:

$$sin\ [(Hour \times \frac{2\pi}{24}) + \frac{3\pi}{2}]$$

where Hour is the hour of the day (0-24);

- SAL – Salinity, continuous variable, no units;
- TEMP - temperature, continuous variable, in ºC.

The decapod larval vertical distribution, in relation to these 4 explanatory variables, was analysed in two steps, for each species and larval stage separately. As referred in the anterior chapter, the two steps procedure is used to deal with the problem of zero inflated data (Wenger & Freeman 2008). In the first step, the presence or absence was predicted using a Logistic Model (LM) with explanatory variables the midpoint of depth stratum (MID_DEPTH), the hour of the day (HOUR_WAVE), the salinity (SAL) and the temperature (TEMP). The results from this model were used in the second step for situations where at least one of the variables was significant and for data points where "presence" was predicted. For these data points, measured values of abundance were modelled with a Generalized Addictive Model (GAM), using linear predictors for the midpoint of depth stratum (MID_DEPTH), the hour of the day (HOUR_WAVE), the salinity (SAL) and the temperature (TEMP) and a spline for temperature (TEMP, with 2 degrees of freedom). Statistical analysis was made using SAS 9.2 (SAS Institute, 2008) using PROC LOGIT for fitting the logistic model (stepwise option) and PROC GAM for the generalized additive models.

3.4- Results

3.4.1- Oceanographic conditions

For the complete description of the oceanographic conditions during the survey please see dos Santos et al. (2007). The vertical distribution of the hydrological fields showed the arrival of a

less saline water lens at the surface from the 12 h of the second day (19th May), consequence of the change in the oceanographic conditions in the sampling area (Fig. 2a). At the end of the sampling this less saline water lens was distributed in the first 15 to 20 m of the water column. Considering the hydrological conditions during sampling, probably this lens was advected from the north near coast. It was registered a second salinity minimum between the 15 and 35 m depth, flanked by two pycnoclines (Fig. 2b). Salinity maximum was 35.71 registered at 10h of the third sampling day, at 47 m depth; and the minimum was 35.06 registered at 16h of the third day sampling, at 5 m depth (Fig. 2a).

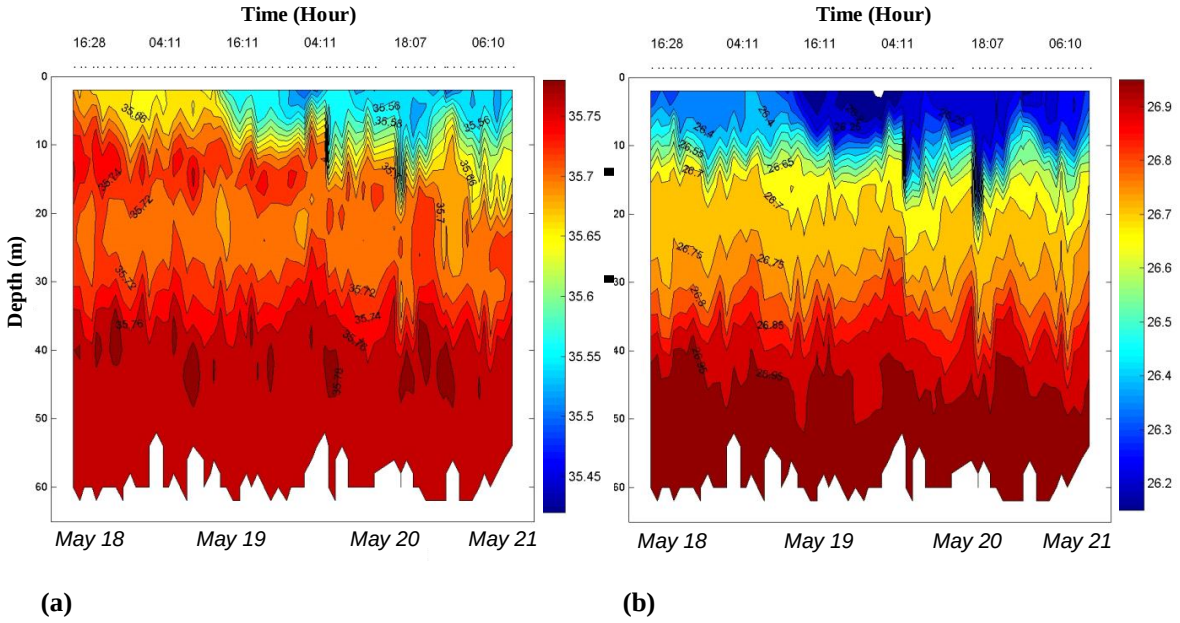

Figure 2- Sequence of CTD measurements of: (a) salinity and (b) density (σ_t, kg.m^{-3}), during sampling at the 69 h fixed station, 18-21 May 2002. X axis represents the sampling date; Y axis the depth (m).

A thermocline was recorded between the 10 and 20 m depth (Fig. 3). During the sampling the temperature varied between the 13.14 ºC at 22h of the third sampling day at 53 m depth, and the 16.04 ºC at 18h of the same day at 2.5 m depth.

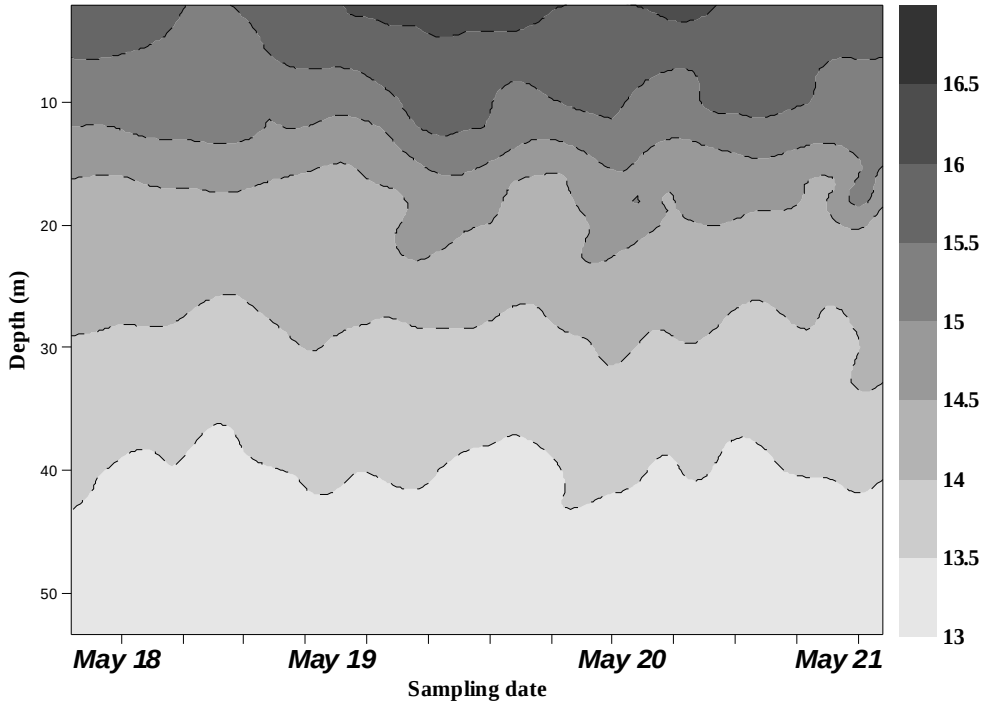

Figure 3- Temperature (º C) measurement during sampling at the 69 h fixed station, 18-21 May 2002. X axis represents the sampling date; Y axis the depth (m).

3.4.2- Decapod larval stages vertical distribution

The vertical larval distributions of the 9 selected species, *Processa nouveli*, *Pandalina brevirostris*, *Philocheras bispinosus*, *Anapagurus* spp., *Pagurus bernhardus*, *Pisidia longicornis*, *Atelecyclus rotundatus*, *Liocarcinus* spp. and *Necora puber* were analysed by developmental stage.

The two steps approach used modelled the decapod larval stages vertical distribution in relation to the environmental variables (Tables 2 to 4), and showed that the hour of the day (HOWR_WAVE) as expected was the variable that explained the larval distribution of the majority of the studied larval stages, being always negatively related with the zoeal stages presences, reflecting the vertical migration to the surface during the night.

Table 2- Coefficients of significant explanatory variables of the two steps model (presence or absence predicted using a Logistic Model in the first step, values of abundance modelled with a Generalized Addictive Model in the second step) describing the larval vertical distribution of the Caridea selected species collected with the Neuston and LHPR nets in the fixed station. Levels of significance: ***p<0.001, **p<0.01,*p<0.05; n.s. not significant.

Species	Stage	Model phases	n	Cofficients of significant explanatory variables				
				MID_DEPTH	HOUR_WAVE	SAL	TEMP (linear)	TEMP (spline)
Processa nouveli	ZI	presence	297	n.s.	-0.788 ***	4.205 *	n.s.	-
		abundance	-	-	-	-	-	-
	ZII	presence	297	n.s.	-0.566 **	n.s.	-0.375 **	-
		abundance	58	n.s.	n.s.	n.s.	n.s.	n.s.
	ZIII	presence	297	n.s.	-1.329 ***	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZIV	presence	297	n.s.	-0.796 *	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZV	presence	297	n.s.	-1.313 **	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZVI	presence	297	n.s.	-1.069 **	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZVII	presence	297	-	-	-	-	-
		abundance	-	-	-	-	-	-
	ZVIII	presence	297	-	-	-	-	-
		abundance	-	-	-	-	-	-
	ZIX	presence	297	0.060 ***	0.934 ***	-7.759 *	n.s.	-
		abundance	31	n.s.	n.s.	n.s.	n.s.	n.s.
	M	presence	297	-	-	-	-	-
		abundance	-	-	-	-	-	-
Pandalina brevirostris	ZI	presence	297	0.044 *	-1.294 *	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZII	presence	297	0.059 ***	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZIII	presence	297	0.043 ***	-0.812 ***	n.s.	n.s.	-
		abundance	9	-	n.s.	n.s.	n.s.	n.s.
	ZIV	presence	297	n.s.	-0.881 ***	11.320 ***	n.s.	-
		abundance	43	n.s.	n.s.	n.s.	n.s.	n.s.
	ZV	presence	297	n.s.	-1.331 ***	7.208 **	n.s.	-
		abundance	27	n.s.	n.s.	n.s.	n.s.	n.s.
	ZVI	presence	297	n.s.	-1.253 ***	8.498 ***	n.s.	-
		abundance	85	n.s.	n.s.	n.s.	0.067 ***	n.s.
	ZVII	presence	297	0.031 *	-1.534 ***	n.s.	n.s.	-
		abundance	119	n.s.	n.s.	0.758 *	n.s.	0.046
	M	presence	297	n.s.	-3.357 ***	9.876 **	n.s.	-
		abundance	8	n.s.	-	n.s.	n.s.	n.s.
Philocheras bispinosus	ZI	presence	297	n.s.	-0.909 ***	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZII	presence	297	n.s.	-0.957 ***	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZIII	presence	297	n.s.	-1.204 ***	6.532 *	n.s.	-
		abundance	-	-	-	-	-	-
	ZIV	presence	297	n.s.	-2.140 ***	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZV	presence	297	n.s.	-1.802 ***	n.s.	n.s.	-
		abundance	81	0.003 **	n.s.	n.s.	0.070 **	0.035
	M	presence	297	n.s.	-4.566 ***	n.s.	n.s.	-
		abundance	-	-	-	-	-	-

Table 3- Coefficients of significant explanatory variables of the two steps model (presence or absence predicted using a Logistic Model in the first step, values of abundance modelled with a Generalized Addictive Model in the second step) describing the larval vertical distribution of the Anomura selected species collected with the Neuston and LHPR nets in the fixed station. Levels of significance: ***p<0.001, **p<0.01,*p<0.05; n.s. not significant.

Species	Stage	Model phases	n	Cofficients of significant explanatory variables				
				MID_DEPTH	HOUR_WAVE	SAL	TEMP (linear)	TEMP (spline)
***Anapagurus* spp.**	ZI	presence	297	n.s.	-1.504 ***	-7.537 **	n.s.	-
		abundance	-	-	-	-	-	-
	ZII	presence	297	n.s.	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZIII	presence	297	n.s.	-2.026 ***	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZIV	presence	297	n.s.	-1.730 ***	n.s.	-0.926 ***	-
		abundance	206	n.s.	-0.163 ***	4.509 ***	0.444 ***	
	M	presence	297	n.s.	-3.588 ***	n.s.	n.s.	-
		abundance	126	n.s.	n.s.	3.649 ***	0.288 **	n.s.
Pagurus bernhardus	ZI	presence	297	n.s.	-3.419 ***	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZII	presence	297	n.s.	-2.089 *	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZIII	presence	297	0.059 ***	-1.113 ***	n.s.	n.s.	-
		abundance	17	n.s.	n.s.	n.s.	0.799 *	n.s.
	ZIV	presence	297	0.059 ***	-1.318 ***	n.s.	n.s.	-
		abundance	81	0.010 *	0.267 ***	n.s.	n.s.	n.s.
	ZV	presence	297	n.s.	n.s.	n.s.	-1.194 ***	-
		abundance	-	-	-	-	-	-
	M	presence	297	0.043 ***	-1.246 ***	n.s.	n.s.	-
		abundance	9	-	n.s.	n.s.	n.s.	n.s.
Pisidia longicornis	ZI	presence	297	n.s.	-1.112 ***	-10.101 ***	n.s.	-
		abundance	18	n.s.	n.s.	n.s.	n.s.	n.s.
	ZII	presence	297	n.s.	-1.064 ***	-7.726 *	-0.847 ***	-
		abundance	181	0.007 **	-0.067 *	n.s.	0.179 **	-
	M	presence	297	-0.033 ***	-3.671 ***	n.s.	n.s.	-
		abundance	111	n.s.	n.s.	n.s.	n.s.	n.s.

The midpoint of depth stratum (MID_DEPTH) reflected the positioning of the larvae in a certain water stratum, and it was important for almost all the studied taxa. It was always positively related with the caridean shrimps (Table 2) and hermit crabs (Table 3) larval stages distribution, with the exception of *P. longicornis* megalopa whose presence was negatively related with this predictor. Brachyuran crabs larvae (Table 4) were always negatively related with the midpoint of depth stratum, except *A. rotundatus* third zoeae presence that was positively related. The last zoeal stages of the caridean shrimps, ninth, seventh and fifth zoeae

for *P. nouveli*, *P. brevirostris* and *P. bispinosus* respectively, were always related with the midpoint of depth stratum (Table 2).

Table 4- Coefficients of significant explanatory variables of the two steps model (presence or absence predicted using a Logistic Model in the first step, values of abundance modelled with a Generalized Addictive Model in the second step) describing the larval vertical distribution of the Brachyura selected species collected with the Neuston and LHPR nets in the fixed station. Levels of significance: ***p<0.001, **p<0.01,*p<0.05; n.s. not significant.

Species	Stage	Model phases	n	Cofficients of significant explanatory variables				
				MID_DEPTH	HOUR_WAVE	SAL	TEMP (linear)	TEMP (spline)
Atelecyclus rotundatus	ZI	presence	297	n.s.	-1.665 ***	-13.319 ***	n.s.	-
		abundance	11	n.s.	n.s.	n.s.	n.s.	0.0326
	ZII	presence	297	n.s.	-1.493 ***	n.s.	0.896 ***	-
		abundance	58	n.s.	n.s.	-	n.s.	n.s.
	ZIII	presence	297	0.078 *	-0.589 **	16.630 ***	2.310 ***	-
		abundance	10	n.s.	n.s.	-	n.s.	n.s.
	ZIV	presence	297	n.s.	-0.647 ***	n.s.	0.288 *	-
		abundance	58	n.s.	n.s.	0.829 *	n.s.	n.s.
	ZV	presence	297	n.s.	-0.445 **	n.s.	n.s.	-
		abundance	180	n.s.	-0.062 *	1.118 ***	0.142 **	n.s.
	M	presence	297	n.s.	-2.819 ***	n.s.	n.s.	-
		abundance	27	n.s.	-	-	n.s.	n.s.
***Liocarcinus* spp.**	ZI	presence	297	-0.036 **	-1.026 ***	-8.051 **	n.s.	-
		abundance	97	-0.094 ***	-0.537 **	n.s.	n.s.	n.s.
	ZII	presence	297	-0.042 ***	-1.093 ***	-5.619 *	n.s.	-
		abundance	145	-0.074 ***	-0.463 ***	n.s.	n.s.	n.s.
	ZIII	presence	297	-0.077 ***	n.s.	6.485 *	n.s.	-
		abundance	19	n.s.	-0.532 **	-	n.s.	n.s.
	ZIV	presence	297	n.s.	-0.863 ***	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZV	presence	297	n.s.	n.s.	-10.754 ***	n.s.	-
		abundance	293	n.s.	n.s.	-1.027 ***	0.157 ***	n.s.
	M	presence	297	n.s.	n.s.	-18.498 ***	-0.810 **	-
		abundance	286	-0.005 *	-0.064 ***	n.s.	n.s.	n.s.
Necora puber	ZI	presence	297	n.s.	-1.138 **	n.s.	0.524 *	-
		abundance	-	-	-	-	-	-
	ZII	presence	297	-0.093 ***	-1.454 ***	15.598 **	n.s.	-
		abundance	-	-	-	-	-	-
	ZIII	presence	297	-0.100 *	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZIV	presence	297	-0.120 *	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	ZV	presence	297	-1.303 **	n.s.	n.s.	n.s.	-
		abundance	-	-	-	-	-	-
	M	presence	297	n.s.	-0.562 **	n.s.	0.567 ***	-
		abundance	-	-	-	-	-	-

Salinity (SAL) was always explaining some larval stages distribution, except for *P. bernhardus*, and was in general related with the early and the late zoeae, as well as with the megalopal presences and/or abundances.

Finally, the temperature (TEMP) influenced some larval stages of all species, and was in general significantly related with the last zoeal stages and the megalopa distribution.

3.4.2.1- Vertical distribution of the caridean shrimps larvae

The modelling results (Table 2) confirmed that the three selected species had almost all the zoeal and megalopal stages presences negatively related with the hour of the day (HOUR_WAVE) (dos Santos et al. 2008). The three selected species had their last zoeal stage presence positively related with the midpoint of depth stratum (MID_DEPTH). The first and last zoeal stages presences of *P. nouveli* were significantly related with salinity (SAL), although with different signals (positive for zoeae I and negative for zoeae IX). *P. brevirostris* older zoeal stages (zoeae IV to VII) were always positively related with the salinity (SAL). *P. brevirostris* and *P. bispinosus* had their late zoeal stages (sixth zoeae and fifth zoeae respectively) positively related with the temperature (TEMP). For the three selected species we present the average depth of distribution of all larval stages (Figs. 4-6). All showed similar patterns of diel vertical distribution, being in a deeper position during the day, migrating to the surface layers of the water column during the night, and returning back to the deeper layers of the water column around the dawn.

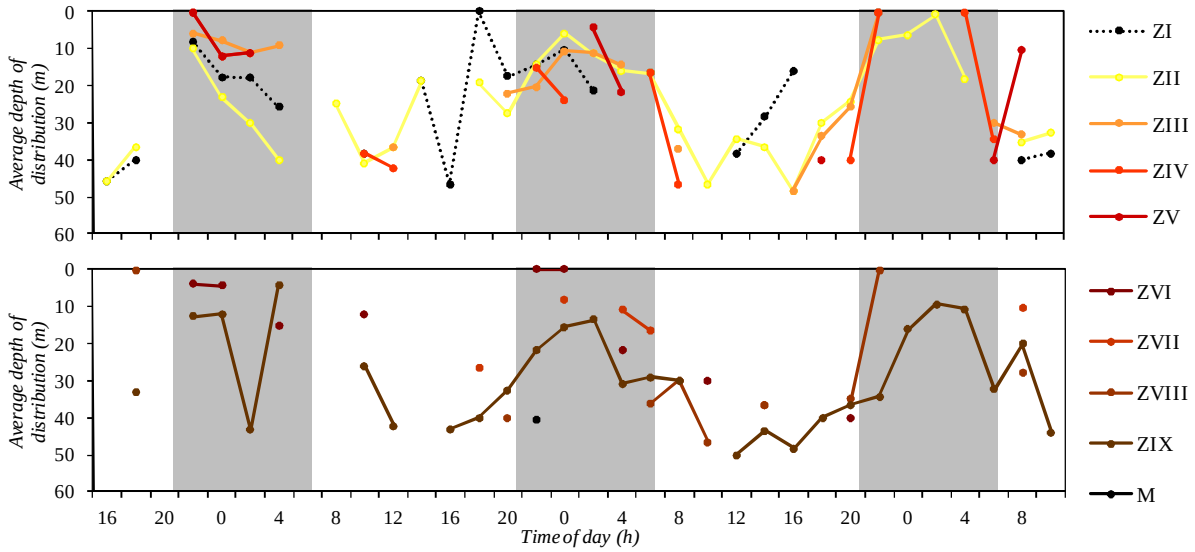

Figure 4- Average depth of distribution of *Processa nouveli* (ind. m^{-3}) zoeal stages: ZI (first zoea) to ZIX (ninth and last zoea), and M (megalopa). The grey areas represent the night period.

P. nouveli second zoeal stage was the more abundant (0.460 ind.m^{-3}), and the megalopa was almost absent from the water column appearing only in the deeper strata, beyond the 25 m, in the second night. The larval vertical migration with the upward movement to near the surface during the night was clear (Fig. 4), for almost all the larval stages, so the higher abundances in the water column were registered in the night period. The larval migration excursion was not evident for the eighth and the megalopal larval stages, although, the first to seventh and the last zoeae had their vertical distribution negatively related with the hour of the day, confirming the migration to the deeper layers of the water column. These species zoeal stages were not common in the neuston layer, and particularly the megalopa was sampled only in the three deeper strata. As referred before, zoeae IX had his presence positively related with the midpoint of depth stratum because of the higher larval concentrations beyond the 25 m during all the sampling period (60% of the sampled larvae were distributed beyond the 25 m depth).

Taking into account the ontogenetic development, the first zoea was in a less profound depth relatively to the second in the first night, but beyond here it was not possible to observe a

distributional pattern for the early zoeal stages. The last zoea was distributed at higher depths during almost all the sampling period.

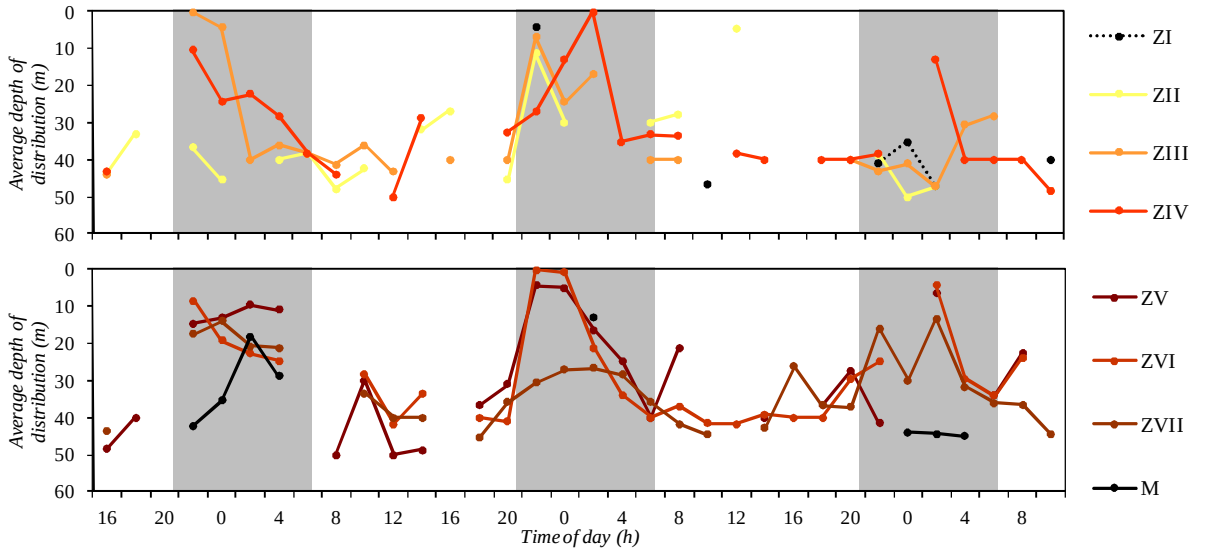

Figure 5- Average depth of distribution of *Pandalina brevirostris* (ind. m^{-3}) zoeal stages: ZI (first zoea) to ZVII (seventh and last zoea), and M (megalopa). The grey areas represent the night period.

The sixth zoeal stage was the more abundant (1.366 ind.m^{-3}), and the first zoea was almost absent from the water column. The megalopa never ascended to the neuston layer, and it was sampled immediately after the sunset in the water column, being absent during the day (Fig.5). The vertical migration was evident for these species larval stages whose presence in the first 15 m of the water column was exclusively nocturnal. The first to third and the last zoeae had their presences positively related with the midpoint of depth stratum because they were concentrated in the deeper strata of the water column (over 70 % of each of the referred larval stages were sampled in the strata above the 35 m). The fourth to sixth zoeae and the megalopa had their presences positively related with the salinity, as well as the last zoea abundance. Temperature only had a signicant effect in the sixth zoeal stage abundance.

Whenever present, the first zoea nocturnal average depth of distribution was more close to the surface than for the second zoea. Fifth and sixth zoeal stages ascended similarly in the water column, describing almost coincident average depths during the sampling period. Similarly to the previous species, the last zoea was distributed at higher depths during almost all the sampling period.

P. bispinosus also presented the diel vertical migration pattern (Fig. 6), so it was sampled in the water column mostly during the night. The second zoeal stage was the most abundant (0.583 ind.m^{-3}), and the megalopa was the only of the three selected caridean shrimps species that migrated to the neuston layer. Similarly to the observed for *P. brevirostris*, the megalopa was absent from the water column during the day. The last zoea was the only stage related with the midpoint of depth stratum (abundance, positively related), and with the temperature (abundance, positively related). The third zoeal stage abundance was the significantly and positively related with the temperature.

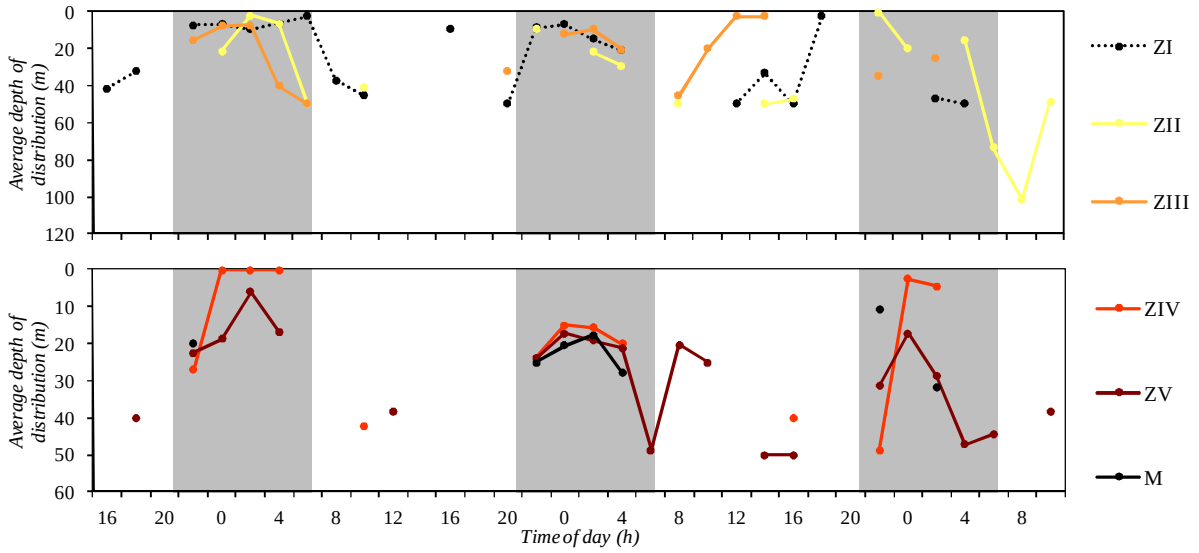

Figure 6- Average depth of distribution of *Philocheras bispinosus* (ind. m^{-3}) zoeal stages: ZI (first zoea) to ZV (fifth and last zoea), and M (megalopa). The grey areas represent the night period.

Similarly to the described for previous species, the last zoeal stage was distributed in a more deep position during almost all the sampling period.

3.4.2.2- Vertical distribution of the anomuran crabs larvae

Anapagurus spp. (Table 3) distribution did not showed any relation with the midpoint of depth stratum (MID_DEPTH), but this predictor was significantly related with the other two species older larval stages (zoeae three to five and megalopa of *P. bernhardus*, zoea two and megalopa of *P. longicornis*). Salinity was important for the distribution of the first, last and megalopal stages of *Anapagurus* spp. with contrary signals: it was positively related with the first zoea presence and negatively related with last zoea and megalopal presence and abundance respectively; *P. bernhardus* did not showed any relation with salinity; *P. longicornis* zoeal stages were both negatively related with this predictor. The three species larval distributions were also influenced by the temperature, having their presences negatively related with this predictor (*Anapagurus* spp. fourth zoeal stage, *P. bernhardus* fifth zoeal stage and *P. longicornis* second zoeal stage) and the abundances positively related (*Anapagurus* spp. fourth zoeal stage, *P. bernhardus* third zoeal stage and *P. longicornis* second zoeal stage). Figures 7 to 9 show the average depth of distribution of all larval stages of the three species. The three species had similar patterns of diel vertical distribution, being almost absent from the water column during the daytime (presences negatively related with the hour of the day).

Anapagurus spp. was the anomuran most abundant species, being the last zoeal stage the more represented during the sampling period (7.285 ind.m^{-3}). The megalopa was almost absent from the water column during the day and when present it was beyond the 35 m depth; the first to third zoeae appeared in the more superficial strata during the night, having, as well

as the megalopa some of their average depth of distribution points in the neuston layer (Fig. 5). The last zoea was always in a more profound distribution relatively to the other zoeal stages, and during the day it was consistently present in the 30 to 50 m depth layer. The first zoeal stage appeared with higher abundances in the second and third nights, probably because it was transported by the less saline water lens advected from nearshore, arrived to the fixed point during the second day of sampling (19th May at 12h). The first zoeae presence in this less saline water lens was indicated by the salinity effect (ZI presence negatively related with the salinity).

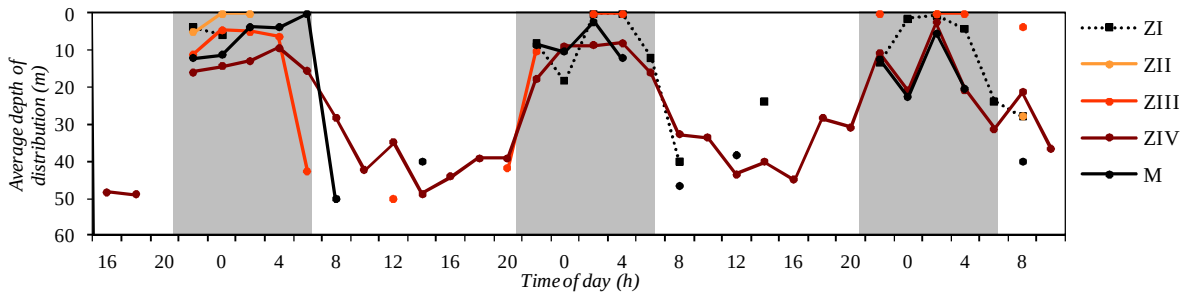

Figure 7- Average depth of distribution of *Anapagurus* spp. (ind. m^{-3}) zoeal stages: ZI (first zoea) to ZIV (fourth and last zoea), and M (megalopa). The grey areas represent the night period.

P. bernhardus was more abundant in the deeper strata than at the surface, and migrated to less deep strata during the night (Fig. 8). The nocturnal migration from close to the bottom to a less deep stratum of the water column, but rarely to the neuston layer, was evident. The larval stages which were related with the midpoint of depth stratum were concentrated in a more deep position (Fig. 8), being 74% of the third zoea and 62% of the fourth distributed beyond the 35 m, and 58 % of the megalopae sampled were distributed deeper than 35 m. Zoeae four and five, as well as the megalopa were distributed at higher depths during the day. The temperature also explained the larval distribution of third and fifth zoeal stages, probably because these were concentrated deeper in the water column, above the thermocline.

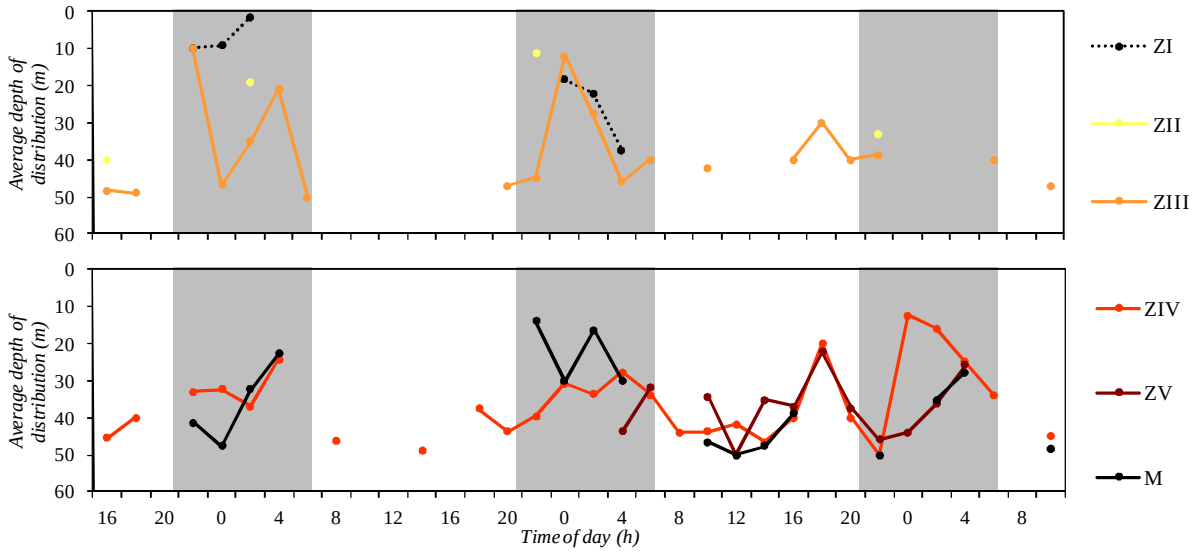

Figure 8- Average depth of distribution of *Pagurus bernhardus* (ind. m^{-3}) zoeal stages: ZI (first zoea) to ZV (fifth and last zoea), and M (megalopa). The grey areas represent the night period.

Finally, *P. longicornis* first and second zoeal stages and the megalopa (Table 3) were negatively related with the hour of the day. The megalopa was the most abundant larval stage during the sampling period (5.268 ind.m^{-3}), and it was present in the superficial waters after the dusk (Fig. 9). The second zoea abundance was positively related with the midpoint of depth stratum, and the megalopa presence was negatively related with this predictor because the second zoea was distributed deeper in the water column, and the megalopa had their higher occurrences in the surface, more precisely in the neuston layer (50% of the megalopae were concentrated in the neuston and in the 0-5 m layers). The first zoea was clearly related with the arriving of the less saline water lens in the second night, reason why it had his presence negatively related with this predictor; also zoea II was negatively related with salinity. Finally, the second zoeal stage had his presence and abundance negatively and positively related with the temperature, probably because this stage was mainly distributed in the first 20 m of the water column were the temperature variation occurred and the thermocline was located.

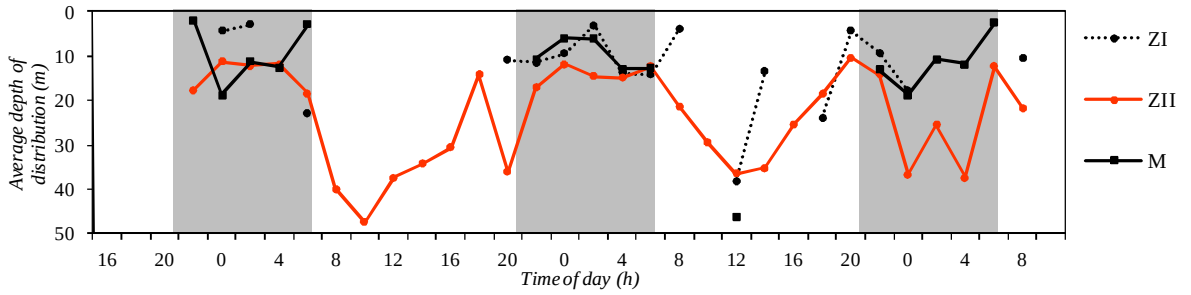

Figure 9- Average depth of distribution of *Pisidia longicornis* (ind. m^{-3}) zoeal stages: ZI (first zoea) to ZII (second and last zoea), and M (megalopa). The grey areas represent the night period.

3.4.2.3- Vertical distribution of the brachyuran crabs larvae

The midpoint of depth stratum was an important predictor in the explanation of brachyuran larval stages distribution, probably because the three selected species were very represented in the neuston layer during the night (Table 4). Salinity explained the distribution of most of the sampled stages of *Liocarcinus* spp. The effect of temperature was important for the second to fifth zoeal stage of *A. rotundatus*, last zoea and megalopa of *Liocarcinus* spp., and the first zoea and megalopa of *N. puber*.

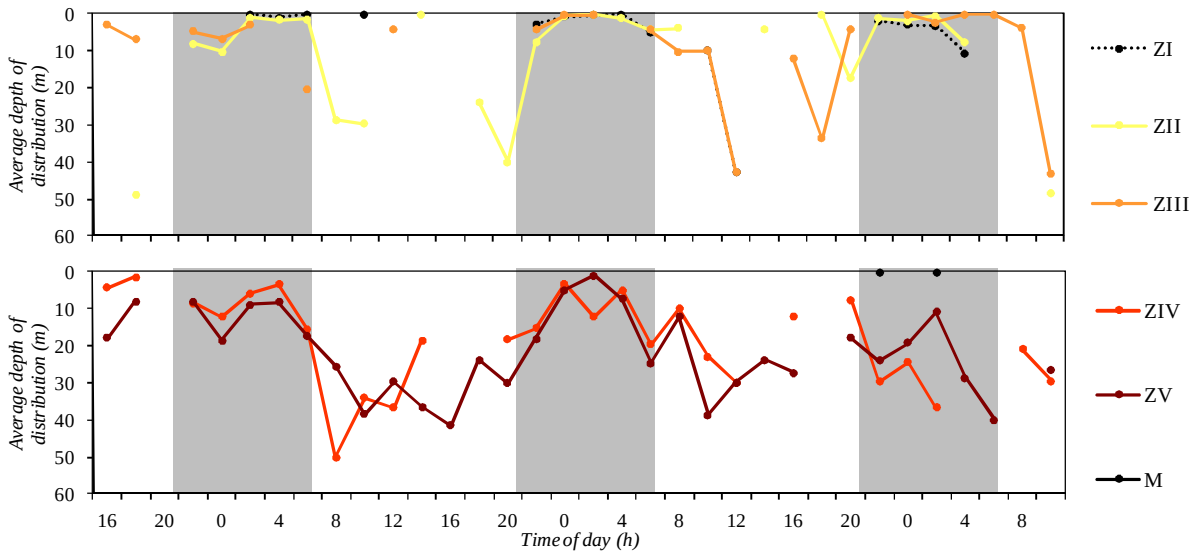

Figure 10- Average depth of distribution of *Atelecyclus rotundatus* (ind. m^{-3}) zoeal stages: ZI (first zoea) to ZV (fifth and last zoea), and M (megalopa). The grey areas represent the night period.

All *A. rotundatus* larval stages were negatively related with the hour of the day, and the higher abundances were always registered in the neuston layer (e.g. the most abundant larval stage was the second in the neuston layer at 2 h of the second night). The megalopa appeared only in two nocturnal neustonic samples collected in the last night (Fig. 10). The first zoeal stage appeared with higher abundances in the second night, because of the probable transport in the less saline water lens advected from nearshore, and it had a more superficial distribution than the other sampled stages in the first and second nights. The midpoint of depth stratum just explained the third zoeal stage presence (Table 4). Observing the significance effects of salinity, zoea I was negatively related with this predictor, and the other stages (third to fifth zoeae) were positively related. We suppose, once again that the first zoea were present in the less saline water lens advected for the fixed point. The temperature was positively related with second to fifth zoeal stages, and non significant for the first zoea and the megalopa.

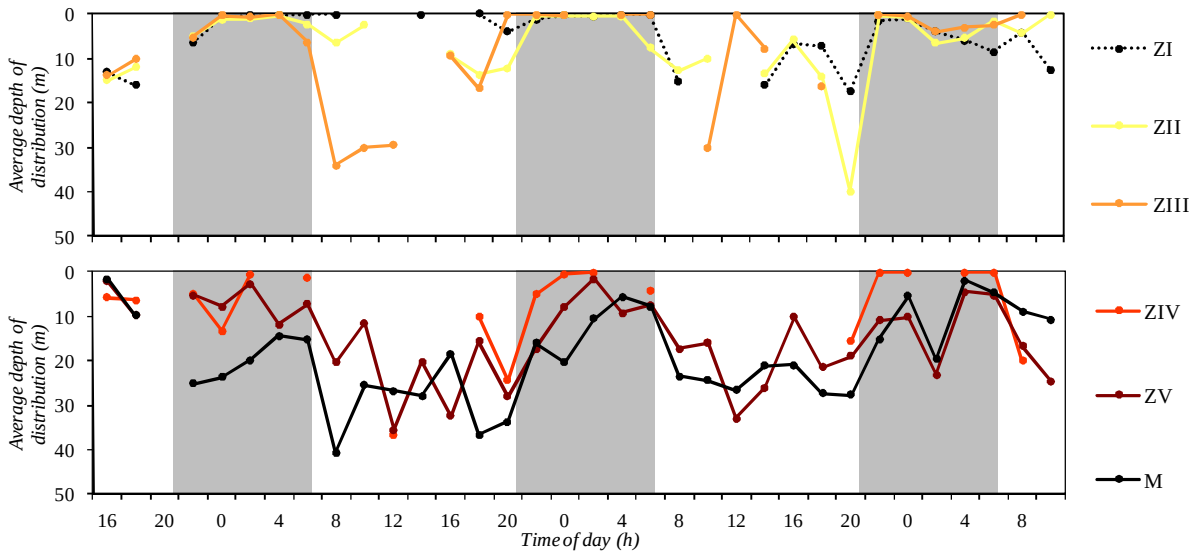

Figure 11- Average depth of distribution of *Liocarcinus* spp. (ind. m^{-3}) zoeal stages: ZI (first zoea) to ZV (fifth and last zoea), and M (megalopa). The grey areas represent the night period.

Liocarcinus spp. was the most abundant species during the fixed point sampling. The first zoea was the most abundant during the sampling period (99.581 ind.m^{-3}, at 0h of the second night, in the neuston layer). All the larval stages were negatively related with the hour of the day with the exception of the fifth zoea, that did not presented a clear vertical migration pattern even having some neustonic presences during the night (Fig. 11). The midpoint of depth stratum was always negatively related with the first to third zoeae and with the megalopal distributions, because these stages were concentrated more close to the surface. The first zoea in the first and second nights was mainly neustonic, and fifth zoea was more deeply distributed in the water column. The salinity was negatively related with the first, second, last and megalopal stages presences, though for the third zoea presence it was positively related. Once again we suppose that the salinity effect reflected the presence of the less saline water lens that probably transported the newly hatched zoeae and for some reason advected the third zoea that in the second night of sampling was almost absent from the water column. The last zoeal stage and the megalopa had their abundance and presence positively and negatively related with the temperature.

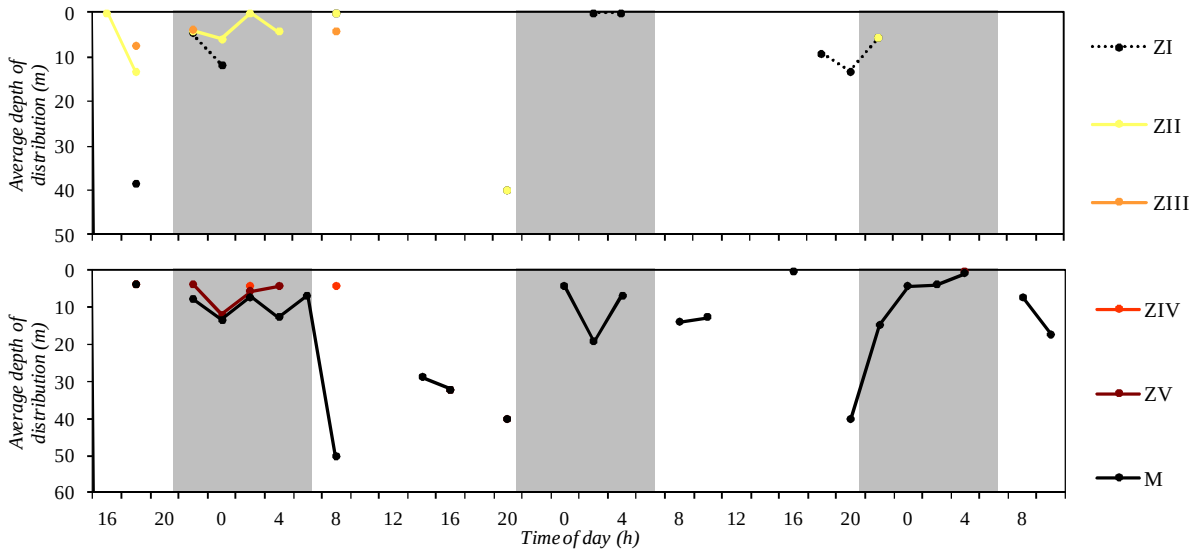

Figure 12- Average depth of distribution of *Necora puber* (ind. m^{-3}) zoeal stages: ZI (first zoea) to ZV (fifth and last zoea), and M (megalopa). The grey areas represent the night period.

N. puber second zoeal stage was the most abundant during the fixed point sampling (58.903 ind.m^{-3}, at 2h of the first night, in the neuston layer). The second to fifth zoeal stages were negatively related with the midpoint of depth stratum, because these stages had their larvae concentrated at the surface (more than 84% of these stages were in the upper 10 m of the water column). The second zoea presence was positively related with the salinity, probably because of the arrived of the less saline water lens that advected it away from the fixed point station. At last, the temperature was positively related with the first zoea and the megalopa, both stages having their maximum in the neuston layer, above the thermocline.

3.5- Discussion

Dos Santos et al. (2008) described the diel vertical migrations of the decapod larvae in the Portuguese upwelling coast, demonstrating that this behaviour was present in the caridean shrimps, the anomuran and the brachyuran crabs. Present work verified the existence of the diel vertical migration (DVM) in almost all the larval stages of the studied species, showing that all the larval stages were capable of swim through the water column, mitigating the seaward transport alternating between the surface and the deeper or bottom layers. According to Peterson (1998) this is the movement responsible for the avoidance of the larvae seaward dispersal. Marta-Almeida et al. (2006) model showed that if crab larvae perform DVM as described, then upwelling may enhance retention of larvae in the inner shelf. Dos Santos et al. (2008) confirmed that the DVM is the mechanism that permits to decapods in general the larval retention in the inner shelf and also over the shelf. Present results demonstrated that the 9 selected taxa had most of the times their newly hatched zoeae distributed in a more

superficial position, and their last zoeae were almost always occupying the deeper strata of the water column.

Knowing that the light (e.g. Sulkin 1975), the salinity (e.g. Latz e Forward 1977) and the temperature (e.g. Dawirs 1985) are determinant factors in the vertical positioning of decapod crustacean larvae, we verified that almost all the selected species larval stages had their presences negatively related with the hour of the day, as expected, indicating that the studied zoeal stages and the megalopa migrated vertically in the water column in response to the diel cycle, and had their higher larval concentrations in the water column during the night (results previously obtained by dos Santos et al. 2008, and studied by stage in present work). Of the four selected environmental cues, the diel cycle was the most important, explaining almost all the larval stages distributions, being the exceptions: the seventh, eighth, and megalopal stages of *P. nouveli*; the second zoea of *P. brevirostris*; the second zoea of *Anapagurus* spp.; the fifth zoea of *P. bernhardus*; the fifth zoea of *Liocarcinus* spp.; and the third to fifth zoeae of *N. puber*. It should be noticed that the hour of the day was many times non significant for the late zoeal stages distributions, probably because these zoeae started to be synchronized with a different environmental cycle. Queiroga & Blanton (2005) affirmed that there are two main types of vertical migration: the cyclic migration and the ontogenetic migration, being the first characterized by the larvae moving up and down in the water column in synchrony with one or more environmental cycles.

The midpoint of depth stratum predictor reflected the positioning of the larvae in a certain depth. All the three caridean shrimps and the anomuran hermit crabs had their last zoeae positively related with this factor, because they were distributed in the deeper layers of the water column, as the average depth of distribution graphs demonstrated. There are at least two

explanantions for the larval positioning in the deeper layers of the water column: it may result of the ontogenetic vertical migration with a change of the average depth of distribution through development (e.g. Queiroga et al. 2005), or it may be describing a strategy to enhance the larval retention over the shelf (e.g. Peterson 1998). It is known that some larvae avoid the surface layer where currents tend to be faster, residing deeper in the water column, retarding the transport, and as a consequence favouring the retention (e.g. Shanks and Brink 2005, Shanks and Eckert, 2005, Morgan et al. 2009). Contrary to these, *P. longicornis* megalopa, *Liocarcinus* spp. zoeal and megalopal stages, and *N. puber* zoeal stages were negatively related with the midpoint of depth stratum, probably because these larvae had more than 40% of the sampled larvae concentrated at the surface, in the neuston layer, during the night. All these three taxa when adults are distributed over the shelf but also inshore (Zariquiey-Álvarez 1968, d'Udekem d'Acoz 1999), so we can suppose that at least for the megalopa this superficial distribution may reflect the transport mechanism to the settlement areas (e.g. Shanks 1985, Tapia et al. 2004). The larval concentrations in the neuston had a peak during the night, and decreased rapidly before sunrise. According to dos Santos et al. (2008) the diurnal distribution of the larvae suggested that both the zoeal and megalopal stages moved to a narrow layer between the 55 m and the bottom at 63 m.

In the present study sometimes the ontogenetic vertical migrations were not so evident, probably because during sampling the hydrological conditions were complex, and the arrival of the less saline water lens to the fixed point lead to the rearrangement of the larval vertical distribution as a response to the hydrological changes. Although, similarly to what have been described (see Lindley et al. 1994 for examples), the first zoeal stage seems to reach the neuston layer more times than the last zoeal stage, and was many times distributed more close to the surface than the second zoeal stage; also the megalopa had many times their maximum

abundances at the neuston layer, but the average depth of distribution was many times more or less coincident with the last zoea distribution, in a more deep position during the day. The megalopal higher abundances were registered in the neuston layer, and as referred by previous authors this position can reflect the transport mechanism. As dos Santos et al. (2008) discussed, this megalopal presence at the surface water layer did not reflected the internal waves transport (Shanks 1985) or the daily sea breezes transport (Tapia et al. 2004). Of the 9 studied species, *Anapagurus* spp., *P. longicornis*, *Atelecyclus rotundatus*, *Liocarcinus* spp. and *N. puber* had their megalopae concentrated in the neuston during the night.

Changes during ontogeny in the osmorregulation of decapod crustacean larvae exist, and are described as an ontogenetic increase in the regulatory capability with the early larval stages in general showing weaker regulatory capabilities than the conspecific juveniles or adults, and so are less tolerant to salinity changes in the environment (Anger 2001). In the literature, the salinity effect is more noticed in the vertical migration excursion than the temperature, and the haloclines involving differences as small as 1 may stop the ascending movement to the surface (Hughes, 1969; Roberts, 1971), inhibiting, rather than promoting, larvae to ascend to surface convergent flows. The effect of salinity was important for the analysed larval stages once that it reflected the presence of the less saline water mass beyond the 12h of the second day. A negative effect of the salinity in the first zoeal stage distribution of *Anapagurus* spp., *P. longicornis*, *A. rotundatus* and *Liocarcinus* spp. probably is reflecting the transport of the newly hatched larvae in the advected water lens from the inner shelf. Whenever the effect was positive, then we had larvae with a deeper distribution in the water column. Also this relation reflected the larval distribution response to the arrival of the less saline water lens, once that the water mass reached the fixed point from the surface in the second day of sampling, it was advected, and through time it showed a wedge form, occupying the first 15-20 m depth of the

water column at the end of the sampling period. As a consequence, the water mass here present in the first day was moved to a more offshore position, and probably the larvae therein sinked in the water column or were advected to a different place.

The temperature effect reflected the distribution of the larvae in relation to the thermocline. It had most of the times a positive effect probably because these larvae had a bimodal distribution showing their maximum abundances at the surface during the night, and remaining in the deeper strata or close to the bottom during the day. Observing the larval distributions of the taxa that had their distributions negatively related with the temperature, we see that *Anapagurus* spp. fourth zoea was more abundant in the 5-20 m depth, *P. bernhardus* fifth zoea was distributed beyond the 20 m, and *P. longicornis* second zoea was more abundant from the surface to the 20 m depth hey have their higher larval concentrations above or below the thermocline.

Taking into account all the assumptions made, we are in accordance with dos Santos et al. (2008). Present study demonstrates that decapod crustacean larvae have their vertical migration movements', ontogenetic and diel, and with this mechanisms are capable of regulate their transport. In fact not only the existence of diel vertical migration permits the larval retention over the shelf (dos Santos et al. 2008), but also the existence of the ontogenetic vertical migration which permits the sinking behaviour in the water column probably to enhance larval survival during the return to the adult habitat, and settlement when competent. The idea of decapod larvae trapped at the frontal convergence zones (e.g. Pineda 1999, Shanks et al. 2000) does not fit in the studied taxa, even considering the larval stages separately. We believe that the larval retention over the shelf results of a constant response to

the environmental conditions. These responses are most of the times revealed as the vertical distribution of the larvae.

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CHAPTER 4

Shedding light over the larval genus *Eretmocaris* – morphological larval features of two closely related trans-isthmian *Lysmata* species using laboratory cultured material*

*****Bartilotti C., Calado R., Rhyne A., dos Santos A. (*Submitted*) **Shedding light over the larval genus *Eretmocaris* – morphological larval features of two closely related trans-isthmian *Lysmata* species using laboratory cultured material**. *Helgoland Marine Research.*

4.1- Abstract

The knowledge of morphological changes during larval development is known to be of vital importance for the understanding of phylogenetic relationships among related species. Of the 37 *Lysmata* valid species worldwide, only three have their complete larval series known from laboratorial studies. Present work analyses the larval development of two closely related trans-isthmian species of *Lysmata* using laboratory cultured material. The morphological larval features of the first four zoeal stages of both species, fifth to seventh stages of *L. galapagensis* (Eastern Pacific Ocean), and the last stage of *L. moorei* (Southwestern Atlantic Ocean) are described and compared with the larval descriptions currently available for the genus. Morphological characters showed a very homogeneous development in both species that hatch in a similar developmental form as *L. seticaudata*, displaying their first and fifth pereiopods as buds. The existing correspondence between the zoeal characters and the phylogenetic results for *Lysmata* genus is very interesting: *L. galapagensis*, *L. moorei* and *L. seticaudata* belong to the "Cosmopolitan Clade" / "*Lysmata* Clade" (Baeza et al. 2009, Fiedler et al. submitted). We hypothesize that all the species within this clade will hatch with the first and fifth pereiopods as buds, and will present a maximum of nine zoeal stages. The resemblance between the zoeal characters described in the present work and those previously described for the composite larval genus *Eretmocaris* are discussed, with emphasis to *Eretmocaris corniger*. The unique larval features of *E. corniger*, namely an extremely long rostrum and a spine in the dorsal surface of third pleomere, are also recorded in *L. galapagensis* enabling us to match the larval forms earlier identified as the Tropical Eastern Pacific *E. corniger* to a known adult *Lysmata* species. The possible identity of *E. corniger* larvae recorded over one century ago from the Tropical Eastern Atlantic is also discussed.

4.2- Introduction

In recent years, caridean shrimp in the genus *Lysmata* have become extremely popular in the marine aquarium industry and have reached high market prices, sometimes ranging over 40 € per specimen for brightly colored species (Calado et al. 2003a, Calado 2008). Presently there are 37 valid species in the genus worldwide (Anker et al. 2009), in habitats ranging from tropical coral reefs to rocky shores in warm-temperate waters (Debelius 2001).

Lysmata culture efforts have been an excellent opportunity to increase the knowledge on its ecology and reproductive biology. Given the key-role played by larviculture for the successful production of *Lysmata* in captivity, mainly to supply the marine aquarium trade, it is surprising to record how little information has been published on their larval development and morphology. Apart from the recent work by Calado et al. (2004) with the redescription of the larval stages of *L. seticaudata*, the complete larval morphology and development patterns of *Lysmata* are only known for two more species in the genus, *L. ensirostris* (Pillai 1974) and Kurata's *L. wurdemanni* (whose adult species identity is not possible to determine) (Kurata 1970). The knowledge of the morphological changes occurring during larval development are essential for the understanding of phylogenetic relationships (e.g. Clark 2009), as well as species geographical differentiation (e.g. Schubart et al. 2005), and ecology (e.g. Levin 2006).

In the "Report of the Scientific Results of the Voyage of H.M.S. Challenger during the years 1873-76" Bate (1888) founded a new genus, *Eretmocaris*, on which he included four larval types, *E. remipes*, *E. longicaulis*, *E. stylorostris* and *E. corniger*. All these larvae shared a common and remarkable morphological feature: a long and slender ocular peduncle. Caroli

(1918), when studying the larvae of *L. seticaudata* (Risso, 1816), acknowledged the similarity of those larval stages with *Eretmocaris* larvae and hypothesized that they could actually be the larval forms of two or more caridean shrimp genera, including *Lysmata* Risso, 1816. Several years later, Gurney (1937) effectively demonstrated that the great elongation of the eyestalks, as well as the precocious development of leg 5 before the appearance of legs 3 and 4 into an enormously long appendage with a paddle-like enlargement of the propodus in *Eretmocaris* specimens, were in fact larval features displayed by hippolytid shrimp in the genus *Lysmata* and allied genus such as *Exhippolysmata* Stebbing, 1915 and *Lysmatella* Borradaile, 1915. Although *Eretmocaris* has never been validated, the name has been continually used by several researchers to classify larval forms collected from the plankton presenting the characters described above (e.g. Lindley et al. 2002, Brightdoom et al. 2006).

One of the most remarkable *Eretmocaris* is certainly *E. corniger*. This larval form displays unique morphological characters among known *Eretmocaris*, namely a prominent spine on dorsal surface of the third pleomere and a remarkably long rostrum with several dorsal teeth (Gurney 1937). Gopalakrishnan & Laurs (1971) reported and described as *E. corniger* several specimens from the Eastern Tropical Pacific and suggested a relationship between the occurrences of these larvae and the eastern ocean tropical islands. The adult form of *Lysmata* corresponding to *E. corniger* larvae remained unknown, both from the Atlantic as from the Pacific oceans, and so far no *Lysmata* species has ever been reported to occur simultaneously in both of these oceans.

The very recent work of Baeza et al. (2009) presents the molecular phylogeny of *Lysmata* shrimps based on a segment of the 16S RNA mitochondrial gene analysis. Their results define a phylogenetic tree with three main clades: the “Tropical American Clade”, the “Cleaner

Clade", and the "Cosmopolitan Clade". Fiedler et al. (submitted) presents the mitochondrial and the nuclear ribosomal phylogeny of the genus, and considers only two main clades: the "*Hippolysmata* clade" which includes the "Tropical American" and the "Cleaner" clades, and the "*Lysmata* Clade" that corresponds to the "Cosmopolitan Clade". *Lysmata moorei* (Rathbun, 1901) and *L. galapagensis* Schmitt, 1924 are two closely related species occurring on the Eastern and Western sides of the Panama Isthmus. In both phylogenetic trees (Baeza et al. 2009, Fiedler et al. submitted) these species are grouped together with *L. nilita*, *L. holthuisi, L. intermedia* and *L. seticaudata*.

The present work describes the available zoeal characters of two closely related species, *Lysmata moorei* (Rathbun, 1901) and *L. galapagensis* Schmitt 1924, using laboratory cultured material. Their descriptions are compared with the larval features previously described for the only species in the "Cosmopolitan Clade" with their larvae known, *L. seticaudata* (Calado et al. 2004), and with the *Eretmocaris* larval genus (Bate 1888) given the evident resemblances between *L. galapagensis* larvae and *E. corniger* from the Tropical Eastern Pacific.

4.3- Material and Methods

4.3.1- Larval culture techniques

Two ovigerous *L. galapagensis* were collected on the Pacific coast of Nicaragua and imported to the USA where they have entered the marine aquarium trade. These specimens were purchased directly from the importer and, apart from the country of origin and ocean, there are no further details on their exact geographic collection site. Three ovigerous *L. moorei* females were collected under a rocky ledge in a tide pool at 2 m depth in Salvador, Bahia, Brazil. Newly hatched larvae were mass cultured according to the methods described in detail

by Calado et al. (2003b). The Nicaragua material was reared in the U.S. and the Brazilian material in Brazil. For each species the larvae were haphazardly sampled every day from the culture tanks in order to determine their zoeal stage. Whenever possible, at least ten individuals were sampled for each species (exceptions: *L. galapagensis* fifth and seventh zoeae; *L. moorei* first, second and third zoeae). The sampled larvae were fixed in 4% formaldehyde for posterior morphological analysis.

4.3.2- Larval drawings and measurements

Drawings and measurements were made with the aid of a *camera lucida* on a binocular Wild M8 and on a Zeiss microscope. The preparation of slides was temporary. Larval description followed the method proposed by Clark et al. (1998). Setal terminology is according to Garm (2004), while aesthetascs are classified according to Gurney (1937). Whenever necessary, aesthetascs were drawn truncated to facilitate the illustration of the antennule. Similarly, the long plumose setae on maxilliped exopods and on the pleopods exopods were also drawn truncated and setules from setae were omitted whenever necessary. The exopods of pereiopods were also cut to facilitate preparation.

The following larval measurements were taken: total length (TL) - measured from the tip of the rostrum to posterior end of the telson; carapace length (CL) - measured from the tip of the rostrum to posterior margin of the carapace; and rostrum length (RL) - measured from the tip of the rostrum to the eye socket (except in zoea I where it was measured from the tip of the rostrum to an imaginary line crossing the orbital margin).

Identity of taxa sampled is available from 16S mtDNA sequence data for spent females catalogued in GenBank; *L. moorei* (000000,000000,000000) *L. galapagensis*

(000000,000000). Both larval series have been deposited in the Instituto Nacional de Recursos Biológicos- IPIMAR in Lisbon, Portugal (IPIMAR/H/Ll/11/2006 and IPIMAR/H/Lb/11/2007).

4.4- Results

Under laboratory conditions *L. galapagensis* and *L. moorei* larvae hatch as typical caridean zoeae. Due to laboratory constraints, it was not possible to obtain the complete larval series for both species.

Concerning *L. galapagensis*, the larval series zoea I to zoea VII was obtained, lacking the last zoeae and the decapodid stages. The first and the seventh zoeal stages are described in detail, while for the second to fifth stages only differences of previous ones are described.

For *L. moorei*, zoea I to IV and the last zoeal stage were obtained. The first and the last zoeal stages are described in detail, and as in *L. galapagensis*, for the second to fourth zoeae only the differences of previous stages are assigned.

***Lysmata galapagensis* Schmitt, 1924**

Figures 1-6

First zoea (Figure 1)

Dimension: TL = 2.46–2.89 mm; CL = 0.92–1.00 mm.

Carapace (Figure 1A): rostrum slender and pointed, longer than the antennular peduncle, eyes compound and sessile; 1 pterigostomial spine and 4 denticles along anterior-ventral margin.

Antennule (Figure 1B): peduncle unsegmented, bears terminally 1 long plumose seta and a small process; short outer flagellum with 1 plumose seta, 3 long aesthetascs and 1 short and stout aesthetasc that ends in a spoon shaped membrane with thickened midrib on distal margin.

Antenna (Figure 1C): protopod unsegmented; endopod apically with 1 long plumose seta and 1 short spine; scaphocerite 5-segmented, 4 short segments distally, with 9 plumose setae on inner margin, 2 plumose setae on outer margin, and a simple small seta on apex.

Mandibles (Figure 1D): slightly asymmetrical, palp absent, armature of incisor and molar processes as illustrated.

Maxillule (Figure 1E): coxal endite with 2 serrulate, 2 papposerrate and 2 simple setae, basial endite with 3 cuspidate and 2 simple setae; endopod with 2 strong subterminal and 3 strong terminal papposerrate setae.

Maxilla (Figure 1F): coxal endite bilobed with 7-8+4 serrulate and papposerrate setae, basial endite bilobed with 3-4+4 papposerrate setae; endopod unsegmented bearing 3+2+1+3 papposerrate and simple setae; scaphognathite with 5 marginal plumose setae.

First maxilliped (Figure 1G): coxa with 4-5 papposerrate setae; basis with 10-12 papposerrate setae; endopod 4-segmented with 3, 1, 2, 3 papposerrate and 1 simple setae; exopod unsegmented, bearing subapically 1 shorter seta on lateral margin and 3 long plumose setae terminally.

Second maxilliped (Figure 1H): basis with 1+1+2 papposerrate setae; endopod 4-segmented with 3, 1, 2, 5 papposerrate and 1 simple setae terminally; exopod 4-segmented, bearing 2, 2, 2, 3 plumose setae.

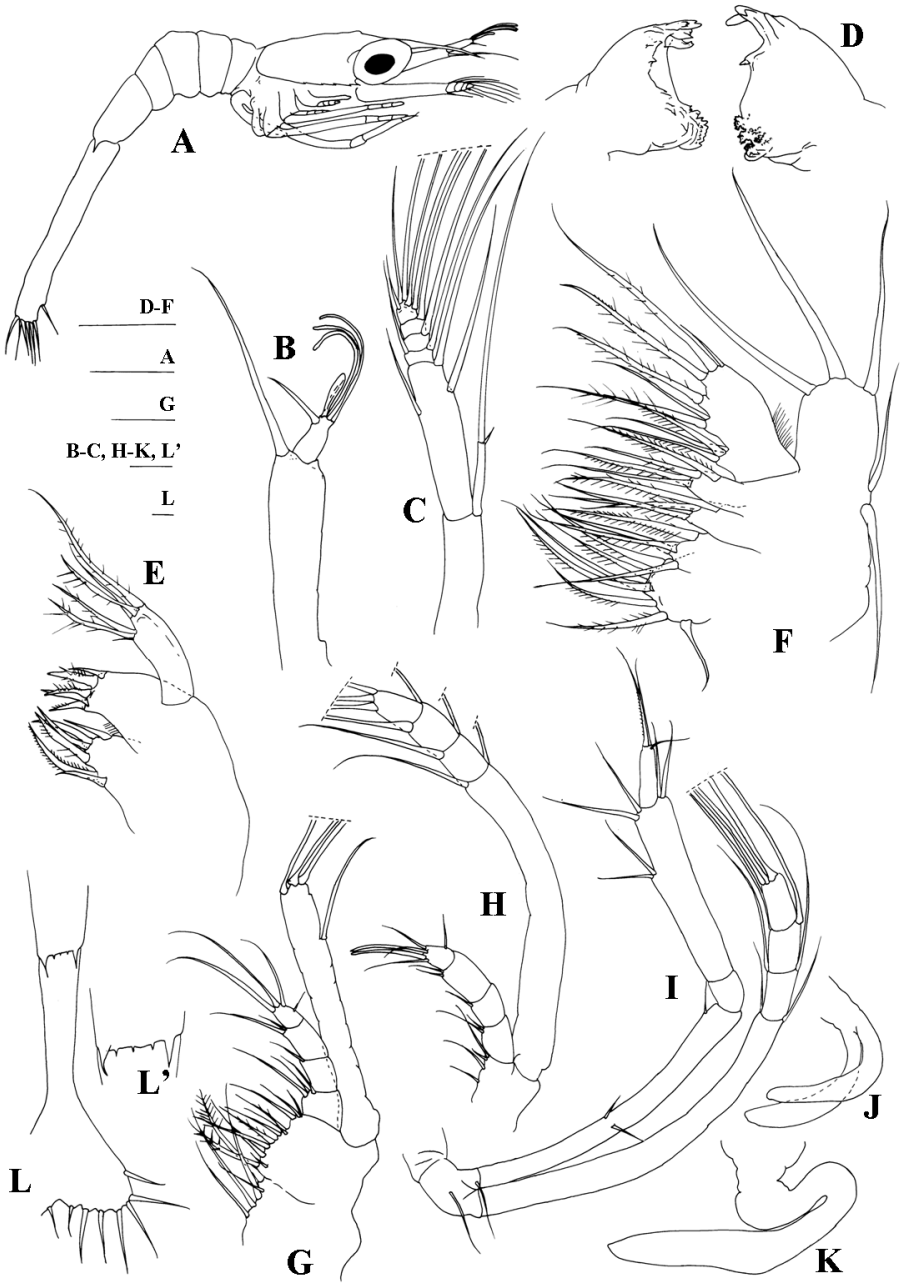

Fig. 1- *Lysmata galapagensis*. First zoea: **A**, total animal, lateral view; **B**, antennule; **C**, antenna; **D**, mandibles; **E**, maxillule; **F**, maxilla; **G**, first maxilliped; **H**, second maxilliped; **I**, third maxilliped; **J**, first pereiopod; **K**, fifth pereiopod; **L**, 5th pleomere and telson; **L'**, detail of the 5th pleomere. Scale bars: 0.5 mm (**A**); 0.1 mm (**B-L'**).

Third maxilliped (Figure 1I): basis with 2-3 papposerrate setae; endopod 4-segmented, with 2+1, 0, 2+4, 2 papposerrate and 1 simple setae; exopod 4-segmented, bearing 2, 2, 2, 3 plumose setae.

First pereiopod (Figure 1J): biramous bud.

Second to fourth pereiopods: absent.

Fifth pereiopod (Figure 1K): uniramous bud.

Pleon (Figures 1A, L, L'): 5 pleomeres; 2 dorso-lateral spines and 3-4 very small dorsal spines on posterior margin of the 5th pleomere.

Pleopods: absent.

Uropods: absent.

Telson (Figure 1L): triangular, broader posteriorly; indented medially with 7+7 setae (inner 5 plumose, outer 2 plumose on proximal axis only); minute spines between and around setae.

Second zoea (Figures 2A–G)

Dimension: TL = 2.81–3.34 mm; CL = 0.89–1.19 mm.

Carapace (Figures 2A, B): eyes stalked, with peduncle shorter than antennal peduncle; rostrum long and slender, almost as long as carapace, with 2-3 dorsal teeth and 1 post rostral spine; with 1 pair of supraorbital, 1 pair of antennal and 1 pair of pterigostomial spines followed by 4-5 denticles on anterior ventral margin.

Antennule (Figures 2B, C): peduncle unsegmented bearing sub distally 3-4 short and distally 2 short+1 long plumose setae; outer flagellum with 4 long+1 short spoon shaped membranous aesthetascs and 1 simple seta.

Antenna (Figures 2B, D): protopod unsegmented; endopod small pointed conical shaped; scaphocerite 4-segmented, 3 short segments distally, with 11-12 plumose setae on inner margin, 2 plumose setae on outer margin and 1 simple small seta on apex.

Mandibles: unchanged.

Maxillule: coxal endite with 6-7 serrulate, papposerrate and simple setae; basial endite with 6-7 strong cuspidate and simple setae; endopod with 2 papposerrate and 1 short simple subterminal setae and 3 strong terminal papposerrate setae.

Maxilla: coxal endite bilobed with 7-9+4 serrulate and papposerrate setae; scaphognathite with 8 marginal plumose setae. Otherwise unchanged.

First maxilliped: coxa with 5 papposerrate setae; exopod with 1 subapical shorter seta on lateral margin and 4 long plumose setae terminally. Otherwise unchanged.

Second maxilliped: basis with 1+1+2-3 papposerrate setae; exopod 4-segmented, bearing 2, 2, 2, 4 plumose setae. Otherwise unchanged.

Third maxilliped: basis with 3 papposerrate setae; exopod 4-segmented, bearing 2, 2, 2, 4 plumose setae. Otherwise unchanged.

First pereiopod (Figures 2E, E'): functional; biramous; basis with 1 simple seta; endopod 4-segmented, with 2+1, 0, 4, 2 papposerrate and 1 simple setae; exopod 3-segmented with 2, 2, 4 plumose setae.

Second pereiopod (Figures 2E'', F): present as a very small biramous bud.

Third and fourth pereiopods: absent.

Fifth pereiopod (Figures 2F, F', F''): functional; uniramous; almost as long as the total larva; basis with 1 simple seta; ischium naked; merus with one spinous process and 2 strong simple setae terminally; carpus with 2 simple+ 4 simple terminal setae; propodus measuring approximately one third of whole pereiopod, flattened, and paddle-like enlarged with margins serrated (17-20 and 19-22 spines on each margin), 2-4 small pappose setae along the surface,

and bearing 2 strong simple setae distally; dactylus small with 1 strong spine, 1 simple subterminal and 1 strong simple terminal setae.

Pleon (Figures 2A, A', A''): third pleomere dorsally with a small protuberance and 2 pairs of simple setae; fifth pleomere with 2 dorso-lateral spines and 0-2 very small dorsal spines on the posterior margin.

Pleopods: absent.

Uropods: absent.

Telson (Figure 2G): 8+8 plumose setae, the outer 2 plumose on proximal axis only.

Third zoea (Figures 2H-M'')

Dimension: TL = 3.50–3.77 mm; CL = 1.29–1.56 mm.

Carapace: ocular peduncle as long as antennal peduncle, rostrum as long as carapace, with 3-4 dorsal teeth and 1 post rostral spine; with 1 pair of supraorbital, 1 pair of antennal and 1 pair of pterigostomial spines followed by 3-4 denticles on ventral margin.

Antennule (Figure 2H): peduncle 2-segmented; first segment with 2 small plumose setae proximally, 2-3 small plumose setae positioned at four fifths of the length of the segment, and 5 small plumose setae distally; distal segment with 5-6 plumose+ 1 simple setae; inner flagellum with 1 short plumose seta; outer flagellum with 1 small simple+ 1 long plumose setae+ 2 long aesthetascs.

Antenna (Figure 2I): scaphocerite 3-segmented; proximal segment with 8 plumose setae on inner margin, 1 small simple seta proximally and 1 strong spine followed by a plumose seta distally; second segment with 1 plumose seta on inner margin; distal segment with 4 long plumose setae and 1 strong spine on apex. Otherwise unchanged.

Mandibles: unchanged besides size.

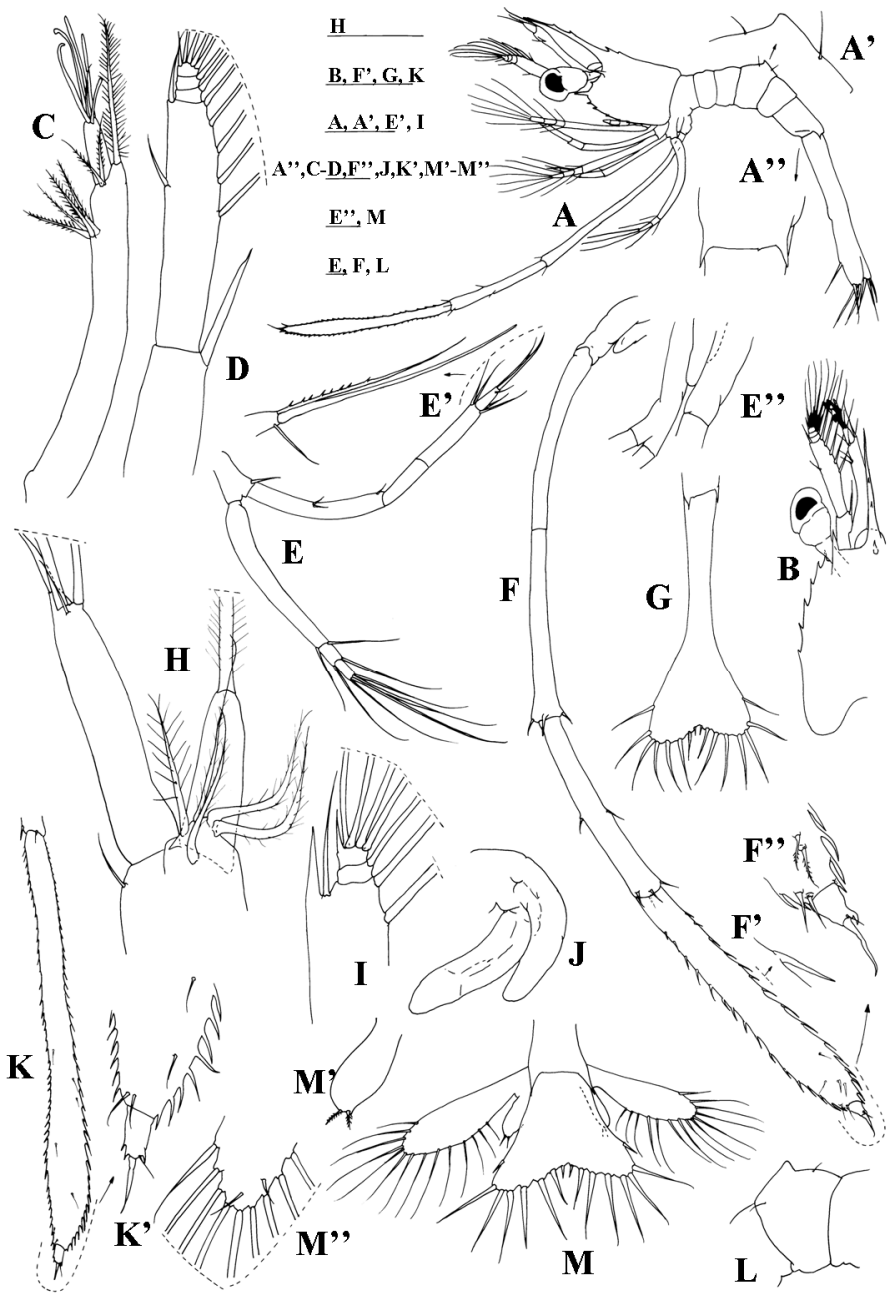

Fig. 2- *Lysmata galapagensis*. Second zoea: **A**, total animal, lateral view; **A'**, detail of 3rd pleomere, lateral view; **A''**, detail of 5th pleomere, dorsal view; **B**, carapace, dorsal view; **C**, antennule; **D**, antenna; **E**, first pereiopod; **E'**, detail of first pereiopod dactylus setae; **E''**, detail of basis of pereiopods; **F**, fifth pereiopod; **F'**, detail of fifth pereiopod propodus lateral spine; **F''**, detail of fifth pereiopod end of propodus and dactylus; **G**, 5th pleomere and telson. Third zoea: **H**, detail of antennule flagella; **I**, detail of scaphocerite distal segments; **J**, second pereiopod; **K**, fifth pereiopod; **K'**, detail of fifth pereiopod propodus and dactylus; **L**, detail of 3rd pleomere, lateral view; **M**, telson and uropods; **M'**, endopod of uropods; **M''**, exopod of uropods. Scale bars: 0.5 mm (**A**, **B**, **G**, **K**); 0.1 mm (**A'-A''**, **C-F''**, **H-J**, **K'-M''**).

Maxillule: coxal endite with 7-8 serrulate, papposerrate and simple setae; basial endite with 7 strong cuspidate and simple setae. Otherwise unchanged.

Maxilla: coxal endite bilobed with 8-9+4 serrulate and papposerrate setae; scaphognathite with 8-9 marginal plumose setae. Otherwise unchanged.

First maxilliped: coxa with 5 papposerrate setae; basis with 11-12 papposerrate setae. Otherwise unchanged.

Second maxilliped: basis with 1+1+3 papposerrate setae. Otherwise unchanged.

Third maxilliped: basis with 2 papposerrate setae. Otherwise unchanged.

First pereiopod: endopod 4-segmented, with 2+1, 1, 4, 2 papposerrate and 1 simple setae; exopod 4-segmented with 2, 2, 2, 4 plumose setae.

Second pereiopod (Figure 2J): present as a biramous bud.

Third and fourth pereiopods: absent.

Fifth pereiopod (Figures 2K, K'): longer than the whole larva; basis without any seta; ischium with 0-2 simple setae; merus with 3-4 simple setae and one spinous process+ 2 strong simple setae terminally; carpus with 14-17 simple setae; propodus measuring one third of whole pereiopod, flattened and paddle-like enlarged with margins serrated (34-43 and 36-47 spines on each margin), 5-8 small pappose setae along the surface, and 2 simple setae distally; dactylus small with 2-3 strong spines, 1 simple subterminal and 1 strong simple terminal setae.

Pleon (Figure 2L): third pleomere dorsally with a small anteriorly curved spine and 2 pairs of simple setae; fifth pleomere with 2 dorso-lateral spines; sixth pleomere separated from telson, with one pair of dorso-lateral spines.

Pleopods: absent.

Uropods (Figures 2M, M', M''): biramous; endopod small with 2 short plumose setae apically; exopod well developed reaching the end of telson, with 13-14 marginal plumose setae and 2 plumose setae on dorsal margin.

Telson (Figure 2M): separated of the sixth pleomere, with one pair of lateral spines followed by 7+7 plumose setae.

Fourth zoea (Figure 3)

Dimension: TL = 4.14–4.63 mm; CL = 1.61–2.15 mm.

Carapace (Figures 3A): eye with two thirds of antennal length, rostrum one and a half times longer than carapace, with 5-6 dorsal teeth and 1 post rostral spine; with 1 pair of supraorbital, 1 pair of antennal and 1 pair of pterigostomial spines followed by 3-4 denticles on ventral margin.

Antennule (Figures 3A, B): peduncle 2-segmented; first segment with 4 plumose setae distributed along inner margin, 5-7 small plumose setae on stylocerite, 4-5 small plumose setae positioned at four fifths of the length of the segment, and 4-5 small plumose setae distally; distal segment with 4-6 plumose+ 1 simple setae; inner flagellum with 1 plumose+ 2 simple setae; outer flagellum with 2 subterminal aesthetascs, and 1 small simple+ 1 long plumose setae terminally.

Antenna (Figures 3A, C): endopod short and triangular shaped; scaphocerite unsegmented; inner margin with 16-17 plumose setae; 1 small simple seta proximally on outer margin; a very strong spine on apex.

Mandibles (Figure 3D): incisor and molar processes as illustrated.

Maxillule: unchanged besides size.

Maxilla: scaphognathite with 12 marginal plumose setae. Otherwise unchanged.

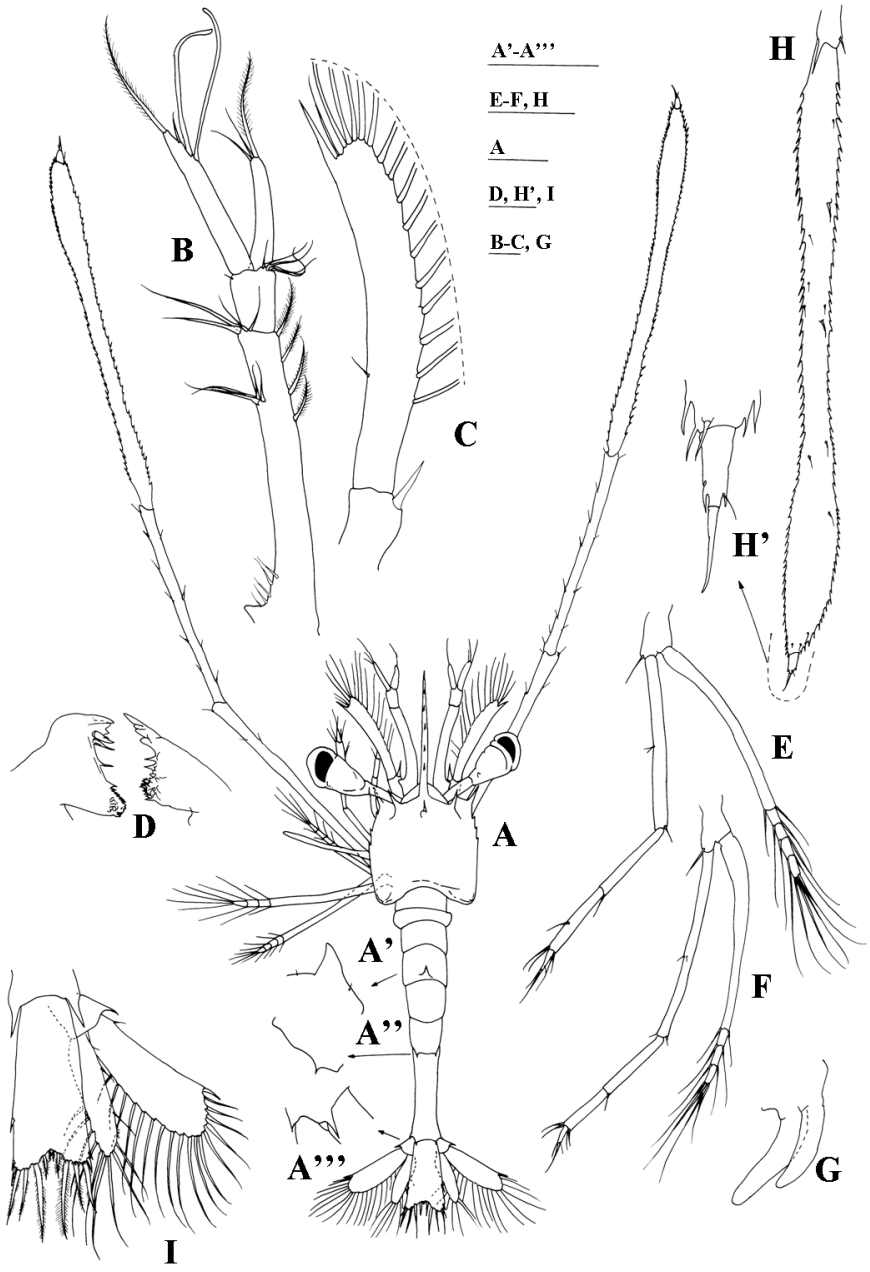

Fig. 3- *Lysmata galapagensis.* Fourth zoea: **A**, total animal, dorsal view; **A'**, third pleomere, lateral view of procurved spine; **A''**, fifth pleomere, lateral view of dorso-lateral spines; **A'''**, sixth pleomere, lateral view of dorsal- and ventral-lateral spines; **B**, antennule; **C**, antenna; **D**, mandibles; **E**, first pereiopod; **F**, second pereiopod; **G**, third pereiopod; **H**, fifth pereiopod, end of propodus and dactylus; **H'**, detail of fifth pereiopod end of propodus and dactylus; **I**, telson and uropods. Scale bars: 0.5 mm (**A-A'''**, **D-F**, **H**, **I**); 0.1 mm (**B-C**, **G**, **H'**).

First maxilliped: basis with 12 papposerrate setae. Otherwise unchanged.

Second maxilliped: unchanged besides size.

Third maxilliped: exopod 5-segmented, bearing 2, 2, 2, 2, 4 plumose setae. Otherwise unchanged.

First pereiopod (Figure 3E): endopod 4-segmented, with 2+1, 1, 1+4, 2 papposerrate and 1 simple setae; exopod 5-segmented with 2, 2, 2, 2, 4 plumose setae.

Second pereiopod (Figure 3F): functional; biramous; basis with 1 simple seta; endopod 4-segmented, with 2+2, 0-1, 4, 2 papposerrate and 1 simple setae; exopod 4-segmented with 2, 2, 2, 4 plumose setae.

Third pereiopod (Figure 3G): present as a very small biramous bud.

Fourth pereiopod: absent.

Fifth pereiopod (Figures 3H, H'): one and a half times longer than the whole larva; basis with 0-1 simple seta; ischium with 1-2 simple setae; merus with 6-10 simple setae and one spinous process; carpus with 18-22 simple setae and one spinous process; propodus measuring two fifhths of the whole pereiopod, flattened and paddle-like enlarged with margins serrated (52-59 and 53-59 spines on each margin), 11-14 small pappose setae along the surface, and 2 strong simple setae distally; dactylus small with 2-3 strong spines, 1 simple subterminal and 1 strong simple terminal setae.

Pleon (Figures 3A, A', A'', A'''): third pleomere dorsally with an anteriorly curved spine and 2 pairs of simple setae; fifth pleomere with 2 dorso-lateral spines; sixth pleomere separated from telson, with one pair of dorso-lateral spines and one pair of small ventral-lateral spines.

Pleopods: absent.

Uropods (Figures 3A, I): protopod without setae, with a spine; endopod with 1-2 short plumose setae proximally on outer margin and 10-12 plumose setae along distal and inner

margins; exopod as long as telson, with 1 short plumose seta proximally on outer margin, 1 spine on apex followed by 16-18 marginal plumose setae along distal and inner margins, and 2-3 short plumose setae on dorsal margin.

Telson (Figures 3A, I): almost rectangular shaped, with one pair of lateral spines, 1 pair of outer spines and 6 pairs of plumose setae on the posterior end.

Fifth zoea (Figures 4 A-G)

Dimension: TL = 6.08–6.40 mm; CL = 2.88–3.12 mm.

Carapace (Figure 4A): rostrum now measuring twice over the carapace, with 6 dorsal teeth, 1 post rostral spine; with 1 pair of supraorbital, 1 pair of antennal and 1 pair of pterigostomial spines. No denticles on ventral margin.

Antennule (Figure 4A): peduncle 2-segmented; first segment with 4-5 plumose setae distributed along inner margin, 6 small plumose setae on stylocerite, 5 small plumose setae positioned at two thirds the length of the segment, and 5 small plumose setae distally; distal segment with 5 plumose+ 1 simple setae. Inner flagellum 2-segmented, proximal segment naked, distal segment with 2 small simple+ 1 plumose setae. Outer flagellum 2-segmented, proximal segment naked, distal segment with 3 subterminal aesthetascs+ 2 small simple and 1 plumose terminal setae.

Antenna (Figure 4A): inner margin with 20-22 plumose setae; 1 small simple seta proximally on outer margin; a very strong spine on apex.

Mandibles: unchanged besides size.

Maxillule (Figure 4B): coxal endite with 8-9 serrulate, papposerrate and simple setae; basial endite with 8 strong cuspidate and simple setae. Otherwise unchanged.

Maxilla (Figure 4C): coxal endite bilobed with 9+4 serrulate and papposerrate setae; scaphognathite with 16-19 marginal plumose setae. Otherwise unchanged.

First maxilliped: basis with 12-13 papposerrate setae; epipod bud present. Otherwise unchanged.

Second maxilliped: unchanged besides size.

Third maxilliped (Figures 4D, D'): basis with 3 papposerrate setae; endopod 4-segmented, with 2+1, 1, 1+2+4, 2 papposerrate and 1 simple setae; exopod 7-segmented, bearing 2, 2, 2, 2, 2, 2, 4 plumose setae. Otherwise unchanged.

First pereiopod: exopod 7-segmented, each segment with 2 and the last one with 4 plumose setae. Otherwise unchanged.

Second pereiopod: exopod 7-segmented, each segment with 2 and the last one with 4 plumose setae. Otherwise unchanged.

Third pereiopod (Figure 4E): functional; biramous; basis with 1 simple seta; endopod 4-segmented, with 1+2, 2, 1+4, 2 papposerrate and 1 simple setae; exopod 5-segmented with 2, 2, 2, 2, 4 plumose setae.

Fourth pereiopod (Figure 4F): present as a biramous bud.

Fifth pereiopod: five thirds longer than the whole larva; carpus with 22-23 simple setae and one spinous process; propodus flattened and paddle-like enlarged with margins serrated (54-56 and 56-67 spines on each margin), 10-14 small pappose and 3-4 short strong plumose setae along the surface, and 2 strong simple setae distally; dactylus small with 3 strong spines, 1 simple subterminal and 1 strong simple terminal setae. Otherwise unchanged.

Pleon: anteriorly curved spine in the third pleomere increased in size; fifth pleomere now without spines; sixth pleomere with one pair of dorso-lateral spines and one pair of small ventral-lateral spines.

Pleopods: absent.

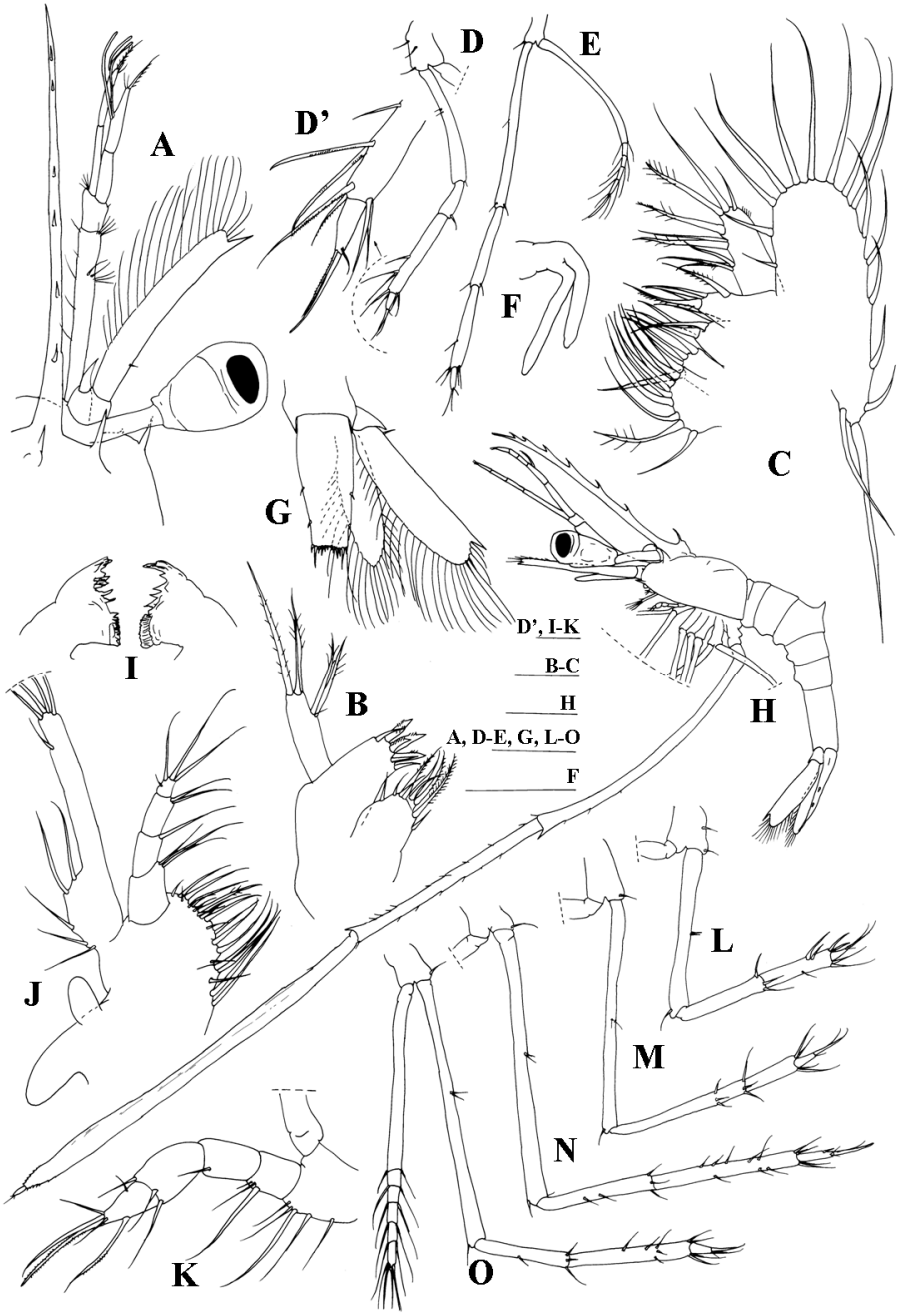

Fig. 4- *Lysmata galapagensis*. Fifth zoea: **A**, carapace, dorsal view; **B**, maxillule; **C**, maxilla; **D**, third maxilliped; **D'**, third maxilliped, detail of propodus and dactylus; **E**, third pereiopod; **F**, fourth pereiopod; **G**, telson and uropods. Sixth zoea: **H**, total animal, lateral view; **I**, mandibles; **J**, first maxilliped; **K**, second maxilliped; **L**, first pereiopod; **M**, second pereiopod; **N**, third pereiopod; **O**, fourth pereiopod. Scale bars: 1 mm (**H**); 0.5 mm (**A**, **D**, **E-G**, **L-O**); 0.1 mm (**B-C**, **D'**, **I-**K).

Uropods (Figure 4G): endopod longer than telson, endopod with 3 short plumose setae proximally on outer margin and 20-22 plumose setae along distal and inner margins; exopod with 5 short plumose setae proximally on outer margin, 1 spine on apex followed by 24-26 marginal plumose setae along distal and inner margins, and 4-6 short plumose setae on dorsal margin.

Telson (Figure 4G): margins laterally parallel, slightly narrower posteriorly, with two pairs of lateral spines, and 6 pairs of short plumose setae on the posterior end.

Sixth zoea (Figures 4 H-O)

Dimension: TL = 6.64–7.68 mm; CL = 3.28–4.00 mm.

Carapace (Figure 4H): eyes still with two thirds of antennal length, presence of one pair of cervical carinae, rostrum measuring twice over the carapace with 6-8 dorsal teeth, 1 post rostral spine; with 1 pair of supraorbital, 1 pair of antennal and 1 pair of pterigostomial spines.

Antennule: peduncle 2-segmented, first segment with 6-7 plumose setae distributed along inner margin, 7-9 small plumose setae on stylocerite, 5-6 small plumose setae positioned at two thirds of the length of the segment, and 4-5 small plumose setae distally; distal segment with 5-6 plumose+1 simple setae. Inner flagellum 4-segmented, proximal segment with 1-2 small simple setae, second segment with 1-2 small simple setae, third segment with 1-3 small simple setae, and distal segment with 3-4 short simple+1 plumose setae. Outer flagellum 5-segmented, proximal segment with 0-1 seta, second segment with 1-2 short simple setae and 3 aesthetascs, third segment with 0-1 simple seta and 4 aesthetascs, fourth segment with 2-3 short simple setae, and distal segment with 3 short simple+1 plumose setae.

Antenna: endopod reaching half the length of antennal scale, 2-segmented, proximal segment naked, distal segment with 3 short simple setae terminally; inner margin with 24-25 plumose setae. Otherwise unchanged.

Mandibles (Figure 4I): unchanged besides size.

Maxillule: coxal endite with 9-10 serrulate, papposerrate and simple setae; basial endite with 9 strong cuspidate and simple setae. Otherwise unchanged.

Maxilla: coxal endite bilobed with 8-9+4 serrulate and papposerrate setae; scaphognathite with 26-28 marginal plumose setae. Otherwise unchanged.

First maxilliped (Figure 4J): coxal endite with 4-5 papposerrate setae; basial endite with 14-15 papposerrate setae; exopod unsegmented with 3-5 plumose setae on proximal lobe, 1 short subterminal+ 4 long plumose setae on distal margin. Otherwise unchanged.

Second maxilliped (Figure 4K): basis with 1+1+2-3 papposerrate setae; endopod 4-segmented with 3, 1, 3, 5 papposerrate and 1 simple setae terminally. Otherwise unchanged.

Third maxilliped: basis with 3 papposerrate setae; endopod 4-segmented, with 2+1, 2, 7+4, 2 papposerrate and 1 simple setae; exopod 7-segmented, 1^{st} segment with 1+2, 2^{nd} to 7^{th} segments with 2 and the last one with 4 plumose setae. Otherwise unchanged.

First pereiopod (Figure 4L): endopod 4-segmented, with 2+1, 3, 3-4+4, 2 papposerrate and 1 simple setae; exopod 9-segmented, each with 2 and the last one with 4 plumose setae. Otherwise unchanged.

Second pereiopod (Figure 4M): endopod 4-segmented, with 2+2, 3+3, 4, 2 papposerrate and 1 simple setae; exopod 9-segmented, each with 2 and the last one with 4 plumose setae. Otherwise unchanged.

Third pereiopod (Figure 4N): endopod 4-segmented, with 2+2, 5, 8-10+4, 3 papposerrate and 2 simple setae; exopod 8-segmented, each with 2 and the last one with 4 plumose setae. Otherwise unchanged.

Fourth pereiopod (Figure 4O): functional; biramous; basis with 1 simple seta; endopod 4-segmented, with 2+1, 1+3, 5-6+4, 2 papposerrate and 1 simple setae; exopod 6-segmented, each with 2 and the last one with 4 plumose setae.

Fifth pereiopod (Figure 4H): five thirds longer than the whole larva; merus with 10-12 simple setae and one spinous process; carpus with 21-27 simple setae and one spinous process; propodus length remains as two fifths of the whole pereiopod, flattened and paddle-like enlarged with margins serrated (56-62 and 58-73 spines on each margin), 7-10 small pappose setae along the surface, and 2 strong simple setae distally; dactylus small with 2-3 strong spines, 2 simple subterminal and 1 strong simple terminal setae. Otherwise unchanged.

Pleon (Figure 4H): anteriorly curved spine in the third pleomere increased in size. Otherwise unchanged.

Pleopods (Figure 4H): presence of small protuberances in pleomeres.

Uropods: endopod with 3 short plumose setae proximally on outer margin and 24-27 plumose setae along distal and inner margins; exopod with 3-5 short plumose setae proximally on outer margin, 1 spine on apex followed by 29-31 marginal plumose setae along distal and inner margins, and 5-6 short plumose setae on dorsal margin.

Telson: narrowing towards the posterior end, with one pair of lateral spines followed by 1 pair of outer spines and 6 pairs of short setae of which the inner 4 pairs are plumose and the outer 2 pairs are simple.

Seventh zoea (Figure 5)

Dimension: TL = 8.24–8.48 mm; CL = 4.08–4.32 mm.

Carapace (Figure 5A, B): rostrum measuring more than twice over the carapace with 7 dorsal teeth; otherwise unchanged besides size.

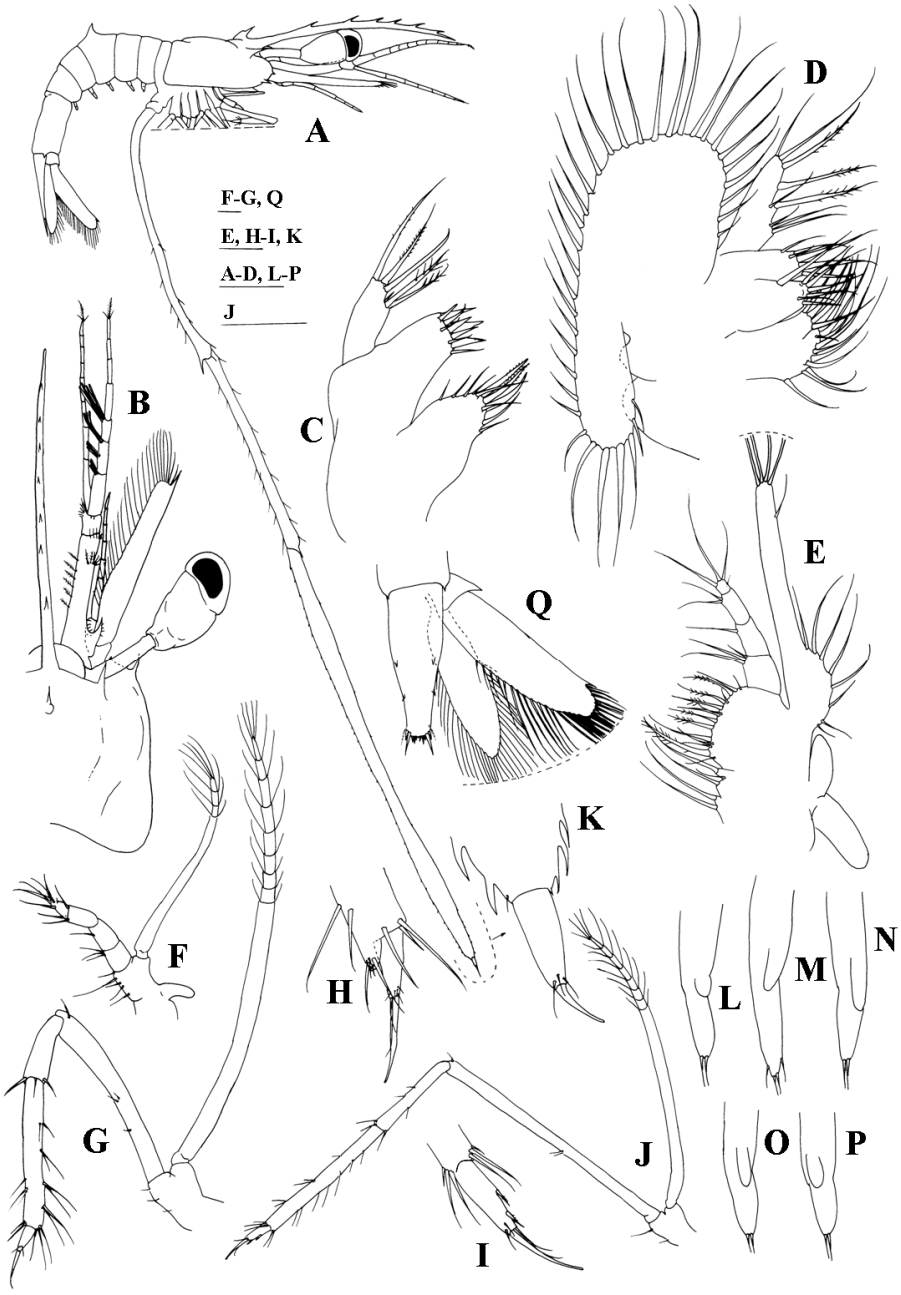

Fig. 5- *Lysmata galapagensis.* Seventh zoea: **A**, total animal, lateral view; **B**, carapace, dorsal view; **C**, maxillule; **D**, maxilla; **E**, first maxilliped; **F**, second maxilliped; **G**, third maxilliped; **H**, first pereiopod; **I**, third pereiopod; **J**, fourth pereiopod; **K**, fifth pereiopod, propodus and dactylus; **L**, first pleopod; **M**, second pleopod; **N**, third pleopod; **O**, fourth pleopod; **P**, fifth pleopod; **Q**, telson and uropods. Scale bars: 1 mm (**A**); 0.5 mm (**B**, **J**); 0.1 mm (**C-I**, **K-Q**).

Antennule (Figure 5B): peduncle 2-segmented, first segment with 6 plumose setae distributed along inner margin, 9 small plumose setae on stylocerite, 6 small plumose setae at two thirds of the length of the segment, and 6 small plumose setae distally; distal segment with 5 plumose+2 simple setae. Inner flagellum 8-segmented, proximal and second segments with 4 small simple setae each, third segment with 3 simple+1 plumose short setae, fourth and fifth segments with 3 and 4 small simple setae respectively, sixth segment with 4 simple+1 plumose short setae, seventh segment with 4 short simple setae, and distal segment with 2 short+2 longer simple+1 plumose setae. Outer flagellum 8-segmented, proximal segment with 2 simple setae and 3 aesthetascs, second segment with 3 aesthetascs in a middle position+3 short simple setae and 3 aesthetascs terminally, third segment with 3 simple setae and 4 aesthetascs, fourth to sixth segments with 4, 5 and 3 short simple setae respectively, and distal segment with 3 short simple+1 plumose setae.

Antenna (Figure 5B): flagellum 8-segmented, shorter than scaphocerite, with 2 smaller segments proximally, 3rd segment the longer one followed by 5 short segments distally, all bearing 3-5 simple setae; scaphocerite now with 28 plumose setae.

Mandibles: enlarged in size.

Maxillule (Figure 5C): coxal endite with 9-11 serrulate, papposerrate and simple setae; basial endite with 10-11 strong cuspidate and simple setae. Otherwise unchanged.

Maxilla (Figure 5D): coxal endite bilobed with 10-11+4 serrulate and papposerrate setae; basial endite with 7+6 papposerrate setae; scaphognathite with 36 marginal plumose setae. Otherwise unchanged.

First maxilliped (Figure 5E): coxal endite with 5 papposerrate setae; basial endite with 15-17 papposerrate setae; exopod unsegmented with 7-9 plumose setae on proximal lobe, 1 short subterminal+ 4 long plumose setae on distal margin; epipod bilobed. Otherwise unchanged.

Second maxilliped (Figure 5F): basis with 1+1+3 papposerrate setae; epipod bud present. Otherwise unchanged.

Third maxilliped (Figure 5G): endopod 4-segmented, with 4, 3, 15-16, 2 papposerrate and 2 simple setae; exopod 9-segmented, each segment with 2 and the last one with 4 plumose setae. Otherwise unchanged.

First pereiopod (Figure 5H): endopod subchelate, with internal distal margin of propodus produced forward to about one third of dactylus, 4-segmented, with 2+1, 1+3, 13, 7 papposerrate and simple setae; exopod 10-segmented, each segment with 2 and the last one with 4 plumose setae. Otherwise unchanged.

Second pereiopod: endopod 4-segmented, with 4, 7, 5, 5 papposerrate and simple setae; exopod 10-segmented, each segment with 2 and the last one with 4 plumose setae. Otherwise unchanged.

Third pereiopod (Figure 5I): endopod 4-segmented, with 5, 5, 21, 6 papposerrate and simple setae; exopod 10-segmented, each segment with 2 and the last one with 4 plumose setae. Otherwise unchanged.

Fourth pereiopod (Figure 5J): basis with 2 simple seta; endopod 4-segmented, with 2+1, 6, 18, 5 papposerrate and simple setae; exopod 8-segmented, each segment with 2 and the last one with 4 plumose setae.

Fifth pereiopod (Figure 5K): propodus flattened and paddle-like enlarged with margins serrated (60 and 80 spines on each margin), 7 small pappose setae along the surface, and 7 simple setae distally; dactylus small with 3 simple subterminal and 1 strong simple terminal setae. Otherwise unchanged.

Pleon (Figure 5A): anteriorly curved spine in the third pleomere increased in size. Otherwise unchanged.

Pleopods (Figure 5L-P): endopod rudimentary, bud-like; exopod with 2-4 apical simple setae.

Uropods (Figure 5Q): endopod with 6 short plumose setae proximally on outer margin and 32 plumose setae along distal and inner margins; exopod with 4 short plumose setae proximally on outer margin, 1 spine on apex followed by 33 marginal plumose setae along distal and inner margins, and 5 short plumose setae on dorsal margin.

Telson (Figure 5Q): narrowing towards the posterior end, bears one pair of lateral spines followed by 1 pair of plumose setae and 6 pairs of strong short setae.

Lysmata moorei (Rathbun, 1901)

Figures 6-9

First zoea (Figure 6)

Dimension: TL = 2.77–2.81 mm; CL = 0.92–0.96 mm.

Carapace (Figure 6A): rostrum slender and pointed, longer than the antennular peduncle, eyes compound and sessile; 1 pterigostomial spine and 4-5 denticles along anterior ventral margin.

Antennule (Figures 6B, B'): peduncle unsegmented, with 1 long plumose seta and a small process terminally; short outer flagellum with 1 plumose seta, 3 long aesthetascs and 1 short and stout aesthetasc that ends in a spoon shaped membrane with thickened midrib at distal margin.

Antenna (Figure 6C): protopod unsegmented; endopod apically with 1 long plumose seta and 1 short spine; scaphocerite 5-segmented, 4 short segments distally, with 9 plumose setae on inner margin, 2 plumose setae on outer margin, and a simple small seta on apex.

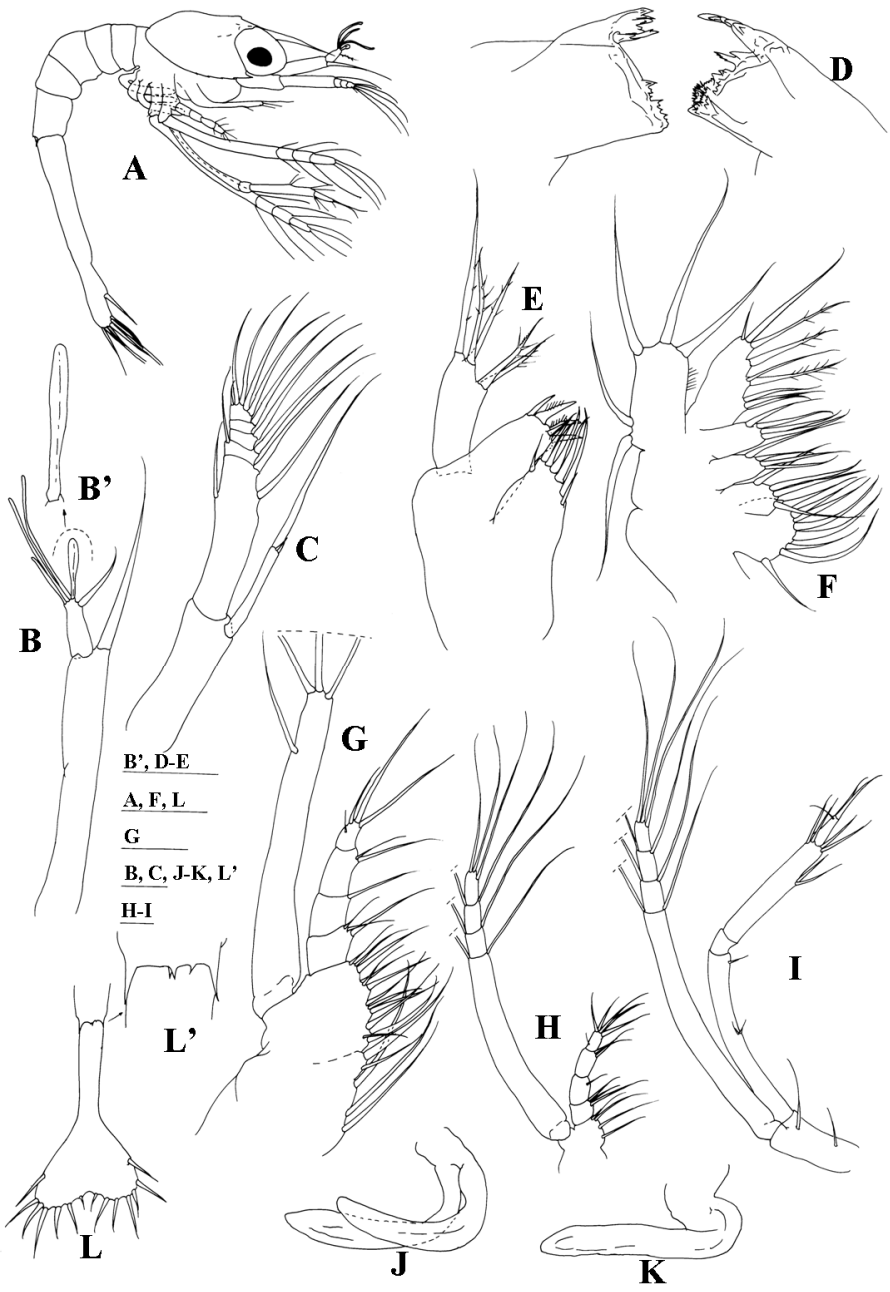

Fig. 6- *Lysmata moorei*. First zoea: **A**, total animal, lateral view; **B**, antennule; **B'**, antennule, detail of short aesthetasc; **C**, antenna; **D**, mandibles; **E**, maxillule; **F**, maxilla; **G**, first maxilliped; **H**, second maxilliped; **I**, third maxilliped; **J**, first pereiopod; **K**, fifth pereiopod; **L**, 5th pleomere and telson; **L'**, detail of the 5th pleomere. Scale bars: 0.5 mm (**A**, **L**); 0.1 mm (**B-K**, **L'**).

Mandibles (Figure 6D): asymmetrical, palp absent, armature of incisor and molar processes as illustrated.

Maxillule (Figure 6E): coxal endite with 2 serrulate, 3 papposerrate and 2 simple setae, basial endite with 3 cuspidate and 2 simple setae; endopod with 2 strong subterminal and 3 strong terminal papposerrate setae.

Maxilla (Figure 6F): coxal endite bilobed with 8+4 serrulate and papposerrate setae, basial endite bilobed with 4+4 papposerrate setae; endopod unsegmented bearing 3 + 2 + 1 + 3 papposerrate and simple setae; scaphognathite with 5 marginal plumose setae.

First maxilliped (Figure 6G): coxa with 5 papposerrate setae; basis with 12 papposerrate setae; endopod 4-segmented with 3, 1, 2, 3 papposerrate and 1 simple setae; exopod unsegmented, bearing subapically 1 shorter seta on lateral margin and 3 long plumose setae terminally.

Second maxilliped (Figure 6H): basis with 1+1+2-3 papposerrate setae; endopod 4-segmented with 3, 1, 2, 5 papposerrate and 1 simple setae terminally; exopod 4-segmented, bearing 2, 2, 2, 3 plumose setae.

Third maxilliped (Figure 6I): basis with 2 papposerrate setae; endopod 4-segmented, with 2+1, 0, 2+4, 2 papposerrate and 1 simple setae; exopod 4-segmented, bearing 2, 2, 2, 3 plumose setae.

First pereiopod (Figure 6J): biramous bud.

Second to fourth pereiopods: absent.

Fifth pereiopod (Figure 6K): uniramous bud.

Pleon (Figures 6L, L'): 5 pleomeres; 2 dorso-lateral spines and 3-4 very small dorsal spines on posterior margin of the 5th pleomere.

Pleopods: absent.

Uropods: absent.

Telson (Figure 6L): triangular, broader posteriorly; indented medially with 7+7 setae (inner 5 plumose, outer 2 plumose on proximal axis only); minute spines between and around setae.

Second zoea (Figures 7A-G')

Dimension: TL = 3.08–3.23 mm; CL = 1.00–1.04 mm.

Carapace (Figure 7A): eyes stalked, with peduncle longer than antennal peduncle; rostrum long and slender, as long as carapace, with 2-3 dorsal teeth and 1 post rostral spine; with 1 pair of supraorbital, 1 pair of antennal and 1 pair of pterigostomial spines followed by 3-4 denticles on anterior ventral margin.

Antennule (Figure 7B): peduncle unsegmented bearing proximally 1 short, followed by 4-5 short, and 2 short+1 long plumose setae distally; outer flagellum with 1 very small subterminal spine, 1 short simple seta, and 1 short+ 4 long aesthetascs.

Antenna (Figure 7C): protopod unsegmented; endopod small pointed conical shaped; scaphocerite 4-segmented, 3 short segments distally, with 12 plumose setae on inner margin, 2 plumose setae on outer margin and 1 simple small seta on apex.

Mandibles (Figure 7D): unchanged.

Maxillule: basial endite with 6-7 strong cuspidate and simple setae. Otherwise unchanged.

Maxilla: scaphognathite with 8 marginal plumose setae. Otherwise unchanged.

First maxilliped: exopod with 1 subapical shorter seta on lateral margin and 4 long plumose setae terminally. Otherwise unchanged.

Second maxilliped: exopod 4-segmented, bearing 2, 2, 2, 4 plumose setae. Otherwise unchanged.

Third maxilliped: exopod 4-segmented, bearing 2, 2, 2, 4 plumose setae. Otherwise unchanged.

First pereiopod (Figures 7E): functional; biramous; basis with 1 simple seta; endopod 4-segmented, with 2+1, 1, 4, 2 papposerrate and 1 simple setae; exopod 3-segmented with 2, 2, 3 plumose setae.

Second pereiopod (Figures 7E', F): present as a very small biramous bud.

Third and fourth pereiopods: absent.

Fifth pereiopod (Figures 7G): functional; uniramous; longer than the whole larva; basis without seta; ischium with 0-1 seta; merus with one spinous process and 1-3 simple setae; carpus with 7 simple setae; propodus measuring approximately one third of whole pereiopod, flattened and paddle-like enlarged with margins serrated (14-16 and 16-19 spines on each margin), and 5-6 small pappose setae along the surface; dactylus small with 1 simple subterminal and 1 strong simple terminal setae.

Pleon (Figure 7A): fifth pleomere with 2 dorso-lateral spines and 1-2 very small dorsal spines on the posterior margin.

Pleopods: absent.

Uropods: absent.

Telson (Figure 7A): 8+8 plumose setae, the outer ones plumose on proximal axis only.

Third zoea (Figures 7H-M')

Dimension: TL = 3.23–3.44 mm; CL = 1.02–1.24 mm.

Carapace (Figure 7H): eyes with two thirds of antennal length; rostrum a little shorter than carapace. Otherwise unchanged.

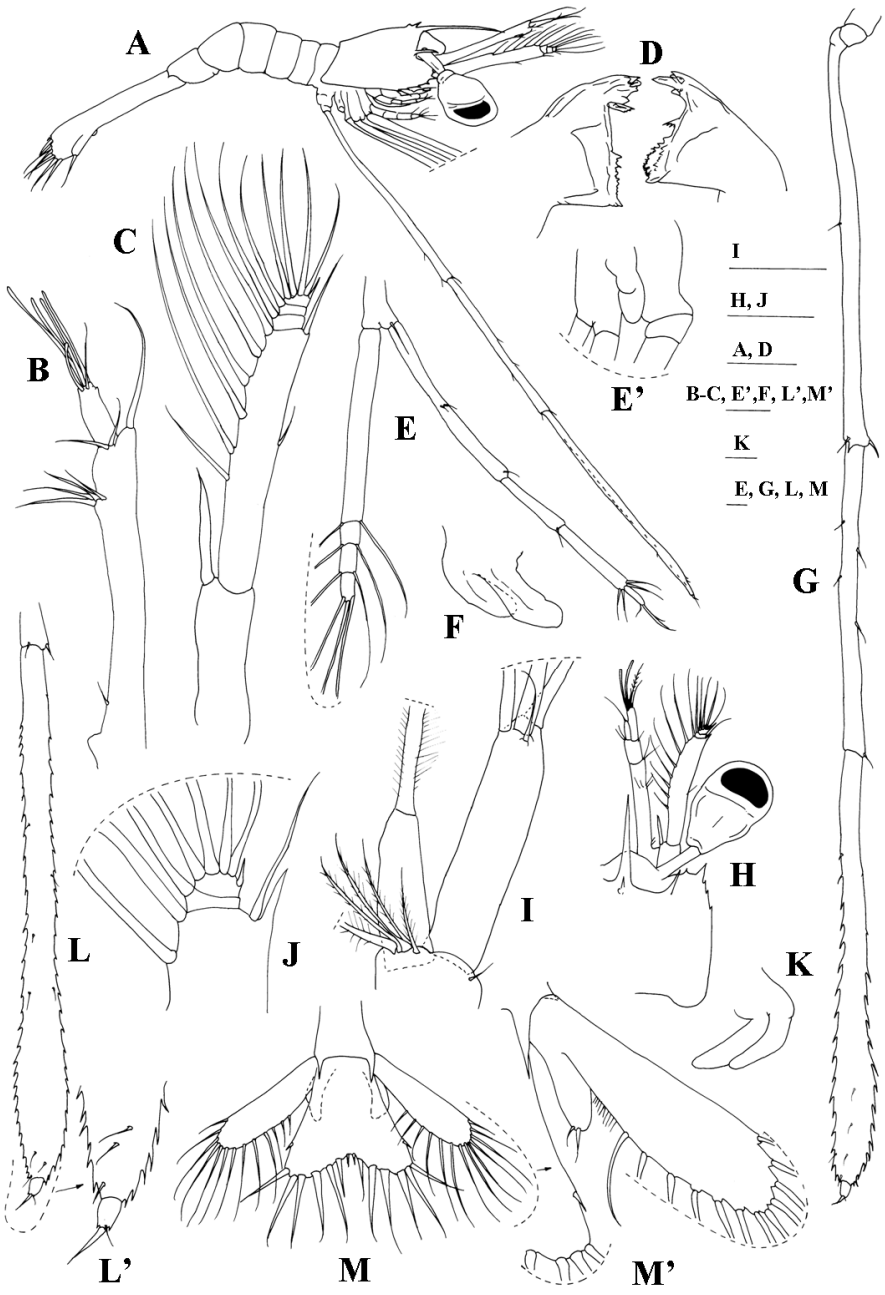

Fig. 7- *Lysmata moorei*. Second zoea: **A**, total animal, lateral view; **B**, antennule; **C**, antenna; **D**, mandibles; **E**, first pereiopod; **E'**, detail of basis of pereiopods; **F**, second pereiopod; **G**, fifth pereiopod. Third zoea: **H**, carapace, dorsal view; **I**, detail of antennule flagella; **J**, detail of scaphocerite distal segments; **K**, second pereiopod; **L**, fifth pereiopod; **L'**, detail of fifth pereiopod propodus and dactylus; **M**, telson and uropods; **M'**, detail of endopod and exopod of uropods. Scale bars: 0.5 mm (**A**, **H**); 0.1 mm (**B-G**, **I-M'**).

Antennule (Figures 7H, I): peduncle 2-segmented; first segment with 2 small plumose setae proximally, 2-3 small plumose setae positioned at one fourth of the length of the segment, and 4-5 small plumose setae distally; distal segment with 5-6 plumose+ 1 simple setae; inner flagellum with 1 short plumose seta; outer flagellum with 1 small simple+ 1 long plumose setae+ 2 long aesthetascs.

Antenna (Figures 7H, J): scaphocerite 3-segmented; proximal segment with 8 plumose setae on inner margin, 1 small simple seta proximally and 1 strong spine followed by a plumose seta distally; second segment with 1 plumose seta on inner margin; distal segment with 4 long plumose setae and 1 strong spine on apex. Otherwise unchanged.

Mandibles: unchanged besides size.

Maxillule: endopod with 2 papposerrate and 1 short simple subterminal setae and 3 strong terminal papposerrate setae. Otherwise unchanged.

Maxilla: unchanged besides size.

First maxilliped: unchanged besides size.

Second maxilliped: unchanged besides size.

Third maxilliped: endopod 4-segmented, with 2+1, 1, 2+4, 2 papposerrate and 1 simple setae. Otherwise unchanged.

First pereiopod: exopod 4-segmented with 2, 2, 2, 4 plumose setae. Otherwise unchanged.

Second pereiopod (Figure 7K): present as a biramous bud.

Third and fourth pereiopods: absent.

Fifth pereiopod (Figures 7L, L'): three halfs longer than the whole larva; merus with 4-5 simple setae and one spinous process; carpus with 10-13 simple setae; propodus measuring approximately two fifths of whole pereiopod, flattened and paddle-like enlarged with margins serrated (24-29 and 26-30 spines on each margin), 6-8 small pappose setae

along the surface; dactylus small with 2 strong spines, 1 simple subterminal and 1 strong simple terminal setae.

Pleon: third pleomere with one pair of dorsal simple setae; fifth pleomere with 2 small dorso-lateral spines; sixth pleomere separated from telson, with one pair of dorso-lateral spines and one pair of small ventral-lateral spines.

Pleopods: absent.

Uropods (Figures 7M, M'): biramous; endopod small with 2 short plumose setae apically; exopod well developed reaching the end of telson, with 12-13 marginal plumose setae and 2 plumose setae on dorsal margin.

Telson (Figure 7M): separated of the sixth pleomere, with one pair of lateral spines and 7 + 7 plumose setae.

Fourth zoea (Figure 8)

Dimension: TL = 3.44–3.66 mm; CL = 1.13–1.29 mm.

Carapace (Figures 8A, B): eyes with two thirds of antennal length, presence of one pair of cervical carinae, rostrum as long as the carapace, pair of pterigostomial spines, followed by 2-3 denticles on ventral margin. Otherwise unchanged.

Antennule (Figures 8A, C): peduncle 2-segmented; first segment with 4 plumose setae distributed along inner margin, 4-5 small plumose setae on stylocerite, 2-3 small plumose setae positioned at four fifths of the length of the segment, and 3-4 small plumose setae distally; distal segment with 5-6 plumose+ 1 simple setae; inner flagellum with 1 plumose seta; outer flagellum with 2 subterminal aesthetascs, and 1 small simple+ 1 long plumose setae terminally.

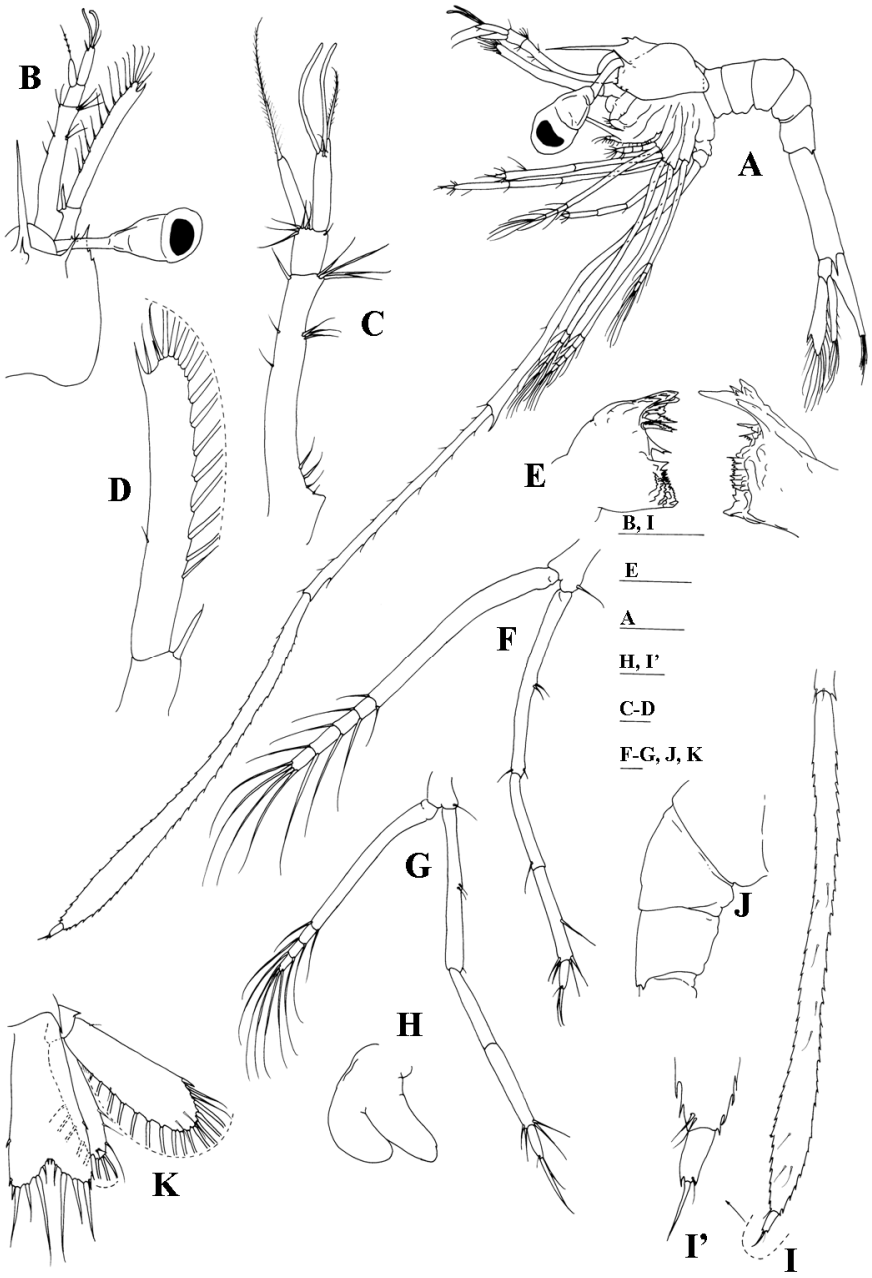

Fig. 8- *Lysmata moorei*. Fourth zoea: **A**, total animal, lateral view; **B**, carapace, dorsal view; **C**, antennule; **D**, antenna; **E**, mandibles; **F**, first pereiopod; **G**, second pereiopod; **H**, third pereiopod; **I**, fifth pereiopod, propodus and dactylus; **I'**, detail of fifth pereiopod propodus and dactylus; **J,** pleon, lateral view; **K**, telson and uropods. Scale bars: 0.5 mm (**A-B**, **I**); 0.1 mm (**C-H**, **I'-J**).

Antenna (Figures 8A, D): scaphocerite unsegmented, with 14 plumose setae on inner margin, followed by 1 very small simple, 1 short plumose setae, and a very strong spine on apex.

Mandibles (Figure 8E): incisor and molar processes as illustrated.

Maxillule: coxal endite with 7-8 serrulate, papposerrate and simple setae, basial endite with 7-8 cuspidate and simple setae. Otherwise unchanged.

Maxilla: coxal endite bilobed with 8-9+4 serrulate and papposerrate setae; scaphognathite with 9-10 marginal plumose setae. Otherwise unchanged.

First maxilliped: unchanged besides size.

Second maxilliped: unchanged besides size.

Third maxilliped: exopod 5-segmented, bearing 2, 2, 2, 2, 4 plumose setae. Otherwise unchanged.

First pereiopod (Figure 8F): endopod 4-segmented, with 2+2, 1, 1+4, 2 papposerrate and 1 simple setae; exopod 5-segmented with 2, 2, 2, 2, 4 plumose setae.

Second pereiopod (Figure 8G): functional; biramous; basis with 1 simple seta; endopod 4-segmented, with 2+1, 0, 4, 2 papposerrate and 1 simple setae; exopod 4-segmented with 2, 2, 2, 4 plumose setae.

Third pereiopod (Figure 8H): present as a very small biramous bud.

Fourth pereiopod: absent.

Fifth pereiopod (Figures 8I, I'): two times longer than the whole larva; merus with 8-10 simple setae and one spinous process; carpus with 20-28 simple setae and one spinous process; propodus length is one third of the whole pereiopod, flattened and paddle-like enlarged with margins serrated (31-38 and 32-39 spines on each margin), 11-14 small pappose setae along the surface, and 2 strong simple setae distally. Otherwise unchanged.

Pleon (Figures 8A, J): unchanged besides size.

Pleopods: absent.

Uropods (Figures 8A, K): protopod without setae, with a spine; endopod with 8-9 plumose setae along distal and inner margins and 4-5 short plumose setae on dorsal margin; exopod longer than telson, with 1 spine on apex followed by 15-16 marginal plumose setae along distal and inner margins, and 3 short plumose setae on dorsal margin.

Telson (Figures 8A, K): almost rectangular shaped, with one pair of lateral spines followed by 2 pairs of outer spines and 5 pairs of plumose setae on the posterior end.

Last zoea (Figure 9)

Dimension: TL = 7.81–9.35 mm; CL = 3.85–4.84 mm.

Carapace (Figures 9A, B): eyes with four fifths of antennal length; presence of one pair of cervical carinae; rostrum measuring almost twice over the carapace, with 7-9 dorsal teeth, 1 post rostral spine; with 1 pair of supraorbital, 1 pair of antennal and 1 pair of pterigostomial spines.

Antennule (Figure 9B): peduncle 2-segmented, proximal segment with 6-7 plumose setae distributed along inner margin, 8-11 small plumose setae on stylocerite, 6-9 small plumose setae positioned at four fifths of the length of the segment, and 5-7 plumose+1 simple small setae distally; distal segment with 5-6 plumose+3-4 simple small setae. Inner flagellum 12-segmented, proximal to eleventh segments with 2-6 small simple setae, and distal segment with 4-5 short simple+1 plumose setae. Outer flagellum 10-segmented, proximal segment with 5 aesthetascs, second and third segments with 4 aesthetascs each, fourth segment with 2-4 simple setae and 5 aesthetascs, fifth segment with 3-4 short simple setae and 2 aesthetascs, sixth to ninth segments with 2-4 short simple setae, and distal segment with 3 short simple+2 plumose setae.

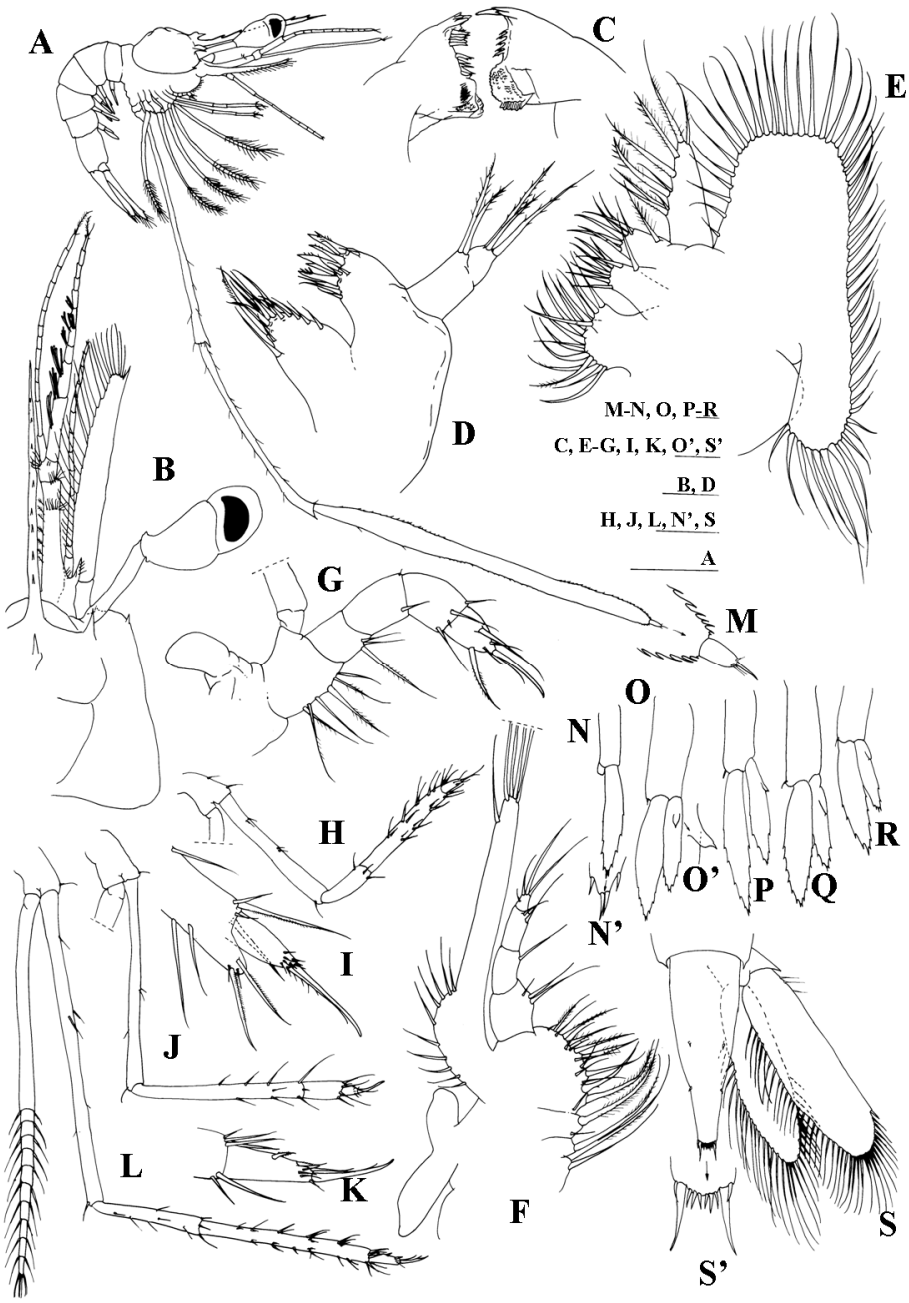

Fig. 9- *Lysmata moorei*. Last zoea: **A**, total animal, lateral view; **B**, carapace, dorsal view; **C**, mandibles; **D**, maxillule; **E**, maxilla; **F**, first maxilliped; **G**, second maxilliped; **H**, third maxilliped, propodus and dactylus; **I**, first pereiopod, propodus and dactylus; **J**, second pereiopod; **K**, third pereiopod, propodus and dactylus; **L**, fourth pereiopod; **M**, fifth pereiopod, propodus and dactylus; **N**, first pleopod; **N'**, first pleopods, detail of spines; **O**, second pleopod; **O'**, second pleopod, detail of appendix interna; **P**, third pleopod; **Q**, fourth pleopod; **R**, fifth pleopod; **S**, telson and uropods. Scale bars: 1 mm (**A**); 0.5 mm (**B**, **H**, **J**, **L**, **S**); 0.1 mm (**C-G**, **I**, **K**, **M-R**).

Antenna (Figure 9B): flagellum long and slender, with 10-segments bearing 2-5 short simple setae each; scaphocerite now with 30-32 plumose setae. Otherwise unchanged.

Mandibles (Figure 9C): armature of incisor and molar processes as illustrated

Maxillule (Figure 9D): coxal endite with 13-16 serrulate, papposerrate and simple setae; basial endite with 12-14 strong cuspidate and simple setae. Otherwise unchanged.

Maxilla (Figure 9E): coxal endite bilobed with 11-13+4 serrulate and papposerrate setae; basial endite with 8-10+7-10 papposerrate setae; scaphognathite with 47-51 marginal plumose setae. Otherwise unchanged.

First maxilliped (Figure 9F): coxal endite with 5 papposerrate setae; basial endite with 17-19 papposerrate setae; exopod unsegmented with 6-10 plumose setae on proximal lobe, 1 short subterminal+ 4 long plumose setae on distal margin; epipod bilobed. Otherwise unchanged.

Second maxilliped (Figure 9G): basis with 3+1+2 papposerrate setae; endopod 4-segmented with 3, 2, 4, 5-6 papposerrate and simple setae terminally. Otherwise unchanged.

Third maxilliped (Figure 9H): endopod 4-segmented, with 4-5, 3, 19-22, 5 papposerrate and simple setae; exopod 10-segmented, each segment with 2 and the last one with 4 plumose setae. Otherwise unchanged.

First pereiopod (Figure 9I): endopod subchelate, with internal distal margin of propodus produced forward to about a half of dactylus; 4-segmented, with 4-5, 4-5, 12-14, 11-13 papposerrate and simple setae; exopod 11-segmented, 1st segment with 1+2, 2^{nd} to 10^{th} segments with 2 and the last one with 4 plumose setae. Otherwise unchanged.

Second pereiopod (Figure 9J): endopod 4-segmented, with 4-5, 9-14, 5-9, 7-12 papposerrate and simple setae; exopod 11-segmented, 1^{st} segment with 1+2, 2^{nd} to 10^{th} segments with 2 and the last one with 4 plumose setae. Otherwise unchanged.

Third pereiopod (Figure 9K): endopod 4-segmented, with 6-8, 7-10, 21-24, 7-10 papposerrate and simple setae; exopod 10-segmented, 1st segment with 1+2, 2nd to 9th segments with 2 and the last one with 4 plumose setae. Otherwise unchanged.

Fourth pereiopod (Figure 9L): endopod 4-segmented, with 6-7, 7-10, 26-29, 7-8 papposerrate and simple setae; exopod 11-segmented, each segment with 2 and the last one with 4 plumose setae.

Fifth pereiopod (Figure 9M): two times longer than the whole larva; merus with 11-15 simple setae and one spinous process; carpus with 37-41 simple setae and one spinous process; propodus length is two fifths of the whole pereiopod, flattened and paddle-like enlarged with margins serrated (78-90 and 81-95 spines on each margin), and 11-15 small pappose setae along the surface; dactylus small with 3-4 simple subterminal and 1 strong simple terminal setae. Otherwise unchanged.

Pleon (Figure 9A): sixth pleomere with one pair of dorso-lateral spines and one pair of small ventral-lateral spines.

Pleopods (Figure 9N-R): protopod with 0-1 short simple seta; first pleopod endopod naked and exopod with 9-12 small spines; second to fifth pleopods endopods with 6-12, 6-12, 8-12, 7-9, and 6 small spines respectively; second to fifth pleopods exopods with 12-16, 15-19, 13-16, and 10-14 small spines respectively; small appendix interna present from second to fifth pleopods, with a spinous termination .

Uropods (Figure 9S): endopod with 4-5 short plumose setae proximally on outer margin and 37-42 plumose setae along distal and inner margins; exopod with 3-4 short plumose setae proximally on outer margin, 1 spine on apex followed by 36-43 marginal plumose setae along distal and inner margins, and 3-4 short plumose setae on dorsal margin.

Telson (Figure 9S, S'): rather triangular, narrower distally than proximally, bears one pair of dorso-lateral spines followed by 1 pair of lateral spines and 5 pairs of strong short setae.

4.5- Discussion

4.5.1- Morphological comparisons of the zoeal stages

Beyond the first zoeal stage, the morphological features displayed by *L. galapagensis* and *L. moorei* larvae are those considered to be diagnosing of the genus *Lysmata*: eyes stalked with a long peduncle; 5th pereiopod developing before the 2nd, 3rd and 4th pereiopods and exhibiting an enlarged, flattened and paddle-like propodus with serrated margins. The first zoeal stage of both species is similar to that of *L. seticaudata*, as they hatch with the first and fifth pereiopods as biramous and uniramous buds, respectively (Calado et al. 2004, p. 739, figures 1a, 1j-k). A comparison of the most relevant larval features of the first, fourth, seventh and last zoeal stages of *L. galapagensis* and *L. moorei* with those of *L. seticaudata* is presented in Table 1.

The studied species present a very homogeneous development, and even in the presence of incomplete larval series, we expect that both will have a maximum number of nine zoeal stages as in *L. seticaudata* larval series. *L. galapagensis* and *L. moorei* hatch with the first and fifth pereiopods as buds which become functional in the second zoeal stage; in the third zoeal stage all the three species have the second pereiopod as a biramous bud that becomes functional in the fourth zoeal stage where fourth pereiopod is present as a biramous bud. When comparing the seventh zoea of *L. galapagensis* with the same stage of *L. seticaudata*, the first one clearly presents more advanced larval characters, such as the antennal flagellum

Table 1 Comparison of relevant morphological characters of *L. seticaudata*, *L. galapagensis* and *L. moorei* zoeal stages.

Features	*L. galapagensis* Present study (1); Gopalakrishnan & Laurs, 1971 (2)	*L. moorei* Present study	*L. seticaudata* Calado et al. 2004
Distribution area	E Pacific	SW Atlantic	NE Atlantic
Number of zoeal stages	8- 9	8- 9	9
ZI, total length, mm	2.46-2.89 (1)	2.77-2.81	3.09-3.24
ZI, carapace length, mm	0.92-1.00 (1)	0.92-0.96	0.98-1.03
ZI, rostrum length, mm	0.35-0.42 (1)	0.42	0.46
ZIV, total length, mm	4.14-4.63 (1)	3.44-3.66	3.12-3.22
ZIV, carapace length, mm	1.61-2.15 (1)	1.13-1.29	1.04-1.08
ZIV, rostrum length, mm	0.92-1.35 (1)	0.54-0.70	0.31
ZVII, total length, mm	8.24-8.48 (1)	NA	4.47-4.79
ZVII, carapace length, mm	4.08-4.32 (1)	NA	1.40-1.56
ZVII, rostrum length, mm	2.80-3.04 (1)	NA	0.43-0.54
Last Z, total length, mm	16.10-20 (2)	7.81-9.35	6.40-6.80
Last Z, carapace length, mm	2.12-3.40 (2)	3.85-4.84	2.00-2.20
Last Z, rostrum length, mm	6.13-7.00 (2)	2.31-3.19	0.72-0.96
CARAPACE			
ZI, marginal denticles	4 (1)	4-5	4
ZIV, marginal denticles	3-4 (1)	2-3	3
ZVII, marginal denticles	0 (1)	NA	0
Last, marginal denticles	0 (2)	0	0
ROSTRUM			
ZIV, number of spines	5-6 (1)	0	0
ZVII, number of spines	7 (1)	NA	0
Last, number of spines	7-8 (2)	7-9	1-2
ANTENNA			
Scaphocerite, absence of segments	ZIV (1)	ZIV	ZIV
FIRST PEREIOPOD			
Biramous bud/ Functional	ZI (1)/ ZII (1)	ZI/ ZII	ZI/ ZII
SECOND PEREIOPOD			
Very small biramous bud	ZII (1)	ZII	ND
Biramous bud	ZIII (1)	ZIII	ZIII
Functional	ZIV (1)	ZIV	ZIV
THIRD PEREIOPOD			
Very small biramous bud	ZIV (1)	ZIV	ND
Biramous bud	ZV (1)	NA	ZV
Functional	ZVI (1)	NA	ZVI
FOURTH PEREIOPOD			
Very small biramous bud	-	NA	ND
Biramous bud	ZV (1)	NA	ZV
Functional	ZVI (1)	NA	ZVI
FIFTH PEREIOPOD			
Uniramous bud/ Functional	ZI (1)/ ZII (1)	ZI/ ZII	ZI/ ZII
PLEON			
3rd pleomere: dorsal anteriorly curved spine	ZIV-ZVII (1)	Absent	Absent
5th pleomere: one pair of dorso-lateral spines	ZI-ZV (1)	ZI-ZIV	ZI-ZII
5th pleomere: small dorsal spines	ZI: 3-4; ZII: 0-2; ZIII: 0 (1)	ZI: 3-4; ZII: 1-2; ZIII: 0	Absent
PLEOPODS			
Small buds	ZVI (1)	NA	ZVI
Biramous buds	-	NA	ZVII
Endopod as bud, exopod with setae	ZVII (1)	NA	ZVIII
With appendix interna	ZVIII (2)	Last (ZVIII or IX)	ZIX

Z: zoea. NA: not available in the larval series. ND: not developed.

as long as scaphocerite with 8 segments, biramous pleopods with endopods as buds and exopods with apical setae, and the telson narrower at the posterior end, typical of a penultimate stage (Calado et al. 2004, p. 746, figures 6a-k), leading us to believe that this species larval series will need just one more stage to complete their zoeal development. Accordingly, is also expected that *L. moorei* completes the zoeal development with eight or nine stages since their larvae hatch with the first and fifth pereiopods as buds, and in the fourth zoeal stage all the morphological structures are at the same level of development as in the other two species (Table 1). When summarizing the larval characters of genus *Lysmata*, Gurney (1937) affirmed that probably it would present 9 zoeal stages. Our results confirm his prediction for *L. galapagensis* and *L. moorei*, as it has been previously found for *L. seticaudata* (Calado et al. 2004). The most evident variation between the zoeal developmental series of *L. seticaudata* and *L. galapagensis* occurs during the pleopods development that in the first species takes 4 stages to complete the development and in the second seems to take only 3 stages. Knowlton & Alavi (1995) stated that the most evident differences in the *Lysmata* larval series were the appearance and development of pereiopods and pleopods.

The study of larval *Lysmata* will certainly be of valuable help for researchers addressing phylogeny subjects. Considering the available larval descriptions in the genus, is possible to hypothesize at least two probable scenarios for the developmental pattern of *Lysmata* species: the first one will be similar to *L. galapagensis, L. moorei* and *L. seticaudata* where the larvae hatch with the first and fifth pereiopods as buds becoming functional in the second zoeal stage and with a complete larval development of 8 to 9 zoeae. The second pattern will be similar to *L. amboinensis* (Wunsch 1996), *L. anchisteus* (Knowlton & Alavi 1996), *L. vittata* (Yang 1999) and Kurata's *L. wurdemanni* (Kurata 1970) where the larvae hatch without buds, and consequently will have a longer zoeal development with a maximum of 11 zoeal stages. The

existing correspondence between the larval duration and the phylogenetic results for *Lysmata* genus is very interesting: the first group of species belongs to the "Cosmopolitan Clade"/ "*Lysmata* Clade", while *L. amboinensis* (Wunsch 1996), *L. anchisteus* and *L. wurdemanni* (Kurata 1970), the only species studied by Baeza et al. (2009) and Fiedler et al. (submitted), were included in the other obtained clades. Table 2 presents the more relevant morphological characters for staging the larvae of *Lysmata* species belonging to the "Cosmopolitan Clade"/ "*Lysmata* Clade". It is commonly accepted that marine organisms such as decapod crustaceans having a pelagic mode of larval development with several larval stages corresponds to a phylogenetically ancestral state, while shorter larval series are considered a derived character (Strathmann 1978, Anger 2001). In this way, the information provided by the zoeal developmental series of *Lysmata* will certainly help researchers to validate the current phylogeny of the genus.

Table 2 Staging of the "Cosmopolitan Clade"/ "*Lysmata* Clade" larval morphological characters based on the three studied species.

Larval stage	General morphological characters
First zoea	Eyes fused; one pterigostomial spine followed by 4-5 marginal denticles; rostrum long, slender and pointed. Scaphocerite 5-segmented. 5th pleomere with one pair of dorso-lateral spines. Pereiopods 1 and 5 as buds. Telson with 7+7 plumose setae.
Second zoea	Eyes stalked; antennal and supraorbital spines present; pterigostomial spine followed by marginal denticles; rostrum long and slender with one post rostral spine. Pereiopods 1 and 5 developed and functional. Telson with 8+8 plumose setae.
Third zoea	Antennular peduncle 2-segmented, inner and outer flagella present. Scaphocerite 4-segmented. 6th pleomere separated from telson. Pereiopod 2 present as a biramous bud. Uropods present, with a rudimentary endopod.
Fourth zoea	Scaphocerite unsegmented. Pereiopod 2 functional. Uropods exopods as long as telson.
Fifth zoea	Biramous buds of pereiopods 3 and 4. Uropods exopods longer than telson margin. Telson narrower at his posterior end.
Sixth zoea	Pereiopods 1 to 5 developed and functional. Pleopods present as small buds.
Penultimate Zoea (Seventh or Eighth)	Antennal flagellum as long as scaphocerite with 8 segments. Biramous pleopods with endopods as buds and exopods with apical setae.
Last Zoea (Eighth or Ninth)	Antennal flagellum longer than scale with more than 8 segments. Pleopods with appendix interna. Telson triangular with the migration of the first and second pairs of dorso-lateral spines almost concluded (telson form almost similar to an adult).

The second zoeal stage of *L. galapagensis* presents a small protuberance in the dorsal region of the third pleomere, which in later zoea differentiates to a well defined anteriorly curved spine. This character makes this species easily distinguishable from all *Lysmata* larvae that have been studied. The similarities recorded between the late zoeal stages of *L. galapagensis* and the material described by Gopalakrishnan & Laurs (1971), namely the presence of an extremely long rostrum with several dorsal teeth and the strong anteriorly curved spine in the dorsal surface of the third pleomere, allow us to suggest that the larvae described as *E. corniger* for the Eastern Tropical Pacific probably corresponds to *L. galapagensis*. Gurney (1937) Species A. V, *Eretmocaris corniger,* from the Atlantic Ocean, presents the same general form of *L. galapagensis*, however Gopalakrishnan & Laurs (1971) gave a list of differences between both species, concluding that the Atlantic specimens were much smaller and presented less one spine on rostrum, three inner lobes on maxilla endopod, less six setae on basal enlargement of first maxilliped exopod, and first and second pereiopods not chelate. *L. galapagensis* penultimate stage described in present work is found to be much smaller (TL = 8.24-8.48 mm) than the last stage (TL = 16.10-20.00 mm) described by Gopalakrishnan & Laurs (1971), especially when considering that they differ from one stage only. We suppose that *L. galapagensis* larvae when in the last zoeal stage will have several instars which will allow them to grow without undergoing the morphological changes to decapodid. *Lysmata* larvae are well known for their ability to delay metamorphosis (Calado 2008) by performing mark-time molting, as defined by Gore (1985). In certain laboratory culture trials, *Lysmata* have remained in last zoeal stage for over 80 days (Palmtag & Holt 2007), and during this period these larvae have molted several times, but displayed little or no morphological differences from one instar to the next apart from a significant increase in size. In this way, the last zoea described by Gopalakrishnan & Laurs (1971) could be bigger simply because it was delaying metamorphosis. Gurney (1942) describes these as giant larvae, which after

reaching the last zoeal stage, at which metamorphosis normally occurs, continue to grow without important changes in structure and without advancing to the juvenile form.

Concerning *L. moorei* larvae, and despite their similarity to *L. galapagensis*, these specimens lack the unique morphological feature displayed by *L. galapagensis* on the 3rd pleomere. Regardless of the close phylogenetic relationship suggested by the molecular studies of Baeza et al. (2009) and Fiedler et al. (submitted) between the Eastern Pacific *L. galapagensis* and the Western Atlantic *L. moorei*, the most evident larval character shared by both species is the presence of a very long rostrum with several teeth in late zoeal stages. Therefore it seems evident that *E. corniger* larval forms found in the Eastern Atlantic are not *L. moorei*. We suppose that they belong to an unknown *Lysmata* species that probably is phylogenetically more close to *L. galapagensis* than to *L. moorei*. Nowadays researchers have a golden opportunity to validate the identification of *Lysmata* larvae previously collected from the plankton and after over one century finally start to correlate the forms of the puzzling *Eretmocaris* to present valid species.

4.5.2- Biogeographical considerations on *Eretmocaris corniger*

L. galapagensis occurs in the eastern Pacific and, considering its larval series, the most similar larvae known so far are those of *Eretmocaris corniger* collected in the eastern Atlantic. The occurrence of such similar larvae in two different oceans, separated by the Panama Isthmus and the Atlantic Ocean, lead us to suppose that we are in the presence of two distinct species that, together with *L. moorei* could constitute a species complex. Gopalakrishnan & Laurs (1971) had described *E. corniger* larvae from plankton collected material in the eastern tropical Pacific Ocean and considered that their specimens could belong to a *Lysmata* species associated with the eastern Ocean tropical islands. The rationale

for their assumption was the occurrence of these larvae in the Galapagos (eastern Pacific) and in Cape Verde islands (eastern Atlantic). The Panama Isthmus is a geographic barrier formed three million years ago during the Pliocene, opened in 1914 prior to the record of *L. galapagensis* species in the eastern tropical Pacific by Schmitt (1924). We do not believe that the opening of the Panama Channel was the responsible for the colonization of this species in both Atlantic and the Pacific Oceans. The present work matches the larvae of *L. galapagensis* to the *E. corniger* Pacific type, and Gopalakrishnan & Laurs (1971) presents consistent differences between this and the *E. corniger* Atlantic type, refuting the remote hypothesis of the Atlantic type have dispersed to the Pacific after the opening of the Panama Channel. It is assumed that present populations of some coastal decapod crustaceans from the eastern Atlantic, western Atlantic and eastern Pacific are genetically isolated from each other (e.g. Schubart et al., 2005). Knowlton et al. (1993) suggested that some of the studied species of the snapping shrimp *Alpheus* may have diverged before the final closure of the Panama seaway, in a staggered model. Also, Schubart et al. (2005) stated that the Atlantic and Pacific species of *Pachygrapsus transversus* complex diverged prior to the closure of the Isthmus of Panama, recognizing that the eastern Pacific population was in fact a valid species, *P. socius*. The tropical shrimp larvae are capable of long-distance dispersal (Goy 1990), and this is the explanation for the circumtropical distribution of many shrimp species, implying that the main way of creating new species is by sympatric speciation. Also, the very recent work of Rhyne et al. (2009) proved that these species potential for larval dispersal is unrealized, and a strong reproductive isolation is observed. As a result, the circumtropical and consequently the trans-isthmian species are not typical in the genus *Lysmata*, reason why no species in this genus were simultaneously recorded in the Pacific and Atlantic Oceans. In fact, the only species once thought to occupy both of these oceans, has been split in two: *L. grabhami* is an amphi-Atlantic species, and *L. amboinensis* known to occur from the Western Tropical Indian

Ocean to the Hawaiian Islands but absent from the Eastern Tropical Pacific (Debelius 2001). Having in mind that both, *L. galapagensis* and *E. corniger* Atlantic type, share the same feature on the 3rd pleomere, that is absent in *L. moorei*, the most plausible scenario should be the possibility that the eastern Atlantic *E. corniger* corresponds to the larval form of a *Lysmata* species, eventually still undescribed, which used to have an amphi-Atlantic distribution and formed a trans-isthmian complex with *L. galapagensis* (or with their common ancestral) and is now restricted to the Eastern Atlantic. Phylogenetically, this undescribed species might be more close to *L. galapagensis* than *L. moorei*, what lead to the hypothesis that there is a missing link between these two species, which is probably already extinct. Future efforts to identify the adult form of Atlantic *E. corniger* should focus on the larval culture of *Lysmata* species which are morphologically and/or molecularly close to *L. galapagensis* and are known to occur in the Eastern Atlantic, with emphasis to the oceanic islands.

4.5.3- Biodiversity and conservation issues

The current fishing pressure on ornamental shrimp from the genus *Lysmata*, namely on coral reef species displaying dazzling colorations and/or fish cleaning behaviour, has lead researchers and policy makers to question the sustainability of this practice and urged them to start monitoring potential ecological impacts of this profitable commercial activity (e.g. Calado 2008). The correct identification of different taxa in their larval form is essential to perform studies related to population estimates, connectivity, recruitment and spatiotemporal distributions (e.g. Levin 2006, Rhyne et al. 2009). This highlights the need of not only matching known *Eretmocaris* with their adult forms, but also to describe the complete larval series of *Lysmata* species.

Understanding larval dispersal is vital to successfully manage any commercial fishery, either for human consumption, biotechnological applications or the marine aquarium trade (e.g. dos Santos 1998, Calado 2008, Marta-Almeida et al. 2008), as well as to establish marine natural reserves and/or no take areas (e.g. Almany et al. 2009, Planes et al. 2009). These studies always require a solid knowledge on the larval stages of target species, which commonly is either incomplete or unavailable, even in heavily studied regions (e.g. the North-Eastern Atlantic, González-Gordillo et al. 2001). Although several advances have been achieved in the identification of larval stages using molecular tools (e.g. Pan et al. 2008, Jones et al. 2009), classical larval morphological descriptions are far from being obsolete. In fact, small intraspecific variations in larval morphology induced by environmental gradients (Schubart et al. 2005) will certainly only be detected when employing classical approaches. Managing the collection of *Lysmata* has the additional challenge posed by the occurrence of cryptic species occurring in sympatry (Rhyne & Lin 2006), which apparently can be more common than initially assumed (Baeza et al. 2009). Under this scenario, the knowledge on the larval stages of these cryptic species can be vital for the establishment of an accurate monitoring and management program for the commercial capture of these highly valuable marine organisms.

4.5.4- Conclusions

The present work allows us to conclude that *Eretmocaris corniger* larval type from the eastern Pacific is in fact *Lysmata galapagensis*. According to Baeza et al. (2009) and Fiedler et al. (submitted) *L. moorei* is phylogenetically the closest known species to *L. galapagensis*, and both of these are included in the same clade of *L. seticaudata*. All these three species hatched with the first and fifth pereiopods as buds, and present a similar first to fourth zoeal development. *L. galapagensis* and *L. seticaudata* will have a maximum of 9 zoeal stages, so,

we can suppose that all the species from the "Cosmopolitan Clade"/ "*Lysmata* Clade" will also present a maximum of nine zoeal stages.

The similarity between *L. galapagensis* larvae and *Eretmocaris corniger* larval type from the Eastern Atlantic, hypothesize the existence of a third, probably still undescribed species, distributed along the Eastern Tropical Atlantic. Focusing our research efforts on the "Cosmopolitan Clade"/ "*Lysmata* Clade" species, it will not be certainly necessary to wait another century to finally find the adult form of the puzzling Atlantic *E. corniger*. We believe that the almost unexplored Western African Coast holds most of the answers to the questions raised concerning the identity of the adult forms of several *Eretmocaris* larvae.

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CHAPTER 5

Complete larval development of the hermit crabs *Clibanarius aequabilis* and *Clibanarius erythropus* (Decapoda: Anomura: Diogenidae), under laboratory conditions, with a revision of the larval features of the genus *Clibanarius**

*****Bartilotti C., Calado R., dos Santos A. (2007) **Complete larval development of the hermit crabs *Clibanarius aequabilis* and *Clibanarius erythropus* (Decapoda: Anomura: Diogenidae), under laboratory conditions, with a revision of the larval features of genus *Clibanarius***. *Helgoland Marine Research* **62**, 103- 121.

5.1- Abstract

The complete larval development (four zoeae and one megalopa) of *Clibanarius aequabilis* and *C. erythropus*, reared under laboratory conditions, is described and illustrated. The larval stages of the two northeastern Atlantic *Clibanarius* species cannot be easily differentiated. Their morphological characters are compared with those of other known *Clibanarius* larvae. The genus *Clibanarius* is very homogeneous with respect to larval characters. All *Clibanarius* zoeae display a broad and blunt rostrum, smooth abdominal segments and an antennal scale without a terminal spine. Beyond the second zoeal stage, the fourth telson process is present as a fused spine, and the uropods are biramous. In the fourth larval stage all species display a mandibular palp. The *Clibanarius* megalopa presents weakly developed or no ocular scales, symmetrical chelipeds, apically curved corneous dactylus in the second and third pereiopods, and 5-11 setae on the posterior margin of the telson. Apart from the number of zoeal stages, *Clibanarius* species may be separated, beyond the second zoeal stage, by the telson formula and the morphology of the fourth telson process.

5.2- Introduction

The larval development of *Clibanarius* species has deserved special attention and has been described for over 13 species. Brossi-Garcia (1987) compiled the larval morphological characters of most *Clibanarius* species described, and highlighted the conservative developmental pattern of the genus. Siddiqui et al. (1991, 1993) confirmed the existence of this developmental pattern among *Clibanarius* species, but also noticed the occurrence of small intraspecific variations.

The hermit crabs *Clibanarius aequabilis* Dana, 1851 and *Clibanarius erythropus* (Latreille, 1818) are both present in the eastern Atlantic. The first occurs around Madeira and the Canary and Cape Verde islands; the second occurs on European Atlantic shores, in the Mediterranean Sea and at the Azores (Udekem d'Acoz 1999, González-Gordillo et al. 2001). In the present paper we describe the complete larval series of *C. aequabilis* and compare it with previously described larval stages of other species of the genus, particularly with those of *C. erythropus*. Despite the fact that these two eastern Atlantic species do not co-occur in the same area, a careful comparison seemed to be required. In this way, the larval morphology of *C. erythropus* was redescribed following modern standards. The general features of *Clibanarius* larvae are revised and discussed.

5.3- Material and Methods

Five ovigerous *Clibanarius aequabilis* females were hand-collected during March 2003 in the intertidal region at Reis Magos (32º38.40' N, 16º50.70' W), Madeira Island, Portugal, and transported to the laboratory. Until hatching of their larvae, females were kept in darkness, in a 180 l tank connected to a larval collector, at a salinity of 34 ± 1 and a temperature of 24 ± 1º C. The most active larvae (those displaying pronounced positive phototactic responses) from five different ovigerous hermit crabs were randomly selected and stocked at a density of 20 larvae l^{-1} in four 10 l small research scale-rearing tanks described by Calado et al. (2003). Seawater was UV-sterilized and 1 µm-filtered, had a salinity of 34 ± 1, and the temperature was kept stable at 24 ± 1º C through a heating/cooling system. The tanks were illuminated from above with fluorescent light, with a photoperiod of LD 14:10. Ammonia and nitrite were monitored daily and maintained below detectable levels. Nitrate and pH showed average values (± standard deviation) of 4 (±1) and 8.1 (±0.1) mg l^{-1}, respectively. *Artemia* cysts

(Unibest® 020730) were hatched according to the procedures described by Sorgeloos et al. (1986). All larval stages were provided with live *Artemia* nauplii as prey at a density of 5,000 items l^{-1}. Ten haphazardly selected larvae were sampled daily and staged to determine stage duration. The sampled larvae were fixed in 4% formaldehyde.

For an adequate comparison, *C. erythropus* larvae were also cultured. Five ovigerous females were hand-collected during July 2005 in Ria do Alvor (37º08' N; 08º37' W), Algarve, Portugal, and transported to the laboratory. *Clibanarius erythropus* larval culture followed the same methods as described for *C. aequabilis*.

Drawings and measurements were made with the aid of a camera lucida on a binocular Wild M8. Setal observations and drawings were made using a Zeiss microscope with camera lucida. Slides with appendages were prepared with Faure's liquid (Reyne 1949). Larval description followed the method proposed by Clark et al. (1998), and setal terminology is according to Ingle (1993) and Garm (2004). The long plumose setae on distal exopod segments were drawn truncated. Setal counts refer from proximal to distal sequence. The measurements taken were: the distance between the tip of the rostrum and the posterior end of the telson (TL), the carapace length from the tip of the rostrum to the posterior margin of the carapace (CL), and the rostrum length from the tip of the rostrum to the eye socket (R).

The spent females and complete larval series have been deposited in the Instituto Nacional de Investigação Agrária e Pescas (IPIMAR) in Lisbon, Portugal (numbers IPIMAR/D/Ca/12/2003 and IPIMAR/D/Ce/09/2005).

5.4- Results

Under laboratory conditions, *C. aequabilis* hatches as zoea and then passes through four zoeal stages and one megalopa. The latter is reached after 8 days. The first and final larval stages are described in detail, while for the other larval stages only deviating characters are described.

Under laboratorial conditions, *Clibanarius erythropus* has a larval development similar to that of *C. aequabilis.* A detailed description of the first zoeal stage, following modern standards, is presented.

Clibanarius aequabilis Dana, 1851

Figures 1–5

First zoea (Figure 1)

Dimension: TL = 2.39– 2.62 mm; CL = 1.04– 1.12 mm; R = 0.46– 0.54 mm

Carapace (Fig. 1a, a'): without processes or spines, with the postero-lateral border smoothly rounded; rostrum broad, rather blunt with triangular pointed tip extending beyond antennal scale, rostrum about 0.89 ± 0.06 times the carapace; eyes compound and sessile.

Antennule (Fig. 1b): peduncle unsegmented, with 1 long plumose seta sub-terminally, 3 aesthetascs and 3 unequal simple setae terminally.

Antenna (Fig. 1c): protopod unsegmented with 1 small strong cuspidate seta; endopod extending beyond more than half the scaphocerite, with 3 long plumose setae on the posterior end; scaphocerite unsegmented, with 11 plumose setae.

Fig. 1- *Clibanarius aequabilis.* First zoea: **a**, total animal, dorsal view; **a'**, detail of rostrum, lateral view; **b**, antennule; **c**, antenna; **d**, mandible; **e**, maxillule; **f**, maxilla; **g**, first maxilliped; **h**, second maxilliped; **i**, third maxilliped; **j**, telson; **j'**, detail of posterior margin of telson. Scale bars: 0.1 mm.

Mandible (Fig. 1d): asymmetrical; palp absent.

Maxillule (Fig. 1e): coxal endite with 3 serrulate, 2 pappose and 2 simple setae; basial endite with 2 cuspidate and 2–3 simple setae; endopod with 1 + 1 + 2 simple setae.

Maxilla (Fig. 1f): coxal endite bilobed with 7 + 4 serrulate and papposerrate setae, basial endite bilobed with 5 + 4 papposerrate and simple setae; endopod unsegmented with 3 + 2 long simple setae and microtricha on outer margin; scaphognathite with 5 marginal plumose setae.

First maxilliped (Fig. 1g): coxa unarmed; basis with 1 + 1 + 1 + 3 + 3 papposerrate setae; endopod 5-segmented with 2, 2, 2, 2 and 1 + 4 papposerrate setae, microtricha on outer margin of second and third segments; exopod unsegmented, bearing 4 long plumose setae terminally.

Second maxilliped (Fig. 1h): coxa unarmed; basis with 1 + 2 papposerrate setae; endopod four-segmented with 2, 2, 2 and 1 + 4 papposerrate setae terminally. Exopod unsegmented, bearing 4 long plumose setae terminally.

Third maxilliped (Fig. 1i): long uniramous bud.

Pereiopods: absent.

Abdomen (Fig. 1a): five somites with margins rounded; with a pair of dorso-lateral marginal minute setae on somites 2–5; sixth somite feebly segmented.

Pleopods and uropods: absent.

Telson (Fig. 1a, j, j'): triangular, broader posteriorly with deep median cleft; with 7 + 7 processes; first process is a very short blunt spine, second process an anomuran hair, third to seven processes plumose setae, with the fourth being the longest.

Second zoea (Figures 2a–f)

Dimension: TL = 3.00– 3.23 mm; CL = 1.31– 1.39 mm; R = 0.58– 0.65 mm

Carapace (Fig. 2a): eyes stalked; otherwise unchanged.

Antennule (Fig. 2a, b): peduncle unsegmented with 2 long plumose and 2 short simple setae terminally, 1 long plumose seta at site of future endopod; exopod bud clearly delineated, with 3 simple setae and 3 aesthetascs.

Antenna (Fig. 2a): unchanged except for size.

Mandible (Fig. 2c): molar process with several small denticles and without teeth; otherwise unchanged.

Maxillule: basial endite with 4 cuspidate and 2 simple setae; otherwise unchanged except for size.

Maxilla: scaphognathite with 7 marginal plumose setae; otherwise unchanged except for size.

First maxilliped (Fig. 2d): endopod five-segmented with 1 + 2, 1 + 2, 1 + 1, 2 papposerrate and 1 + 4 papposerrate setae; exopod bearing six long plumose setae terminally; otherwise unchanged.

Second maxilliped: endopod four-segmented with 2, 1 + 2, 1 + 2 papposerrate and 1 + 4 papposerrate setae terminally. Exopod bearing 6 long plumose setae terminally; otherwise unchanged.

Third maxilliped (Fig. 2e): biramous; endopod represented by a small bud; exopod unsegmented, bearing 6 plumose setae.

First to third pereiopods (Fig. 2f): present as small uniramous buds.

Abdomen: unchanged except for size.

Pleopods and uropods: absent.

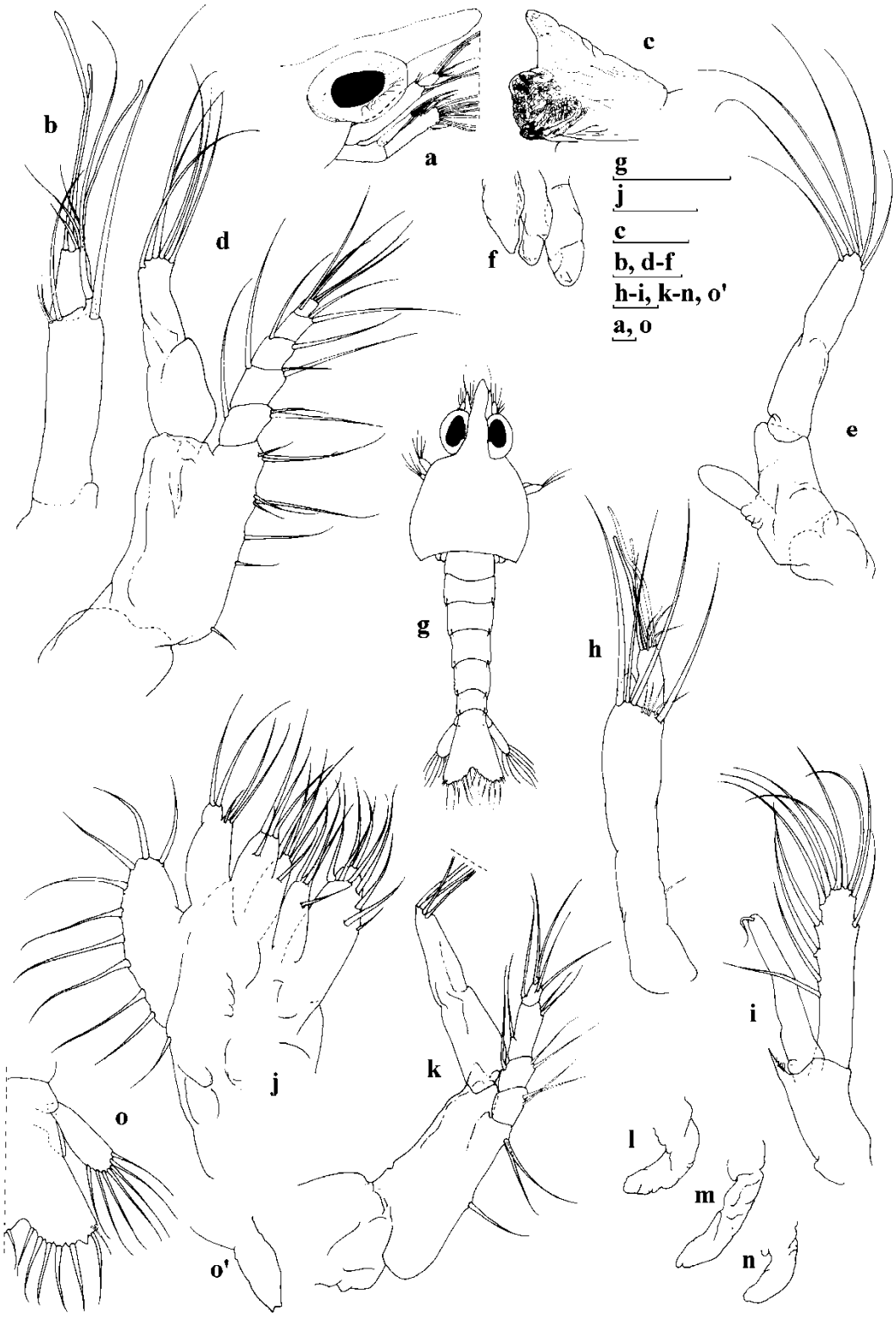

Fig. 2- *Clibanarius aequabilis.* Second zoea: **a**, detail of rostrum, lateral view; **b**, antennule; **c**, mandible; **d**, first maxilliped; **e**, third maxilliped; **f**, first to third pereiopod. Third zoea: **g**, total animal, dorsal view; **h**, antennule; **i**, antenna; **j**, maxilla; **k**, second maxilliped; **l**, third pereiopod; **m**, fourth pereiopod; **n**, fifth pereiopod; **o**, telson and uropods; **o'**, detail of endopod of uropods. Scale bars: 1.0 mm (**g**); 0.1 mm (**b-f**, **h-o'**).

Telson: 8 + 8 processes, with an additional inner pair of telson plumose setae; otherwise unchanged.

Third zoea (Figures 2g–o')

Dimension: TL = 3.44– 3.77 mm; CL = 1.61– 1.72 mm; R = 0.75– 0.81 mm

Carapace (Fig. 2g): unchanged except for size.

Antennule (Fig. 2h): peduncle unsegmented with 1 basial simple seta, 4 long plumose setae and 4 short simple setae terminally; inner flagellum small, bud-like; outer flagellum with 3 aesthetascs, 1 long plumose seta and 3 shorter simple setae.

Antenna (Fig. 2i): endopod as long as the scale, with 1 short simple seta in apical region; scaphocerite with 12 plumose setae. Otherwise unchanged.

Mandible and maxillule: unchanged except for size.

Maxilla (Fig. 2j): coxal endite with 9 + 4 serrulate and papposerrate setae; scaphognathite with 10–11 marginal plumose setae; otherwise unchanged except for size.

First, second (Fig. 2k) and third maxillipeds: unchanged except for size.

First pereiopod: uniramous bud showing its chelate nature.

Second and third (Fig. 2l) pereiopods: unchanged.

Fourth (Fig. 2m) and fifth (Fig. 2n) pereiopods: uniramous buds.

Abdomen (Fig. 2g): abdominal somite 6 fully separated from telson, bearing a pair of minute dorso-lateral setae; otherwise unchanged.

Pleopods: absent.

Uropods (Figs. 2o, o'): biramous; exopods well developed, not reaching the end of telson, with 7–8 plumose setae; endopod small bud with 2 minute projections at its tip.

Telson (Fig. 2o): triangular, broader posteriorly with median cleft less pronounced; number of telson processes: 9–10 + 9–10 (first process as a very short blunt spine, second as an anomuran hair, third as a plumose seta, fourth as a very reduced fused spine and fifth to ninth or tenth processes as plumose setae). One individual in seven observed with 9 + 9 processes (additional inner pair of plumose telson setae, without reduced fused spine).

Fourth zoea (Figure 3)

Dimension: TL = 4.08– 4.40 mm; CL = 1.92– 2.08 mm; R = 0.88– 0.96 mm

Carapace (Fig. 3a): unchanged except for size.

Antennule (Fig. 3b): peduncle unsegmented with 2 + 1 simple setae along lateral margin, 4 short simple and 4 long plumose setae distally; inner flagellum unsegmented, enlarged in size; outer flagellum with 2 + 2 + 2 + 1 aesthetascs, 2 long and 2 short plumose setae.

Antenna (Fig. 3c): endopod longer than antennal scale, two-segmented: first segment unarmed, second segment with a short simple seta and a pointed tip; scaphocerite with 12–13 plumose setae. Otherwise unchanged.

Mandible (Fig. 3d): differs from the previous stage by the presence of an unsegmented palp.

Maxillule (Fig. 3e): coxal endite with 3 papposerrate, 2 pappose and 3 simple setae; otherwise unchanged.

Maxilla (Fig. 3f): coxal endite with 11–12 + 4 serrulate and papposerrate setae; basial endite with 7 + 6 papposerrate and simple setae; endopod unsegmented, bilobed, terminally bearing 3 + 2 long simple setae; scaphognathite with 15–17 marginal plumose setae, inferior lobe without setae.

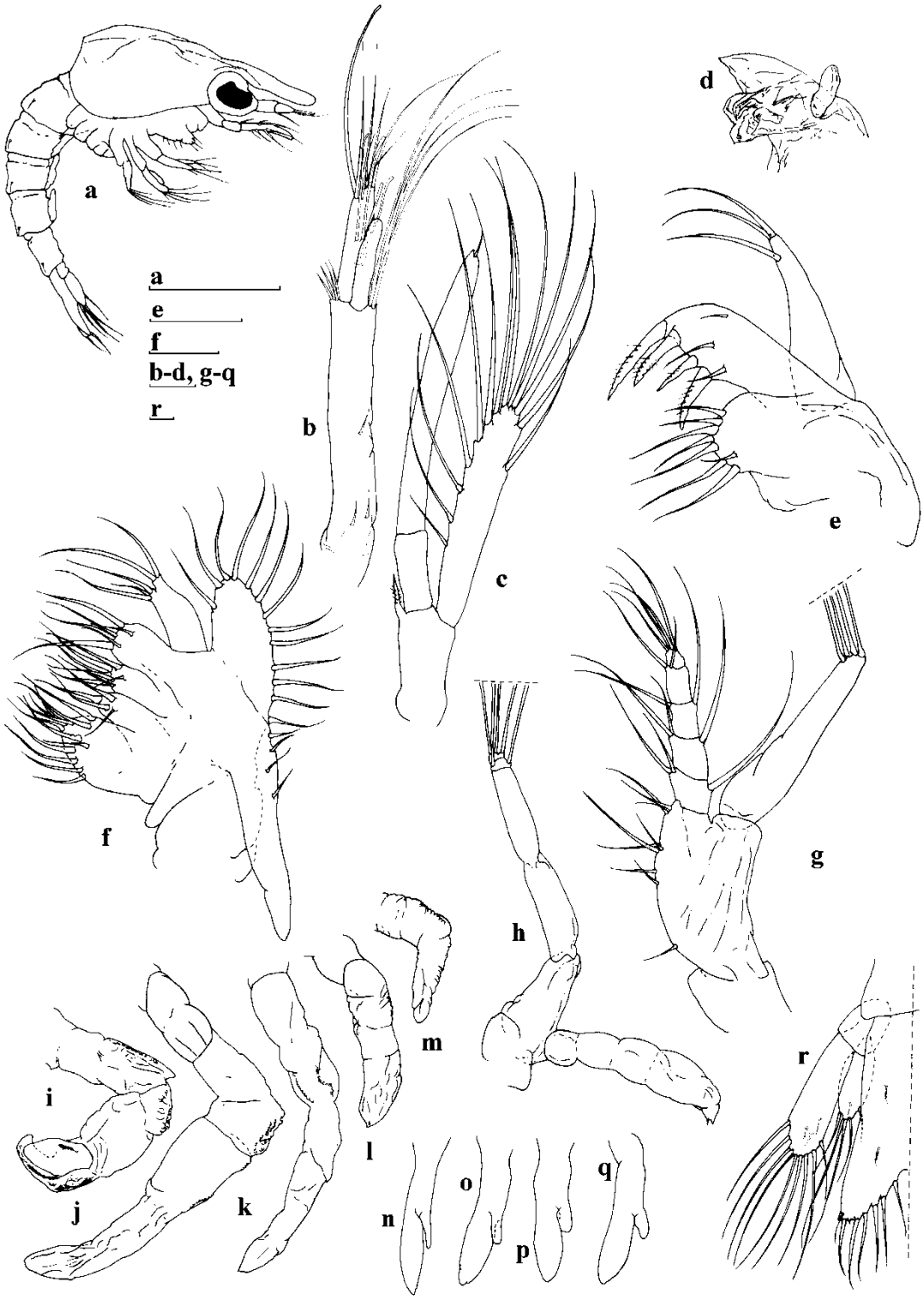

Fig. 3- *Clibanarius aequabilis.* Fourth zoea: **a**, total animal, lateral view; **b**, antennule; **c**, antenna; **d**, mandible; **e**, maxillule; **f**, maxilla; **g**, first maxilliped; **h**, third maxilliped; **i**, first pereiopod; **j**, second pereiopod; **k**, third pereiopod; **l**, fourth pereiopod; **m**, fifth pereiopod; **n- q**, pleopods of abdominal somites 2-5; **r**, telson and uropods. Scale bars: 1.0 mm (**a**); 0.1 mm (**b**-**r**).

First (Fig. 3g) and second maxillipeds: unchanged.

Third maxilliped (Fig. 3h): endopod two-segmented, incomplete 2–5 segments with two-minute projections at its tip; otherwise unchanged.

First pereiopod (Fig. 3i): cheliped with chela enlarged, dactyl distinct, coxa, and merus clearly delineated, with tubercles on surface.

Second and third pereiopods (Fig. 3j, k): long, the first six segments clearly delineated, coxa and merus with some tubercles on surface.

Fourth and fifth pereiopods (Fig. 3l, m): short, with some segments clearly delineated.

Abdomen (Fig. 3a): unchanged except for size.

Pleopods (Fig. 3n–q): present as biramous buds on segments 2–5.

Uropods (Fig. 3r): total individualization of protopod, exopod and endopod; exopod with 8–9 plumose setae on apical region and 3–4 papposerrate setae on ventral surface; endopod smaller with 5 plumose setae on apical region and 1 pappose seta on ventral surface.

Telson (Fig. 3r): almost rectangular in shape; number of telson processes: 9 + 9–10 (first process as a very short blunt spine, second as an anomuran hair, third as a plumose seta, fourth as a very reduced fused spine and fifth to ninth or tenth processes as plumose setae); presence of two pairs of pappose setae on dorsal surface.

Megalopa (Figures 4, 5)

Dimension: TL = 2.85– 3.04 mm; CL = 1.08– 1.15 mm; R = 0.15– 0.19 mm

Carapace (Fig. 4a, a'): smooth, slightly broader than long; rostrum broad and blunt; ocular peduncles stout reaching the end of the antennular peduncle; ocular acicles not developed; surface and margins with proboscate setae distributed as figured.

Antennule (Fig. 4b): peduncle three-segmented, proximal segment with 12–13 papposerrate and simple setae; second segment with 1 long and 3–4 short simple setae and 1 papposerrate seta; distal segment with 6 simple setae; outer flagellum five-segmented, proximal segment unarmed, second segment with 5 aesthetascs, third segment with 5 aesthetascs and 3 simple setae, fourth segment with fourth aesthetascs and 2–3 simple setae terminally, distal segment with three simple setae; inner flagellum three segmented with two, four, and seven simple setae.

Antenna (Fig. 4c): basis with 3–4 simple setae, peduncle three-segmented with 1, 2, 3–4 simple setae; flagellum composed of 10 segments, each with 0–6 simple setae on distal margin, arranged as figured, except for the distal segment which has 9 simple setae on distal margin; acicle with 1 spine-like projection on lateral margin, 4 simple setae and bifid spinous tip.

Mandibles (Fig. 4d): reduced and simplified, lower extremities of the incisor part with processes as illustrated; palp two-segmented, with 4–5 simple setae and 4 serrulate setae on the distal segment.

Maxillule (Fig. 4e): coxa with 18–21 papposerrate and simple setae; basis with 2 simple setae on the lateral inner margin and 5 cuspidate, 4 serrulate, 5–7 papposerrate, 3–4 simple and 1 long plumose setae on the distal margin; endopod unsegmented with 1 + 1 simple setae; 1 plumose seta present on the inner margin of basis.

Maxilla (Fig. 4f): coxa bilobed with 25–32 + 7–9 papposerrate and simple setae; basis bilobed with 8–10 + 11–13 papposerrate and simple setae; endopod unsegmented without seta; scaphognathite with 50–58 plumose setae on the distal margin and 1 simple seta on the dorsal surface of the upper lobe.

First maxilliped (Fig. 4g): coxa with 3–4 simple, 3 pappose and 1 papposerrate setae; basis with 4 simple, 3 serrulate and 11–14 papposerrate setae; endopod unsegmented without

seta; exopod unsegmented with 7–9 plumose setae on the outer margin.

Second maxilliped (Fig. 4h): coxa with 1 simple seta; basis with 3 pappose setae; endopod four-segmented, proximal segment with 1 + 1 simple seta, second segment with 3 simple setae, third segment with 8 simple and papposerrate setae, and 7–8 papposerrate setae on the distal segment; exopod two-segmented, proximal segment with 2 simple setae and the distal segment with 6–8 plumose setae terminally.

Third maxilliped (Fig. 4i): basis with 6–7 simple setae and 3–4 sub-acute processes; endopod five-segmented, ischium with 8–10 simple seta and 5–6 sub-acute processes on crista dentata, merus with 4–6 simple setae, carpus with 9–10 simple setae, propodus with 5 simple, 2 cuspidate, and 13–14 papposerrate setae, and dactyl with 4 serrate, 7–8 simple, 2 cuspidate and 6 papposerrate setae; exopod 2- segmented with 1 simple seta on proximal segment and 6–8 (2 + 4 or 2 + 2 + 4) plumose setae terminally on distal segment.

First pereiopod (Fig. 5a, a'): chelae equal, coxa with 6–8 simple setae; basis with 4–5 simple setae; ischium with 6–7 simple setae; merus, longest segment, with 14–15 simple setae; carpus with 6–8 simple setae; propodus with 31–35 simple setae, distal upper extremity corneous; dactyl length about half palm + propodal prolongation, with 24–26 simple setae and distal extremity corneous.

Second pereiopod (Fig. 5b, b'): coxa with 7–8 simple setae; basis smaller with 2–3 simple setae; ischium with 8–9 simple setae; merus, longest segment, with 14–16 simple setae; carpus less than half the ischium length, with 11–12 simple setae; propodus longer than carpus, with 23–25 simple setae and 1 stout cuspidate seta; dactylus very stout, apically curved and corneous, with 28–30 simple setae and 3 spines.

Third pereiopod (Fig. 5c): coxa with 6–7 simple setae; basis with 3 simple setae and 1 tooth; ischium with 9–11 simple setae; merus longer than ischium and with 14–15 simple setae; carpus with 12–13 simple setae; propodus longer than carpus, with 23–24 simple setae

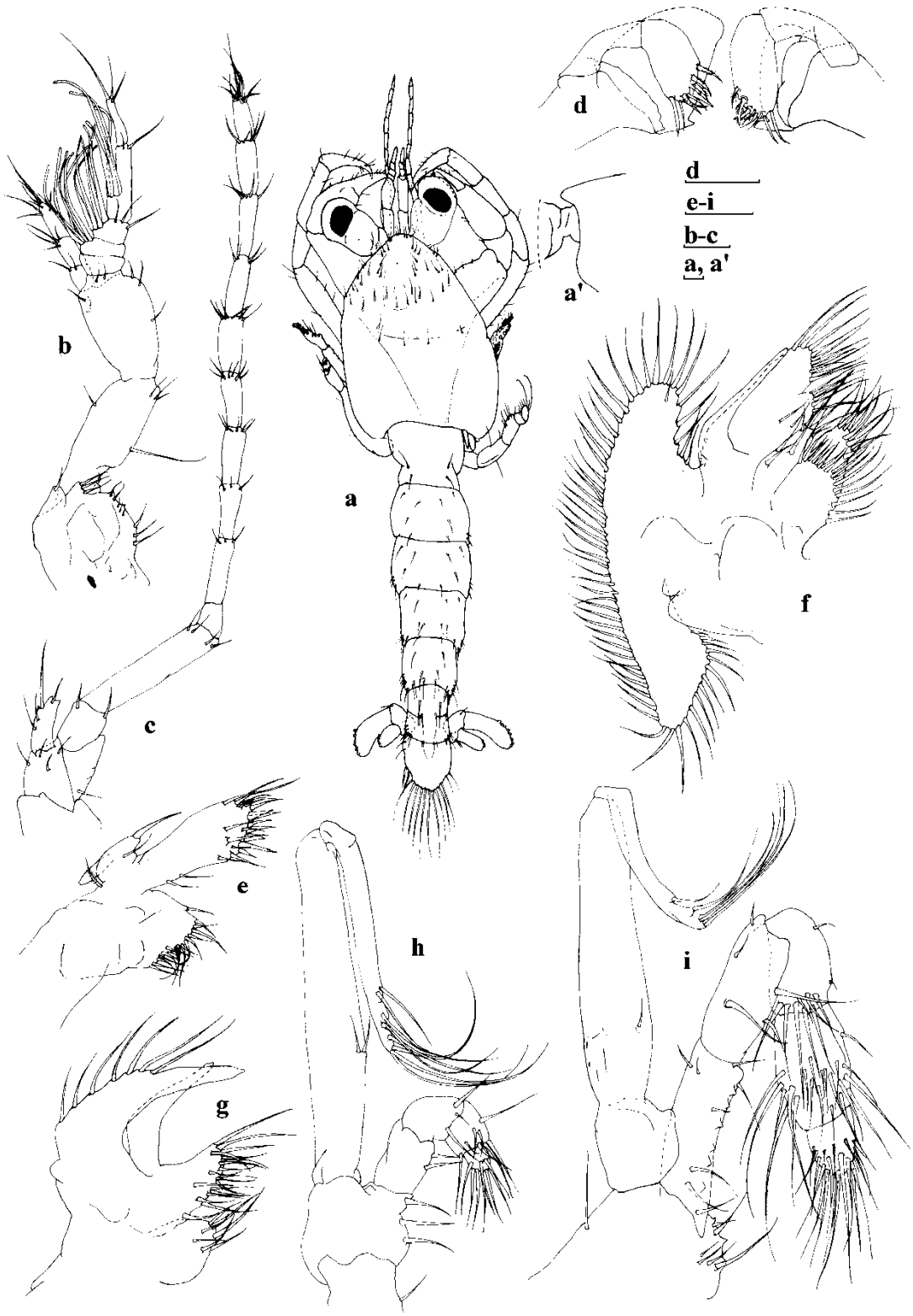

Fig. 4- *Clibanarius aequabilis*. Megalopa: **a**, total animal, dorsal view; **a'**, detail of rostrum, lateral view; **b**, antennule; **c**, antenna; **d**, mandibles; **e**, maxillule; **f**, maxilla; **g**, first maxilliped; **h**, second maxilliped; **i**, third maxilliped. Scale bars: 0.1 mm.

and 1 stout cuspidate seta; dactylus very stout, apically curved and corneous, with 24–26 simple setae and 3 spines.

Fourth pereiopod (Fig. 5d, d'): basis with 5–7 simple setae; ischium with 5–8 simple setae; merus with 8–9 simple setae; carpus with 5 simple setae; propodus not chelate with 9 simple setae and 14–16 pseudochaetae as figured; dactyl with 1 long papposerrate seta, 8–9 simple setae and 2 teeth on the distal extremity.

Fifth pereiopod (Fig. 5e, e'): coxa with 6–7 simple setae; basis with 3 simple setae; ischium with 2–3 simple setae; merus with 2 long papposerrate setae and 6–7 small simple setae; carpus with 3 papposerrate and 5–6 simple setae; propodus not chelate with 3 long papposerrate, 16–18 simple setae and 17–18 pseudochaetae as figured; dactyl with 9–11 simple setae and 3–4 spatulate-shaped pseudochaetae distributed as figured.

Abdomen (Fig. 4a): somites with postero-lateral angles rounded; somite 1 with 2 pairs of dorsal simple setae, somites 2–3 each with 4 pairs of dorsal simple setae and 3 pairs of simple setae on dorso-lateral margin, somites 4–5 each with 5 pairs of dorsal simple setae and 4–5 pairs of simple setae on dorso-lateral margin, somite 6 with 5 pairs of dorsal simple setae and 7 pairs of simple setae on dorsolateral margin.

Pleopods (Fig. 5f–i): well developed, biramous, decreasing in size posteriorly; outer ramus with 8–9 plumose setae; inner ramus shorter, with 2 small apical hooks.

Uropods (Fig. 5j): right pair slightly longer than left pair; protopod with 1 simple seta on outer margin; exopod with 4–5 simple setae dorsally, 3–4 simple setae on dorsal margin, 17–19 plumose setae and 10–13 pseudochaetae marginally; endopod with 4–5 simple setae dorsally, 4 simple setae on dorsal margin, 10–11 plumose setae and 7–8 pseudochaetae marginally.

Telson (Fig. 5j): telson with terminal margin rounded, 10–11 pairs of simple setae on the dorsal surface, 2 pairs of pappose setae on the lateral margin and 5–6 pairs of long

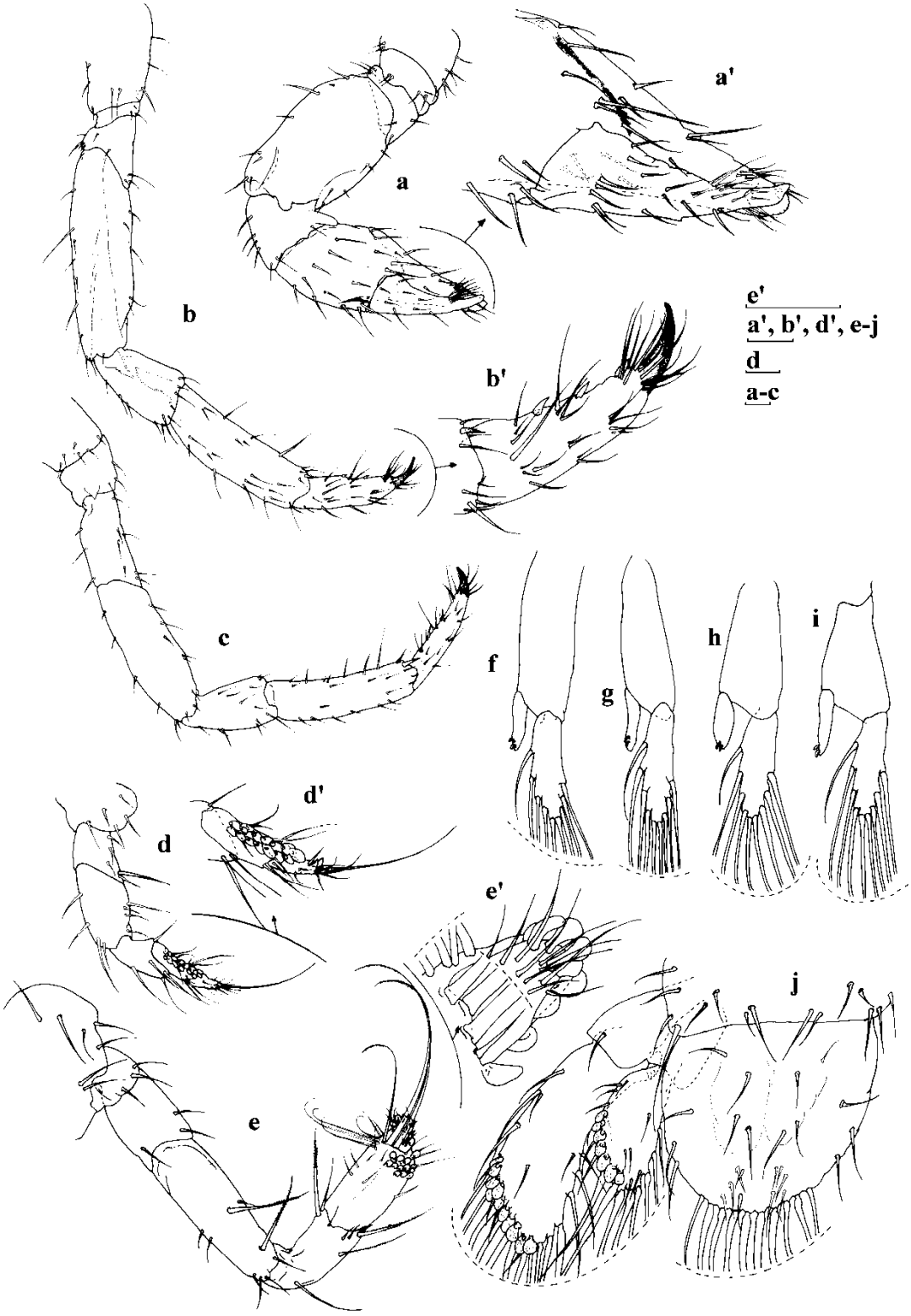

Fig. 5- *Clibanarius aequabilis*. Megalopa: **a**, first pereiopod; **a'**, detail of chela of first pereiopod; **b**, second pereiopod; **b'**, detail of dactylus of second pereiopod; **c**, third pereiopod; **d**, fourth pereiopod; **d'**, detail of dactylus of fourth pereiopod; **e**, fifth pereiopod; **e'**, detail of propodus and dactylus of fifth pereiopod; **f- i**, pleopods of abdominal somites 2-5; **j**, telson and uropods. Scale bars: 0.1 mm (**a-e**, **f-j**); 0.05 mm (**e'**).

plumose setae on the distal margin, distributed as illustrated. One of the observed individuals presented an unpaired number (11) of plumose setae.

Clibanarius erythropus (Latreille, 1818)

Figure 6

Larval references: Pike and Williamson, 1960: p. 503, Fig. 2 (zoea I, zoea II and zoea III); Le Roux, 1966: p. 227, Fig. 1 (zoea IV); Dechancé and Forest, 1958: p. 277, Fig. 2; p. 283, Figs. 16–23; p. 284, Figs. 24–29 (megalopa)

First zoea (Figure 6)

Dimension: TL = 1.83– 1.94 mm; CL = 0.81– 0.91 mm; R = 0.27– 0.38 mm

Carapace (Figs. 6a, a'): without processes or spines, with the postero-lateral border smoothly rounded; rostrum broad, rather blunt, with triangular pointed tip extending beyond antennal scale, rostrum about 0.66 ± 0.08 times the carapace; eyes compound and sessile.

Antennule (Fig. 6b): peduncle unsegmented, with 1 long plumose seta sub-terminally, 3 aesthetascs and 3 unequal simple setae terminally.

Antenna (Fig. 6c): protopod unsegmented with 1 small strong cuspidate seta; endopod with more than half-length the scale, with 3 long plumose seta apically; scaphocerite unsegmented, with 11 plumose setae.

Mandible (Fig. 6d): asymmetrical; palp absent.

Maxillule (Fig. 6e): coxal endite with 3 serrulate, 2–3 pappose and 2 simple setae, basial endite with 2 cuspidate setae and 2 simple setae; endopod with 1 + 1 + 2 simple setae.

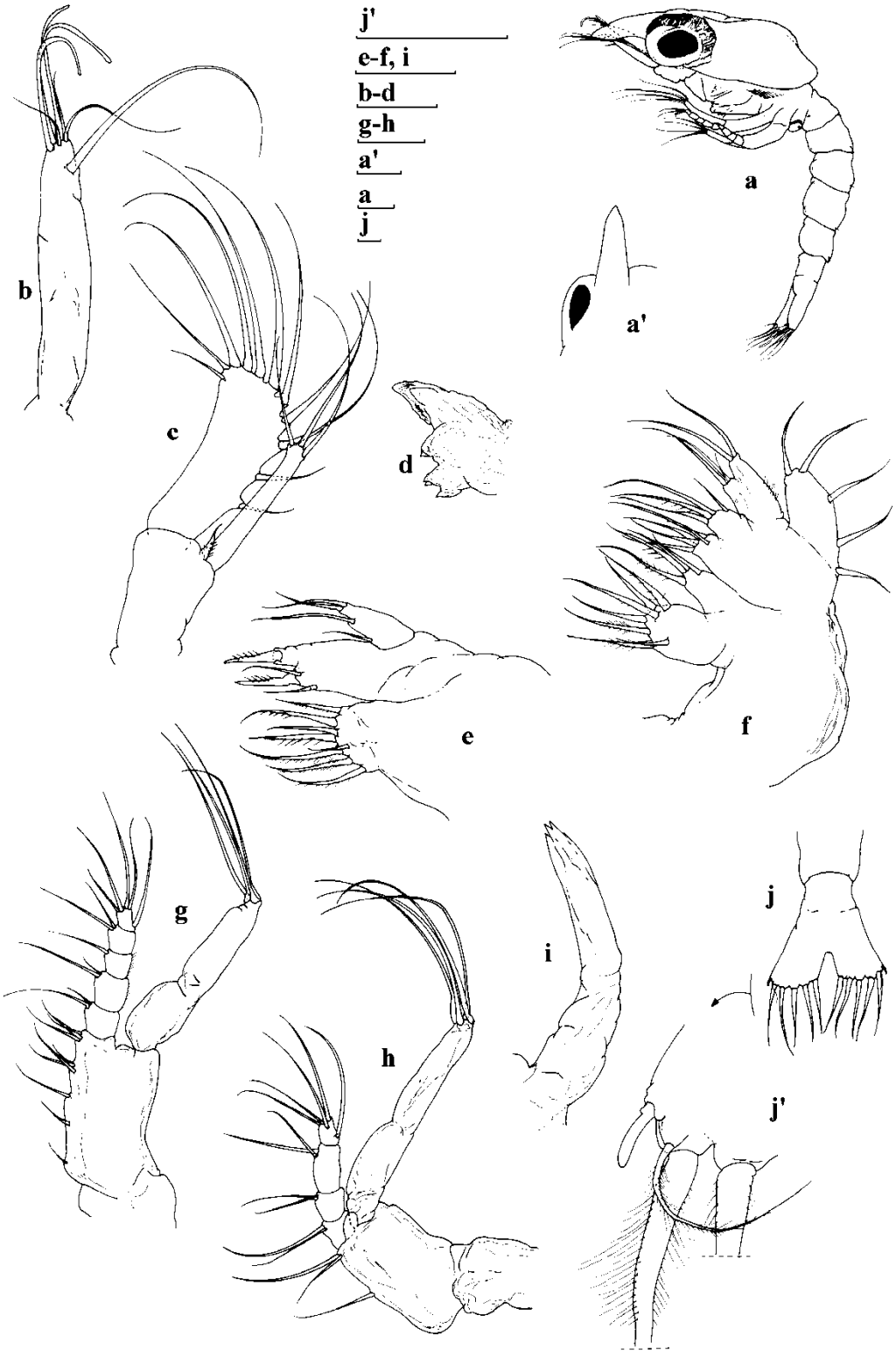

Fig. 6- *Clibanarius erythropus.* First zoea: **a**, total animal, lateral view; **a'**, detail of rostrum, dorsal view; **b**, antennule; **c**, antenna; **d**, mandible; **e**, maxillule; **f**, maxilla; **g**, first maxilliped; **h**, second maxilliped; **i**, third maxilliped; **j**, telson; **j'**, detail of posterior margin of telson. Scale bars: 1.0 mm (**a'**); 0.1 mm (**a-j'**).

Maxilla (Fig. 6f): coxal endite bilobed with 7–8 + 4 serrulate and papposerrate setae, basial endite bilobed with 5 + 4–5 papposerrate and simple setae; endopod unsegmented bilobed terminally, bearing 3+2 long simple setae and microtricha on outer margin; scaphognathite with 5 marginal plumose setae.

First maxilliped (Fig. 6g): coxa unarmed; basis with 1 + 1 + 1 + 3 + 3 papposerrate setae; endopod 5-segmented with 2, 2, 1, 2 papposerrate and 1+4 papposerrate setae, microtricha on outer margin of second and third segments; exopod unsegmented, with 4 long plumose setae terminally.

Second maxilliped (Fig. 6h): coxa unarmed; basis with 1 + 2 papposerrate setae; endopod 4-segmented with 2, 2, 2 papposerrate and 1 + 4 papposerrate setae terminally. Exopod unsegmented, with 4 long plumose setae terminally.

Third maxilliped (Fig. 6i): long uniramous bud.

Pereiopods: absent.

Abdomen (Fig. 6a): somites with margins rounded, with a pair of dorso-lateral marginal minute setae on somites 2– 5; sixth somite feebly segmented.

Pleopods and uropods: absent.

Telson (Fig. 6j, j'): triangular, broader posteriorly with deep median cleft; with 7 + 7 processes, the first process is a very short blunt spine, the second process an anomuran hair, the third to the seventh processes plumose setae, being the fourth the longest.

Second zoea

Dimension: TL = 2.10– 2.26 mm; CL = 1.02– 1.13 mm; R = 0.43– 0.48 mm

Carapace: eyes stalked; otherwise unchanged.

Antennule: peduncle unsegmented with 2 long plumose setae sub-terminally and 2

short simple setae terminally, 1 long plumose seta at site of future endopod; exopod bud clearly delineated, with 3 simple setae and 3 aesthetascs.

Antenna: unchanged except for size.

Mandible: molar process with several small denticles and without teeth; otherwise unchanged.

Maxillule: basial endite with 4 cuspidate setae and 2 simple setae; otherwise unchanged except for size.

Maxilla: basial endite bilobed with 5 + 4 papposerrate and simple setae; scaphognathite with 7 marginal plumose setae; otherwise unchanged except for size.

First maxilliped: endopod 5-segmented with 1 + 2, 2–3, 2, 2 papposerrate and 1 + 4 papposerrate setae. Exopod bearing 6 long plumose setae terminally; otherwise unchanged.

Second maxilliped: endopod four-segmented with 2, 2 + 1, 1 + 2 papposerrate and 1 + 4 papposerrate setae terminally. Exopod bearing 6 long plumose setae terminally; otherwise unchanged.

Third maxilliped: biramous; endopod represented by a small bud basally situated; exopod unsegmented, bearing 5 plumose setae.

First to third pereiopods: present as small uniramous buds.

Abdomen: unchanged except for size.

Pleopods and uropods: absent.

Telson: 8 + 8 processes, with an additional inner pair of telson plumose setae; broader posteriorly with the median cleft less pronounced; otherwise unchanged.

Third zoea

Dimension: TL = 2.74– 2.85 mm; CL = 1.35– 1.51 mm; R = 0.48– 0.59 mm

Carapace: unchanged except for size.

Antennule: peduncle unsegmented with 1 small basial simple seta, 4 long plumose setae and 4 short simple setae distally; the outer flagellum with 3 aesthetascs, 1 long plumose seta and 3 shorter simple setae; the inner flagellum small, bud-like.

Antenna: endopod as long as the scale, with 1 short plumose seta in apical region; scaphocerite with 11–12 plumose setae. Otherwise unchanged.

Mandible and maxillule: unchanged except for size.

Maxilla: coxal endite bilobed with 8 + 4 serrulate and papposerrate setae; scaphognathite with 9–10 marginal plumose setae; otherwise unchanged except for size.

First and second maxillipeds: unchanged except for size.

Third maxilliped: endopod bud enlarged in size; exopod unsegmented, bearing 6 plumose setae.

First pereiopod: uniramous bud showing its chelate nature.

Second and third pereiopods: unchanged except for size.

Fourth and fifth pereiopods: small uniramous buds.

Abdomen: abdominal somite 6 fully separated from telson, bearing a pair of dorso-lateral marginal minute setae on somites 2–5; otherwise unchanged.

Pleopods: absent.

Uropods: biramous; exopod well developed, not reaching the end of telson, with 7–8 plumose setae; endopod a small bud with 2 minute projections at its tip.

Telson: triangular, broader posteriorly with the median cleft less pronounced; the number of telson processes: 8–9 + 9 (the first process as a very short blunt spine, the second as an anomuran hair, the third as a plumose seta, the fourth as a very reduced fused spine and the fifth to eighth or ninth processes as plumose setae).

Fourth zoea

Dimension: TL = 3.01– 3.17 mm; CL = 1.51– 1.61 mm; R = 0.48– 0.59 mm

Carapace: unchanged except for size.

Antennule: peduncle unsegmented with 2 simple setae on lateral margin, 4 short simple and 4 long plumose setae distally; outer flagellum with 2 + 2 + 2 + 1 aesthetascs, 2 long and 2 short plumose setae; inner flagellum unsegmented, enlarged in size.

Antenna: endopod longer than antennal scale, two-segmented: first segment unarmed, second segment with a short plumose seta and a pointed tip terminally; scaphocerite with 11–13 plumose setae. Otherwise unchanged.

Mandible: presence of an unsegmented palp.

Maxillule: unchanged except for size.

Maxilla: coxal endite bilobed with 9–12 + 4 serrulate and papposerrate setae; basial endite with 6–7 + 4–5 papposerrate and simple setae; endopod unsegmented, bilobed, terminally bearing 3 + 2 long simple setae; scaphognathite with 13–16 marginal plumose setae.

First and second maxillipeds: unchanged.

Third maxilliped: endopod two-segmented, incomplete 2 to 5 segments with two-minute projection at its tip; otherwise unchanged.

First pereiopod: cheliped with chela enlarged, dactyl distinct, coxa and merus clearly delineated, with tubercles on surface.

Second and third pereiopods: long, six segments clearly delineated, coxa and merus with some tubercles on surface.

Fourth and fifth pereiopods: short, with some segments clearly delineated.

Abdomen: unchanged except for size.

Pleopods: present as biramous buds on segments 2 to 5.

Uropods: total individualization of protopod, exopod and endopod; exopod with 9–10 plumose setae on apical region and 2–3 papposerrate setae on ventral surface; endopod smaller, with 4–5 plumose setae on apical region and 1 pappose seta on ventral surface.

Telson: almost rectangular in shape; number of telson processes: 9 + 9 (first process as a very short blunt spine, second as an anomuran hair, third as a plumose seta, fourth as a very reduced fused spine and fifth to ninth processes as plumose setae).

Megalopa

Dimension: TL = 2.80– 2.96 mm; CL = 1.08– 1.24 mm; R = 0.16– 0.22 mm

Carapace: smooth, slightly broader than long; rostrum broad and blunt; ocular peduncles stout, reaching the end of the antennular peduncle; ocular acicles not developed; surface and margins with proboscate setae distributed as figured.

Antennule: peduncle three-segmented, proximal segment with 2 papposerrate, 4–6 simple and 5 papposerrate setae; second segment with 1 long and 3–5 short simple setae and 1 papposerrate seta; distal segment with 5–6 simple setae; outer flagellum five-segmented, proximal segment unarmed, second segment with 5 aesthetascs, third segment with 5 aesthetascs and 4–5 simple setae, fourth segment with 3 aesthetascs and 2–3 simple setae terminally, distal segment with 3–4 simple setae; inner flagellum three-segmented with 2, 2–3 and 6–8 simple setae.

Antenna: basis with 3 simple setae, peduncle 3-segmented with 1–2, 2, 4–5 simple setae; flagellum composed of 9–10 segments, each with 0–6 simple setae on distal margin, except for the distal segment which has 8–9 simple setae on distal margin; acicle with 1 spinelike projection on lateral margin, 4 simple setae and bifid spinous tip.

Mandibles: reduced and simplified, lower extremities of the incisor part with processes as illustrated; palp three-segmented, proximal segment with 1 strong simple seta, second segment unarmed, distal segment with 4–5 simple setae and 4–5 serrulate setae terminally.

Maxillule: coxa with 18–20 papposerrate and simple setae; basis with 2 simple setae on lateral inner margin and 5 cuspidate, 5 serrulate, 6–7 papposerrate, 3 simple and 1 long plumose setae on distal margin; endopod unsegmented with 1 + 1 simple setae; 1 plumose seta present on inner margin of basis.

Maxilla: coxa bilobed with 27–29 + 7–8 papposerrate and simple setae; basis bilobed with 9–12 + 10–13 papposerrate and simple setae; endopod unsegmented without seta; scaphognathite with 47–52 plumose setae on distal margin and 1 simple seta on dorsal surface of the upper lobe.

First maxilliped: coxa with 2–3 simple, 2–3 pappose and 3 papposerrate setae; basis with 3–4 simple, 3 serrulate and 13–14 papposerrate setae; unsegmented endopod without seta; exopod unsegmented with 6–8 plumose setae on outer margin.

Second maxilliped: coxa without any seta or with 1 simple seta; basis with 2–3 pappose setae; endopod four-segmented, proximal segment with 1 + 1 simple setae, second segment with 2–3 simple setae, third segment with 7–9 simple and papposerrate setae, and 6–7 papposerrate setae on distal segment; exopod two-segmented with 2 simple setae on proximal segment and 8 plumose setae terminally on distal segment.

Third maxilliped: basis with 5–7 simple setae and 3–4 sub-acute processes; endopod five-segmented, ischium with 7–9 simple seta and 4–5 sub-acute processes on crista dentata, merus with 4–6 simple setae, carpus with 9–10 simple setae, propodus with 3–4 simple, 2 cuspidate and 13–15 papposerrate setae, and dactyl with 5 serrate, 6–7 simple, 3 cuspidate and 5–6 papposerrate setae; exopod 2-segmented with 1 simple seta on proximal segment and 8 plumose setae terminally on distal segment.

First pereiopod: chelae equal, with segments smooth; coxa with 9–10 simple setae; basis with 4 simple setae; ischium with 8–9 simple setae; merus, longest segment, with 14–16 simple setae; carpus with 6–7 simple setae; propodus with 38–40 simple setae, distal upper extremity corneous; dactyl length about half palm + propodal prolongation, with 23–26 simple setae and distal extremity corneous.

Second pereiopod: coxa with 7–8 simple setae; basis smaller with 2 simple setae; ischium with 7–8 simple setae; merus, longest segment, with 14–16 simple setae; carpus less than half the ischium length, with 12–14 simple setae; propodus longer than carpus, with 22–25 simple setae and 1 stout cuspidate seta; dactylus very stout, apically curved and corneous, with 27–29 simple setae and 3–4 spines.

Third pereiopod: coxa with 7–8 simple setae; basis with 4–5 simple setae; ischium with 10–11 simple setae; merus longer than ischium and with 14–16 simple setae; carpus with 11–12 simple setae; propodus longer than carpus, with 24–27 simple setae and 1 stout cuspidate seta; dactylus very stout, apically curved and corneous, with 25–28 simple setae and 3–4 spines.

Fourth pereiopod: basis with 7 simple setae; ischium with 8–10 simple setae; merus with 9–10 simple setae; carpus with 4–5 simple setae; propodus not chelate, with 8–9 simple setae and 10–14 pseudochaetae; dactyl with 1 long papposerrate seta, 7–9 simple setae and 2 teeth on distal extremity.

Fifth pereiopod: coxa with 5–6 simple setae; basis with 3 simple setae; ischium with 2–3 simple setae; merus with 2 long papposerrate setae and 6–7 small simple setae; carpus with 3 papposerrate and 5–6 simple setae; propodus not chelate, with 5–6 long papposerrate, 16–18 simple setae and 11–13 pseudochaetae; dactyl with 5–7 simple setae and 3 spatulate-shaped pseudochaetae.

Abdomen: somites with postero-lateral angles rounded; somite 1 with 2 pairs of dorsal simple setae, somites 2–3 each with 4–5 pairs of dorsal simple setae and 5 pairs of simple setae on dorsolateral margin, somites 4–5 each with 5 pairs of dorsal simple setae and 4–5 pairs of simple setae on dorsolateral margin, somite 6 with 5 pairs of dorsal simple setae and 6 pairs of simple setae on dorsolateral margin.

Pleopods: well developed, biramous, decreasing in size posteriorly; outer ramus with 8–9 plumose setae; inner ramus shorter, with 2 small apical hooks.

Uropods: right one slightly longer than left one; protopod with 4 simple setae on outer margin; exopod with 3 simple setae dorsally, 4 simple setae on dorsal margin, 16–18 plumose setae and 9–11 pseudochaetae marginally; endopod with 3–5 simple setae dorsally, 4–5 simple setae on dorsal margin, 8–9 plumose setae and 5–8 pseudochaetae marginally.

Telson: telson with terminal margin rounded, 8–9 pairs of simple setae on dorsal surface, 2 pairs of pappose setae on lateral margin and 5 pairs of long plumose setae on the distal margin.

5.5- Discussion

Larval stages of *C. erythropus* cultured in the present study displayed only minor differences to those described previously by Pike & Williamson (1960), Le Roux (1966) and Dechancé & Forest (1958). In our study the first zoeal stage had longer TL and CL than described by Pike & Williamson (1960) and Le Roux (1966), while the megalopa was smaller than recorded by Dechancé & Forest (1958) (Table 1). In the first zoeal stage, the endopod of the maxillule was found unsegmented, whereas in previous studies it had been described as a two-segmented structure. Although Pike & Williamson (1960) and Le Roux (1966) reported the absence of pereiopods in the second zoeal stage, in our study this zoeal stage already

presented the first three pairs of pereiopods as small buds. No significant differences were found in the third zoeal stage, but in zoea IV the segmentation of the third maxilliped differed between our specimens and those previously described (see Table 1). The differences in setation patterns may result from different geographical origins of ovigerous females. In our study and in that of Le Roux (1966), ovigerous females were collected in the coastal waters of the northeastern Atlantic (in Ria do Alvor, Algarve, Portugal and in Île Bailleron, Bretagne, France, respectively), while those used by Pike & Williamson (1960) and Dechancé & Forest (1958) originated from the Mediterranean Sea (Naples, Campania region, Italy and Banyuls-sur-Mer, Languedoc-Roussillon region, France, respectively). Similar intra-specific variations were recorded and discussed by Siddiqui et al. (1991), when comparing larval series of *C. antillensis* from Panamanian and Brazilian populations.

The larval stages of the two northeastern Atlantic *Clibanarius* species, *C. aequabilis* and *C. erythropus*, cannot be easily differentiated (Table 1). However, the zoeal stages of *C. aequabilis* were always 23–30% bigger than those of *C. erythropus*. Besides size, there were only small differences (Table 1). In the megalopa stage, *C. aequabilis* can be distinguished from *C. erythropus* by the number of segments of the mandibular palp: 2 segments in *C. aequabilis* and 3 in *C. erythropus*.

Table 1 summarizes the available data on morphological larval characters of *Clibanarius* species. Concerning larval morphology, the genus *Clibanarius* is very homogeneous and available data allow us to complete Shenoy & Sankolli's (1977) definition of its generic larval features. Thus, *Clibanarius* zoeae have a smooth and rounded carapace with a broad and blunt rostrum; the abdominal segments are also smooth, and dorso-marginal spines are

Table 1 Comparison of relevant larval characters of *Clibanarius* species.

Features	*C. aequabilis* (present study)	*C. erythropus* (present study)	*C. erythropus* (1), (2), (3)	*C. antillensis* (4)	*C. vittatus* (5)	*C. sclopetarius* (6)	*C. albidigitus* (7)	*C. signatus* (8)	*C. virescens* (8)	*C. infraspinatus* (9)	*C. merguiensis* (10)	*C. padavensis* (11)	*C. clibanarius* (12)	*C. longitarsus* (13)	*C. olivaceus* (14)
Females collection area	NE Atlantic	NE Atlantic	NE Atlantic	Caribbean Sea	NW Atlantic	SW Atlantic	Central E Pacific	Arabian Sea	Arabian Sea	Arabian Sea	Arabian Sea	Arabian Sea	Bay of Bengal	Bay of Bengal	Bay of Bengal
Stages: zoea (Z)+megalopa (M)	4Z+1M	4Z+1M	4Z+1M	4Z+1M	5Z+1M	5Z+1M	4Z+1M	4Z+1M	4Z+1M	4Z+1M	4Z+1M	4Z+1M	4Z+1M	4Z+1M	5Z+1M
First Zoea															
Total length, mm	2.39-2.62	1.83-1.94	1.70-1.80	1.20	1.90-2.40	-	-	1.60	1.80	-	-	-	1.90	1.52	1.57
Carapace length, mm	1.04-1-12	0.81-0.91	0.80	0.80	0.90-1.10	-	0.63-0.76	-	-	1.00	1.00	0.90	0.85	0.85	0.90
CARAPACE															
Rostrum/ carapace	0.89±0.06	0.66±0.08	-	-	-	-	-	-	-	-	-	-	-	-	-
ANTENNULE															
Peduncle: segments/ setae	Unseg./ 1+(3)+3	Unseg./ 1+(3)+3	Unseg./ -	Unseg./ 1+(3)+3	Unseg./ 1+(3)+2-3	Unseg./ 1+(2)+2	Unseg./ 1+(2-4)+2-3	Unseg./ 1+(4)+2	Unseg./ 1+(4)+1	Unseg./ 1+(3)+3	Unseg./ 1+(2)+2	Unseg./ 1+(2)+3	Unseg./ 1+(2)+3	Unseg./ 1+-	Unseg./ 1+(3)+3
ANTENNA															
Protopod: segments/ setae	Unseg./1	Unseg./1	Unseg./1	Unseg./1	Unseg./1	Unseg./1	Unseg./1	Unseg./1	Unseg./1	Unseg./1	Unseg./1	Unseg./1	Unseg./1	-	Unseg./1
Endopod: segments/ setae	Unseg./3	Unseg./3	Unseg./3	Unseg./3	Unseg./3	Unseg./3	Unseg./2-3	Unseg./3	Unseg./3	Unseg./3	Unseg./3	Unseg./3	Unseg./3	-	Unseg./3
Scaphocerite	11	11	11	10-11	9-11	11	11	11	11	11	11	10-11	11	-	11
MAXILLULE															
Coxal endite	7	7-8	-	7	6	5	7	7	5	7	6	5	7	-	6
Basial endite	5	4	-	4	3-4	3	4	4	2	3	3	3	3	3	3
Endopod: segments/ setae	Unseg./4	Unseg./4	2/1,2	Unseg./3	Unseg./3	Unseg./3	Unseg./4	2/1,2	2/1,2	-	Unseg./3	Unseg./3	Unseg./3	Unseg./-	Unseg./3
MAXILLA															
Coxal endite	7+4	7-8+4	-	7+3	6+4	3+2-3	7+4-5	6+3	5+3	7+4	6+4	7+3	6+3	-	6+3
Basial endite	5+4	5+4-5	-	4-5+3	5+3-4	5-6+2-3	5-6+4	5+4	4+4	5+3	4+4	4+3	2+4	-	4+4
Endopod: segments/ setae	Unseg./3+2	Unseg./3+2	-	Unseg./2+2	Unseg./2+2	Unseg./4	Unseg./2+2	Unseg./4	Unseg./4	Unseg./2+2	Unseg./2+2	Unseg./2+2	Unseg./2+2	Unseg./-	Unseg./2+2
Scaphognathite	5	5	-	5-6	4-6	5	5	5	5	5	5	5	5	5	5
FIRST MAXILLIPED															
Basial endite	9	9	-	6	7-9	7	7-8	6	9	7	8	6-7	-	6	6
Endopod: segments/ setae	5/2,2,2,2,5	5/2,2,1,2,5	-	5/1,3,2,3,5	5/2,2,1,3,4	5/1,1,1,3,3	5/2,2,1,2,5	5/2,2,2,3,4	-	5/2,2,1,2,5	5/1,2,1,2,5	5/2,2,1,2,5	5/2,2,2,2,5	5/-	5/2,2,2,3,4
Exopod: segments/ setae	Unseg./4	Unseg./4	Unseg./4	2/0,4	Unseg./4	-	2/0,4	Unseg./4	-	Unseg./4	2/0,4	Unseg./4	Unseg./4	Unseg./4	Unseg./4
SECOND MAXILLIPED															
Basial endite	3	3	-	3	3	3-6	3	3	3	3	4	3	3	-	2
Endopod: segments/ setae	4/2,2,2,5	4/2,2,2,5	-	4/2,3,3,5	4/2,2,2,5	4/2,2,2,3	4/2,2,2,5	4/2,2,2,5	4/2,2,2,5	4/2,2,2,5	4/2,2,2,5	4/2,2,1,5	4/2,2,2,5	4/-	4/2,2,3,4
Exopod: segments/ setae	Unseg./4	Unseg./4	Unseg./4	2/0,4	Unseg./4	-	2/0,4	Unseg./4	Unseg./4	Unseg./4	2/0,4	Unseg./4	Unseg./4	Unseg./4	Unseg./4
THIRD MAXILLIPED															
Development	Unir. bud	Unir. bud	Unir. bud	Unir. bud	Unir. bud	Unir. bud	Unir. bud	Unir. bud	Unir. bud	Unir. bud	Unir. bud	Unir. bud	Unir. bud	Unir. bud	Unir. bud
Segments	Unseg.	Unseg.	Unseg.	2	-	-	Unseg.	2	2	-	-	-	Unseg.	Unseg.	Unseg.
TELSON															
Posterior margin processes	7+7	7+7	7+7	7+7	7+7	7+7	7+7	7+7	7+7	7+7	7+7	7+7	7+7	7+7	7+7

Table 1 continued

Features	*C. aequabilis*	*C. erythropus*	*C. erythropus*	*C. antillensis*	*C. vittatus*	*C. sclopetarius*	*C. albidigitus*	*C. signatus*	*C. virescens*	*C. infraspinatus*	*C. merguiensis*	*C. padavensis*	*C. clibanarius*	*C. longitarsus*	*C. olivaceus*
Second Zoea															
Total length, mm	3.00-3.23	2.10-2.26	2.00-2.20	1.91-2.00	2.20-2.90	-	-	-	2.20	-	-	-	2.43	2.21	2.00
Carapace length, mm	1.31-1.39	1.02-1.13	1.10	1.00-1.02	1.10-1.30	-	0.85-1.20	-	-	1.20	1.20	0.90	1.05	1.07	1.08
CARAPACE															
Rostrum/ carapace	0.89±0.06	0.72±0.05	-	-	-	-	-	-	-	-	-	-	-	-	-
ANTENNULE	Unir./2-seg.	Unir/2-seg.	-	Unir/2-seg.	Unir/2-seg.	Unir/2-seg.	Unir/2-seg.	Unir/2-seg.	Unir/2-seg.	Unir/2-seg.	Unir/2-seg.	Unir/2-seg.	Unir/2-seg.	Unir/2-seg.	Unir/2-seg.
Peduncle: setae	5	5	-	5	3-6	3	4-5	6	6	6-7	3	5	3	-	3
Outer flagellum: setae	3+(3)	3+(3)	-	3+(5)	2-3+(4)	3+(2)	3-4+(3-4)	1+(5)	2+(4)	6+(1)	3+(2)	4+(2)	2+(5)	-	2+(4)
ANTENNA															
Scaphocerite	11	11	10-12	10-11	11	12	11-12	12	11	11	11	11	12	-	-
MAXILLULE															
Endopod: segments/ setae	Unseg./4	Unseg./4	-	Unseg./3	Unseg./3-4	Unseg./3	Unseg./4	2/1,2	-	Unseg./3	Unseg./3	Unseg./3	Unseg./3	Unseg./-	Unseg./3
MAXILLA															
Scaphognathite	7	7	-	6-7	7	7	7	6	6	6	6-7	6-7	8	7	7
FIRST MAXILLIPED															
Exopod: segments/ setae	Unseg./6	Unseg./6	Unseg./6	2/0,6	Unseg./6	-/6	2/0,6	Unseg./6	Unseg./6	Unseg./6	2/0,6	Unseg./6	Unseg./6	Unseg./6	Unseg./6
SECOND MAXILLIPED															
Exopod: segments/ setae	Unseg./6	Unseg./6	Unseg./6	2/0,6	Unseg./6	-/6	2/0,6	-	Unseg./6	Unseg./6	2/0,6	Unseg./6	Unseg./6	Unseg./6	Unseg./6
THIRD MAXILLIPED															
Endopod: development	Bud	Bud	Bud	Bud	Absent	-	Bud	Absent	Bud	Bud	Bud	Bud	Bud	Bud	-
Exopod: segments/ setae	Unseg./6	Unseg./5	Unseg./4-5	2/0,5	-/4-5	-/5	2/0,5-6	Unseg./4	Unseg./5	Unseg./5	Unseg./5	Unseg./5	Unseg./5	Unseg./5	Unseg./5
PEREIOPODS	1-3 unir. buds	1-3 unir. buds	Absent	-	-	-	-	Absent	Absent	-	Abse nt	Absent	-	-	Absent
ABDOMEN	5 somites	5 somites	5-6 somites	5 somites	-	5 somites	5 somites	-	-	5 somites	5 somites	5 somites	6 somites	6 somites	-
TELSON															
Posterior margin processes	8+8	8+8	8+8	8+8	8+8	8+8	8+8	8+8	8+8	8+8	8+8	8+8	8+8	8+8	8+8
Third Zoea															
Total length, mm	3.44-3.77	2.74-2.85	2.80	2.90-3.20	2.80-4.10	-	-	2.10	2.50	-	-	-	2.96	2.95	2.43
Carapace length, mm	1.61-1.72	1.35-1.51	1.40	1.50-1.70	1.50-2.00	-	1.20-1.40	-	-	1.50	1.40	1.40	1.33	1.43	1.20
CARAPACE															
Rostrum/ carapace	0.86±0.03	0.60±0.04	-	-	-	-	-	-	-	-	-	-	-	-	-
ANTENNULE	Biram.	Biram.	Unir./2-seg.	Unir./2-seg.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram	Biram.	Biram.	Biram.	
Outer flagellum: setae	4+(3)	4+(3)	-	-+(5)	5+(2)	3+(2)	3-4+(2-4)	2+(4)	1+(5)	6+(1)	4+(3)	4+(2)	4+(4)	-	4+(4)
Inner flagellum: development	Small (bud)	Small (bud)	-	Absent	Absent	Small (bud)	Small (bud)	Small (bud)	Small (bud)	Small (bud)	Small (bud)	Small (bud)	Small (bud)	Small (bud)	Small (bud)
ANTENNA															
Scaphocerite	12	11-12	10-12	12	11-12	11-12	12	12	12	12	12	12	13	12	12
MAXILLA															
Scaphognathite	10-11	9-10	-	10-13	7-10	10-11	9-11	8	11	12-13	10	10	11	9	9
THIRD MAXILLIPED															
Endopod: development	Bud	Bud	Bud	3-seg. bud	Absent	Bud	Bud	Bud	Bud	Bud	Bud	Bud	Bud	Bud	Bud
Exopod: segments/ setae	Unseg./6	Unseg./6	Unseg./4-5	2/0,5	2/0,5-6	-	2/0,5-6	Unseg./6	Unseg./6	Unseg./6	Unseg./6	Unseg./6	Unseg./6	Unseg./6	Unseg./6
PEREIOPODS	All as buds	All as buds	All as buds	-	-	-	Buds	All as buds	All as buds	-	1-4 as buds	All as buds	1-3 as buds	All as buds	1-4 as buds
UROPODS	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.
Exopod: marginal setae	7-8	7-8	6-7	8	6-9	7	6-8	7-8	-	8-9	8	8-9	8	8	7
TELSON															
Posterior margin processes	9-10+9-10	8-9+9	8+8	8+1+8	8+1+8	8+1+8	8+1+8	8+1+8	8+1+8	8+1+8	8+1+8	8+1+8	8+1+8	8+1+8	8+1+8
4th telson process: fused spine	Reduced	Reduced	Reduced	Well developed	Well developed	Reduced	Well developed	Reduced	Well developed	Reduced	Well developed	Reduced	Reduced	Reduced	Reduced

Table 1 continued

Features	*C. aequabilis*	*C. erythropus*	*C. erythropus*	*C. antillensis*	*C. vittatus*	*C. sclopetarius*	*C. albidigitus*	*C. signatus*	*C. virescens*	*C. infraspinatus*	*C. merguiensis*	*C. padavensis*	*C. clibanarius*	*C. longitarsus*	*C. olivaceus*
Fourth Zoea															
Total length, mm	4.08-4.40	3.01-3.17	2.80	3.10-3.30	3.60-5.20	-	-	-	3.80	-	-	-	3.77	3.58	3.19
Carapace length, mm	1.92-2.08	1.51-1.61	1.40	1.60-1.70	1.70-2.60	-	1.50-1.70	-	-	1.60	1.70	1.60	1.72	1.63	1.73
CARAPACE															
Rostrum/ carapace	0.86±0.08	0.54±0.04	-	-	-	-	-	-	-	-	-	-	-	-	-
ANTENNULE	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.	Biram.
Outer flagellum: segments/setae	Unseg./4+(7)	Unseg./4+(7)	Unseg./-	Unseg./4+(9)	Unseg./4+(4-5)	Unseg./4-5+(4-5)	Unseg./3-4+(7)	Unseg./2+(6)	Unseg./1+(7)	Unseg./6+(1)	Unseg./4+(3)	Unseg./3+(3)	Unseg./9+(-)	Unseg./-	Unseg./4+(4)
Inner flagellum: development	Unseg./0	Unseg./0	Unseg./-	Unseg./0	Bud	Unseg./0	Unseg./0	Unseg./1	Unseg./0	Unseg./0	Unseg./0	Unseg./2	Unseg./0	Unseg./0	Unseg./0
ANTENNA															
Scaphocerite	12-13	11-13	10-12	13	11-14	12-13	11-12	10	12	-	13	13-14	14	13	13
MANDIBLE	Palp present	Palp present	-	Palp present	Palp present/Absent	Palp present	Palp present	Palp present	Palp present	Palp present	Palp present	Palp present	Palp present	Palp present	Palp present
MAXILLA															
Endopod: segments/ setae	Unseg./3+2	Unseg./3+2	-	Unseg./2+2	Unseg./2+2	Unseg./2+2	Unseg./2+2	Unseg./4	Unseg./4	-	Unseg./2+2	Unseg./2+2	Unseg./2+2	Unseg./-	Unseg./2+2
Scaphognathite	15-17	13-16	-	12-16	12-14	12	12-18	9	16	-	14	35-38	13	13	16
FIRST MAXILLIPED															
Exopod: segments/ setae	Unseg./6	Unseg./6	Unseg./6	2/0,6	Unseg./6	-/7	Unseg./6-7	Unseg./6	Unseg./6	Unseg./6	2/0,6	Unseg./7	Unseg./6	Unseg./6	-
SECOND MAXILLIPED															
Exopod: segments/ setae	Unseg./6	Unseg./6	Unseg./6	2/0,6	Unseg./6-7	-/7	Unseg./7-8	Unseg./6	Unseg./6	Unseg./6	2/0,6	Unseg./7	Unseg./6	Unseg./6	-
THIRD MAXILLIPED															
Endopod: development	2-seg	2-seg.	Inc. 5-seg.	Inc. 5-seg.	Absent	Unseg.	Inc. 3-5 seg.	-	Unseg.	-	3-seg.	Unseg.	Unseg.	Unseg.	Unseg.
Exopod: segments/ setae	Unseg./6	Unseg./6	Unseg./6	2/0,5	2/0,5-6	-/6	2/0,5-6	-	Unseg./6	Unseg./6	Unseg./6	Unseg./7	Unseg./6	Unseg./6	Unseg./6
PEREIOPODS	All as buds	All as buds	All as buds	All as buds	All as buds	-	All as buds	All as buds	All as buds	-	All as buds	Al l as buds	All as buds	All as buds	All as buds
PLEOPODS	Biram. buds	Biram. buds	Biram. buds	Biram. buds	Absent-small buds	Biram. buds	Biram. buds	Biram. buds	Biram. buds	Biram. buds	Biram. buds	Biram. buds	Biram. buds	Biram. bud	Absent
UROPODS															
Uropod endopod: marginal setae	5	4-5	3-5	5-6	3-6	4-5	4-6	4	4	-	4-6	5-6	5	5	5
Uropod exopod: marginal setae	8-9	9-10	8-9	10	6-9	9-11	8-10	9	9	-	9-10	11-12	12	11	11
TELSON															
Posterior margin processes	9+9-10	9+9	9+9	8+1+8	8+1+8/ 9+9	8+1+8	8+1+8	8+1+7	8+1+8	8+1+8	8+1+8	8+1+8	8+1+8	8+1+8	8+1+8
4th telson process: fused spine	Reduced	Reduced	Reduced	Well developed	Well developed	Reduced	Well developed	Reduced	Well developed	Reduced	Well developed	Reduced	Reduced	Reduced	Reduced
Fifth Zoea															
Total length, mm	-	-	-	-	4.30-5.30	-	-	-	-	-	-	-	-	-	3.76
Carapace length, mm	-	-	-	-	2.30-2.70	-	-	-	-	-	-	-	-	-	2.19
ANTENNULE															
Inner flagellum: development	-	-	-	-	Unseg./0-1	Unseg./0	-	-	-	-	-	-	-	-	Unseg./1
ANTENNA															
Endopod: segments/ setae	-	-	-	-	2/0,1	3/0,0,1	-	-	-	-	-	-	-	-	-
MANDIBLE	-	-	-	-	Palp present	2-seg. palp	-	-	-	-	-	-	-	-	Unseg. palp
SECOND MAXILLIPED															
Exopod: segments/ setae	-	-	-	-	Unseg./8	-/7	-	-	-	-	-	-	-	-	-
THIRD MAXILLIPED															
Endopod: development	-	-	-	-	Bud	Unseg.	-	-	-	-	-	-	-	-	Unseg.
Exopod: segments/ setae	-	-	-	-	2/0,6	-/6	-	-	-	-	-	-	-	-	Unseg./7
PEREIOPODS	-	-	-	-	All as buds	-	-	-	-	-	-	-	-	-	All as buds
PLEOPODS	-	-	-	-	Unir. buds	Biram. buds	-	-	-	-	-	-	-	-	Biram. buds
TELSON															
Posterior margin processes	-	-	-	-	9+9	-	-	-	-	-	-	-	-	-	8+1+8

Table 1 continued

Features	*C. aequabilis*	*C. erythropus*	*C. erythropus*	*C. antillensis*	*C. vittatus*	*C. sclopetarius*	*C. alidigitus*	*C. signatus*	*C. virescens*	*C. infraspinatus*	*C. merguiensis*	*C. padavensis*	*C. clibanarius*	*C. longitarsus*	*C. olivaceus*
Megalopa															
Total length, mm	2.85-3.04	2.80-2.96	3.10-3.30	2.70-2.90	3.20-4.10	-	-	2.50	3.20	-	-	-	2.72	3.14	3.40
Carapace length, mm	1.08-1.15	1.08-1.24	0.90-1.00	0.60-0.80	1.10-1.50	-	0.80-1.00	-	-	1.40	1.20	1.20	1.12	1.09	1.34
CARAPACE															
Rostrum	Broad, blunt	Broad, blunt	Broad, blunt	Triangular	Small, blunt	Small, blunt	Broad, blunt	Triangular	Truncated	-	Broad	Triangular	Broad, rounded	Broad, rounded	Broad, rounded
Rostrum/carapace	0.17±0.02	0.18±0.03	-	-	-	-	-	-	-	-	-	-	-	-	-
Ocular scales	Absent	Absent	Absent	-	Absent	-	Weakly developed	Absent	Absent	Weakly developed	Weakly developed	Weakly developed	Weakly developed	-	Weakly developed
ANTENNULE														UROPODS	
Peduncle: segments/ setae	3/12-13,4-5,6	3/11-13,5-7,5-6	3/-	3/7,1-2,1-2	-	3/9-13,-,-	3/7-10,3-5,3-5	3/-	3/-	3/-,-,4	3/-	3/-	3/-	3/-	3/-
Outer flagellum: segments/	5/	5/	5/	5/	5/	5/	6/	5/	5/	5/	5/	4/	5/	5/	5/
aesthetascs	0,(5),(5),(4),0	0,(5),(5),(3),0	-	0,(6),(5),(3),0	0,(4),(4),(3),0	0,(6),(4),(2),0	0,(6),(4-5),(3),0,0	-	-	(14)	0,(5-6),(4),(2),0	-	-	-	0,(7-9),0
Inner flagellum: segments/setae	3/2,4,7	3/2,2-3, 6-8	3/-	3/1,3,5	3/2,5-6	3/-	3/2-4,2-3,4-5	2-3/-	3/-	3/-,-,4	-	3/1,1,4	3/-	3/-	3/-,-,4
ANTENNA															
Flagellum: segments	10	7-10	7-9	10	11	10-11	10	8	11	11	9	10	13	13	12
MANDIBLE	2-seg. palp	3-seg. palp	3-seg. palp	2-seg. palp	3-seg. palp	3-seg. palp	2-/3-seg. palp	3-seg.	3-seg.	Part. seg.	3-seg.	3-seg.	Part. 3-seg.	Part. Seg.	3-seg.
MAXILLULE															
Coxal endite	18-21	18-20	-	-	18-20	22	-	18	-	-	19	-	-	-	-
Basial endite	21-24	23-24	-	-	19	20	-	19	-	-	17-19	-	-	-	-
Endopod: segments/setae	1+1	1+1	-	1	1	1	1	1+2	-	1+2-3	1+1-2	-	1	1	-
MAXILLA															
Coxal endite	25-32+7-9	27-29+7-8	-	-	27+6	27-30+6-7	-	19	-	-	-	-	-	-	-
Basial endite	8-10+11-13	9-12+10-13	-	-	8+15	10-12+10-15	-	14	-	-	-	-	-	-	-
Endopod: segments/setae	0	0	-	0	0	0	0	0	-	0	-	0	0	-	0
Scaphognathite	50-58+1	47-52+1	-	49-55	60	52	45-60	45	-	-	50	-	-	-	54-56
FIRST MAXILLIPED															
Basial endite	18-21	19-21	-	-	20	15-16	-	15	-	-	-	-	-	-	-
Endopod: segments/setae	Unseg./0	Unseg./0	Unseg./0	Unseg./0	Unseg./0	Unseg./0	Unseg./0	Unseg./3+6	Unseg./0	5/-,-,-,-,4	-	Unseg./1	Unseg./-	Unseg./-	-
Exopod: segments/setae	Unseg./7-9	Unseg./6-8	7	Unseg./9	Unseg./8-10	Unseg./9	Unseg./9-10	2/5,6	2/10,-	Unseg./8-12	-	Unseg./11	Unseg./9+2	Unseg./9+2	Unseg./10
SECOND MAXILLIPED															
Basial endite	3	2-3	-	2	3	0 or 2-3	-	-	-	-	-	-	-	-	-
Endopod: segments/setae	4/2,3,8,7-8	4/2,2-3,7-9, 6-7	-	4/1-2,2,-,-	4/-,-,4,6	4/2,2-3,4-6,7	4/1-3,1-3,1-3,-	5/4,2,3,5,6	-	5(part.7)/-	-	5/-	5/-	5/-	5/-
Exopod: segments/setae	2/2, 6-8	2/2,8	-	2/1-2,7-8	Unseg./6-8	2/-,7	2/1-2,6-7	2/1,6	2/2,-	-/6	-	2/-,4-6	2/-,6	2/-	2/0,10
THIRD MAXILLIPED															
Basial endite	6-7+3-4sub-ac.pr	5-7+ 3-4sub-ac.pr	-	4+-	2-3	4+1-2sub-ac.pr	-+2-4sub-ac.pr	2sub-ac.pr	-	-+4sub-ac.pr	3+-	-	-	-	-
Endopod: segments	5	5	-	5	5	5	5	5	-	7	5/2+2,2+1,4+1,-,-	6/-	5/-	5/-	-
Endopod: *crista dentata*	Present	Present	Present	Present	-	Present	Present	Present	Present	Absent	-	-	-	-	-
Exopod: segments/setae	2/1,6-8	2/1,8	-	2/1,8	Unseg./2-4,6	2/-	2/0,6	2/-,6	-	3/-,-,6	Unseg./6	-	2/-,6	2/-,6	-
PEREIOPODS															
First: chelipeds	Equal	Equal	Equal	Equal	Equal	Equal	Equal	Equal	Equal	Sub-equal	Sub-equal	Equal/sub-equal	Sub-equal	Sub-equal	Sub-equal
Second/ third: dactyl spines	3	3-4	-	3	-	3-4/8-11	3	-	-	3	2-3/4-6	4	-	-	-
Fourth/ fifth: propodal pc	14-16/17-18	10-14/11-15	-	-	-	-	-	-	-	-	-	-	-	-	-
PLEOPODS	Decr. post.	Decr. post.	Decr. post.	Decr. post.	Equal	Equal	Decr. post.	Equal	-	Decr. post.	-				
Endopod: setae	10-11+7-8pc	8-9+7-8pc	7-8+6-8pc	11-14+5-7pc	12-20+6-7pc	8-10+5-6pc	9-13+8-10pc	9+-	-	-	-	-	7+-	12+-	10+4-6pc
Exopod: setae	17-19+10-13pc	16-18+9-11pc	-+10pc	19-21+8-12pc	8-15+-	11-14+5-6pc	16-19+11-13pc	18+-		-	-	-.	14+-	19+-	16+5-6pc
TELSON															
Setae: marginal/lateral/distal	10-11/2 pairs/10-12	8-9/2 pairs/10	-/-/10	10/1-2 pairs/9	-/-/8-9	-/-/9-10	-/-/9	-	-	6/2 pairs/9	6/2 pairs/9	5/-/9	-/2 pairs/9	-	-/-/9

Unseg. unsegmented; *unir.* uniramous; *seg.* segmented; *bir.* biramous; *part.* partially; *sub-ac.pr* sub-acute processes; *pc* pseudochaetae; *decr. post.* decreasing in size posteriorly. Sources: (1) Pike and Williamson (1960); (2) Le Roux (1966); (3) Dechancé and Forest (1958); (4) Siddiqui et al. (1991); (5) Lang and Young (1977); (6) Brossi-Garcia (1987); (7) Siddiqui et al. (1993); (8) Tirmizi and Siddiqui (1979); (9) Shenoy and Sankolli (1977); (10) Nayak (1984); (11) Shenoy and Sankolli (1975); (12) Ajmal Khan et al. (1981); (13) Ajmal Khan and Natarajan (1981a); (14) Ajmal Khan and Natarajan (1981b). Numbers in parenthesis: number of aesthetascs. If not otherwise indicated, the given numbers represent the respective setation formula.

missing. The antennal scale lacks a terminal spine; the eyes are stalked in the second larval stage, with rounded eyes at the top of a short ocular peduncle. From the third zoeal stage on, the formula of the telson processes is usually 8 + 1 + 8, but also 9 + 9 or 9 + 9–10; the outermost process is a very short blunt spine, the second an anomuran hair, the third an articulated plumose seta, the fourth a fused spine (reduced or well developed), and the fifth to ninth or tenth processes are plumose setae. Biramous uropods are already present in the third stage, and in the fourth stage the mandibular palp is present. *Clibanarius* megalopa displays a carapace without processes or spines, with the postero-lateral border smoothly rounded, and a broad and rather blunt rostrum; the abdominal somites have their postero-lateral angles rounded; the telson has a rounded posterior margin, with 5–11 setae. Usually, the megalopa has symmetrical chelipeds, and an apically curved and corneous dactylus in the second and third pereiopods. Although Shenoy & Sankolli (1977) reported the presence of ocular acicles as a generic character for the megalopa, these structures can be reduced or even absent in some species (see Table 1).

Regardless of the general larval morphology homogeneity in *Clibanarius*, there is some variation among congeneric species. These relate to the telson formula and to the morphology of the telson processes beyond the second larval stage, as well as to the number of zoeal stages.

Shenoy & Sankolli (1977) stated that the telson of the fourth zoeal stage always bears 8 + 1 + 8 processes, except for *C. erythropus* where it bears 9 + 9 processes. However, the available data indicate that the latter telson formula can also be displayed by another two species: *C. aequabilis* with 10–9 + 9–10 processes in zoea III and 9 + 9–10 processes in zoea IV, and *C. vittatus* with 8 + 1 + 8 or 9 + 9 processes in zoea III and 9+9 processes in zoea IV (Table 1).

All these three species are from the North Atlantic Ocean (*C. aequabilis* and *C. erythropus* from the northeastern coast and *C. vittatus* from the northwestern coast). Thus, the telson formula may be related to geographic distribution, since only the above three species acquire an inner pair of plumose setae in the third zoeal stage and not only a single median plumose seta. *Clibanarius vittatus* can present both types of telson formula and that *C. aequabilis* can also present an uneven number of telson processes on the terminal margin of the telson. Therefore, telson development in *Clibanarius* apparently shows some intraspecific plasticity, a feature already highlighted by other authors (e.g. Barria et al. 2006).

Concerning the fourth telson process as a fused spine, beyond the second larval stage, this fused spine can be either well developed (*C. albidigitus, C. antillensis, C. merguiensis, C. virescens* and *C. vittatus*), or reduced (*C.aequabilis, C. erythropus, C. clibanarius, C. infraspinatus, C. longitarsus, C. olivaceus, C. padavensis, C. sclopetarius* and *C. signatus*). Shenoy & Sankolli (1977) considered this reduced fused spine a generic feature of *Clibanarius* larvae. However, Nayak (1984) later showed that in *C. merguiensis* the fourth telson process is a well developed used spine. Therefore, we suggest that it is the transformation of the plumose fourth telson process into a fused spine (either well developed or reduced) in the transition from zoea II to zoea III that should be considered a generic character of *Clibanarius* zoeal stages.

Studies on anomuran larvae generally consider telson processes a very important diagnostic character (e.g. McLaughlin et al. 1992, 1993). Based on the different formation of the fourth telson process beyond the second zoeal stage, four groups of species can be differentiated within the Diogenidae: group 1 (genera *Calcinus*, *Dardanus*, *Petrochirus* and *Trizopagurus*) with the fourth telson process as a well developed fused spine; group 2 (genus *Clibanarius*)

with the fourth telson process either as a well develop or a reduced fused spine; group 3 (genus *Diogenes*) with the fourth telson process as a strongly reduced spine which disappears during larval development; and finally group 4 (genus *Paguristes*) where in all larval stages telson processes are represented by setae. These findings do not give any indication of the polarity of the respective evolutionary changes. In pagurids and diogenids early larval stages, the fourth telson process is articulated and later becomes fused, while the opposite sequence has never been recorded so far. McLaughlin et al. (2004) highlighted the fact that telson processes frequently become fused in paguroids, particularly in members of the family Paguridae. The same evolutionary pattern may therefore be suggested for the Diogenidae (with the exception of the genus *Paguristes*), as indicated by our study.

Finally, the occurrence of a fifth zoeal stage as in *C. olivaceus*, *C. sclopetarius* and *C. vittatus*, does not seem to be the rule among *Clibanarius* species. Siddiqui et al. (1991) recorded the occurrence of four or five zoeal stages in *Clibanarius* and the need of 5 zoeal stages to complete the larval sequence in *C. vittatus*. From the larval sequences of the three species with five zoeal stages (Table 1), we can conclude that in *C. olivaceus* and *C. vittatus* a fifth zoea is absolutely necessary to acquire all the features of a final zoeal stage, while a fifth zoea might be unnecessary in *C. sclopetarius*. Lang & Young (1977) also concluded that the most common developmental series in *C. vittatus* involves five zoeal stages, with stage IV being separated from stage V by the lack of mandibular palp. The larval sequence in *C. sclopetarius* commonly includes four zoeal stages, and when a fifth zoeal stage appears, zoea III already exhibits a biramous antennule (Brossi-Garcia 1987). Due to the morphological similarity displayed by the fourth and fifth zoeal stages of *C. sclopetarius*, it seems that the occurrence of a fifth zoeal stage might result from a laboratorial artefact. Gore (1985) reported how unsuitable rearing conditions may favour the appearance of extra and intermediate larval

stages. Another explanation for the occurrence of a fifth zoeal stage may be interspecific variation (Ajmal Khan & Natarajan 1981b). Furthermore, since *Clibanarius* larvae can easily delay metamorphosis (Harms 1992, Harvey 1996), the absence of adequate settlement cues might be another factor favouring the occurrence of extra zoeal stages in laboratory cultures.

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CHAPTER 6

Complete larval development of the crab *Ilia nucleus* (Linnaeus, 1758) (Decapoda, Brachyura, Leucosiidae) reared under laboratory conditions*

*****Bartilotti C., González-Gordillo J.I., dos Santos A. (2009) **Complete larval development of the crab *Ilia nucleus* (Linnaeus, 1758) (Decapoda, Brachyura, Leucosiidae) reared under laboratory conditions**. *Scientia Marina* **73**, 551-562.

6.1- Abstract

The complete larval development of *Ilia nucleus* (Linnaeus, 1758) reared under laboratory conditions was obtained. The four zoeal stages and the megalopa are described and illustrated in detail. The larval features observed in *I. nucleus* fit into the characteristics of the family proposed by Rice (1980) for zoeal stages and by Quintana (1986) for megalopa. The morphological characters of larval stages of *I. nucleus* are compared with previous descriptions, and with those of other known larvae of Leucosiidae (only for subfamilies Ebaliinae and Leucosiinae). Present work supports the hypothesis that subfamily Ebaliinae is a heterogeneous group. For the correct identification of a zoeal stage of a leucosiid crab besides counting the number of setae on maxilliped exopods, the antennule as well as the pereiopods development should be used as additional characters.

6.2- Introduction

Family Leucosiidae Samouelle, 1819 is represented in northeastern Atlantic and Mediterranean by 17 species (d'Udekem d'Acoz 1999), but the larval development is only known for six of these (González-Gordillo et al. 2001). *Ilia nucleus* (Linnaeus, 1758) *sensu lato* is a leucosiid crab, occurring in the Atlantic Ocean from southeast Spain to Cape Verde islands, and in the Mediterranean Sea (d'Udekem d'Acoz 1999). Previous larval descriptions for this species are known from plankton collected material and laboratory reared larval stages (Cano 1891, Boraschi 1921, Bourdillon-Casanova 1960, Heegard 1963), although these do not make a complete larval development description according to modern standards.

Cano (1891) described the first and second zoeal stages as well as the megalopa of *I. nucleus* from laboratory reared material (females collected in the Gulf of Napoli), but the first two zoeal stages were ascribed to *Nautilograpsus*. Later, Boraschi (1921) presented a telson of the first zoea of *Nautilograpsus minutus* collected from plankton along the Italian coast (collection sites: Quarto dei Mille in Genova, Ligurian Sea, and Palermo in Sicily, Tyrrhenian Sea) citing Cano's description (1891), and keeping the name assigned by the previous author. In the same year, Caroli (1921) perceived Cano's mistake. He captured some late zoeae of what he thought to be *Planes minutus* (as *Nautilograpsus minutus*), and reared these in the laboratory. After the moult, a small brachyuran crab with all characters of *I. nucleus* was obtained, results that lead him to suppose that Cano (1891) described both species, *I. nucleus* and *N. minutus*, but during the plates preparation presented *I. nucleus* zoeal description in the place of *N. minutus*, with the correct figure for the megalopa, and *vice versa*. Bourdillon-Casanova (1960) described the first three zoeal stages of *I. nucleus* from plankton captured larvae and discussed her results with previous larval descriptions of this species. She concluded that Boraschi (1921) ignored a correction made by Cano in 1893 to his own mistake. Bourdillon-Casanova (1960) made the same assumptions about Cano (1891) zoeal description: the megalopa attributed to *I. nucleus* was correctly identified; however, the zoeal stages description attributed to *I. nucleus* belong to *N. minutus*. Later, Heegard (1963) presented a description of the first zoea of *I. nucleus*, from laboratory reared material.

Present study describes in detail the four zoeal stages and the megalopa of *I. nucleus* from laboratory reared material. The morphological characters of larval stages of *I. nucleus* are compared with previous descriptions, and with those of other known larvae of Leucosiidae (only for subfamilies Ebaliinae and Leucosiinae).

6.3- Material and Methods

Three ovigerous females of *Ilia nucleus* were caught on March 2003 with a benthic trawl at 6 m depth in Valdelagrana Beach, Cádiz Bay, SW Spain (36º34.24'N, 06º14.19'W). The three specimens were maintained in a 2 l glass beaker, containing well-aerated filtered natural seawater (36) until hatching. No food was added. Females released larvae 72 h after their collection in several pulses for 24 h, in a total amount of approximately 1600 larvae. After hatching, the most actively swimming larvae were gathered in only one pool. The larvae contained in the pool were then transferred to 1 l glass bottles (500 larvae per litre) with aeration at constant temperature (20º C±1) and fed with *Artemia* nauplii. The water was changed daily, and larvae were checked for evidence of moulting. Each time the water was renewed, 3- 4 larvae were preserved in 4% formalin. Rearing was terminated when larvae moulted to the megalopal stage.

Drawings and measurements were made with the aid of a *camera lucida* on a binocular Wild M8. Setal observations and drawings were made using a Zeiss microscope with *camera lucida*. The preparation of slides with appendages was temporary. Larval description followed the method proposed by Clark et al. (1998) and setal terminology is according to Ingle (1992). The aesthetascs of figures 3B' and 5B were drawn truncated to facilitate the illustration of the antennule. Similarly, the long plumose setae on maxilliped exopods in figures 4F-G and on the pleopods exopods in figures 6H-I are drawn truncated. Finally, the megalopal pereiopods are also drawn truncated in figures 5A, 5A', and 6F. The setules from setae were omitted from drawings when necessary.

The sizes are given as the arithmetic mean ± 95% confidence intervals. Measurements taken in zoeal stages were: rostro-dorsal length (RDL) measured from tip of rostral spine to tip of dorsal spine; carapace length (CL) measured from the base of the rostrum (between the eyes) to posterolateral carapace margin; carapace width (CW) the greatest distance across the carapace measured between the bases of carapace spines and, carapace width with lateral spines (CWls) measured between the tips of lateral spines. In megalopa, total length (TL) is the distance from tip of rostrum to posterio-median margin of telson; carapace length (CL) is the distance between the tip of rostrum and the posterior margin of the carapace; carapace width (CW) is the carapace maximum width; propodus length (PL) from an imaginary line across the base of propodus to the distal margin, and dactylus length (DL) from an imaginary line across the base of dactylus to its distal end. The DL and PL measurements proportion of the first pereiopod of the megalopa is presented, once that in the future this might be useful for phylogenetic and systematic purposes.

The complete larval series has been deposited in the *Instituto Nacional de Recursos Biológicos- IPIMAR* in Lisbon, Portugal (number IPIMAR/L/In/02/2007).

6.4- Results

Under laboratory conditions, the complete larval development of *I. nucleus* from hatching to megalopa took 32- 38 days at 20º C. Four zoeal stages and one megalopa were obtained. The first zoeal stage and megalopa are fully described, while for second to fourth zoeae only differences of previous stages are described in detail.

Ilia nucleus (Linnaeus, 1758)

Figures 1-6

First zoea (Figure 1)

Dimensions: RDL= 1.436± 0.041 mm; CL= 0.548± 0.013 mm; CW= 0.400± 0.010 mm; CWls= 0.856± 0.056 mm.

Carapace (Figs. 1A, A'): globose and smooth. Dorsal spine present, long, stout and straight; rostral spine present inward curving, almost as long as dorsal spine; well developed lateral spines, with half the length of rostrum, backwards curving. One pair of dorsolateral setae present. Ventral margin of carapace without any seta. Eyes sessile.

Antennule (Fig. 1B): uniramous, unsegmented, conical shaped. With 4 aesthetascs and 1 short simple seta terminally.

Antenna (Fig. 1B): present as a very small bud.

Mandible (Fig. 1C): asymmetrical, palp absent. Incisor and molar processes differentiated.

Maxillule (Fig. 1D): coxal endite with 5-6 setae (1 simple and 4-5 papposerrate setae); basial endite with 4 strong (3 papposerrate and 1 serrulate) and 1 simple seta. Endopod unsegmented, with 4 terminal setae. Microtrichia as illustrated. Exopod seta absent.

Maxilla (Fig. 1E): coxal endite bilobed with 3+2 sparsely papposerrate setae, basial endite bilobed with 4+4 sparsely papposerrate setae. Endopod unsegmented with 2+2 pappose setae and microtrichia on inner and outer margin. Scaphognathite with 4 marginal plumose setae and a long setose posterior process. Microtrichia arranged as figured.

First maxilliped (Fig. 1F): coxa with 1 seta; basis with 8 medial setae arranged 2+2+2+2 on the ventral side; endopod 5-segmented with 2, 2, 1, 2, 5 (one subterminal and

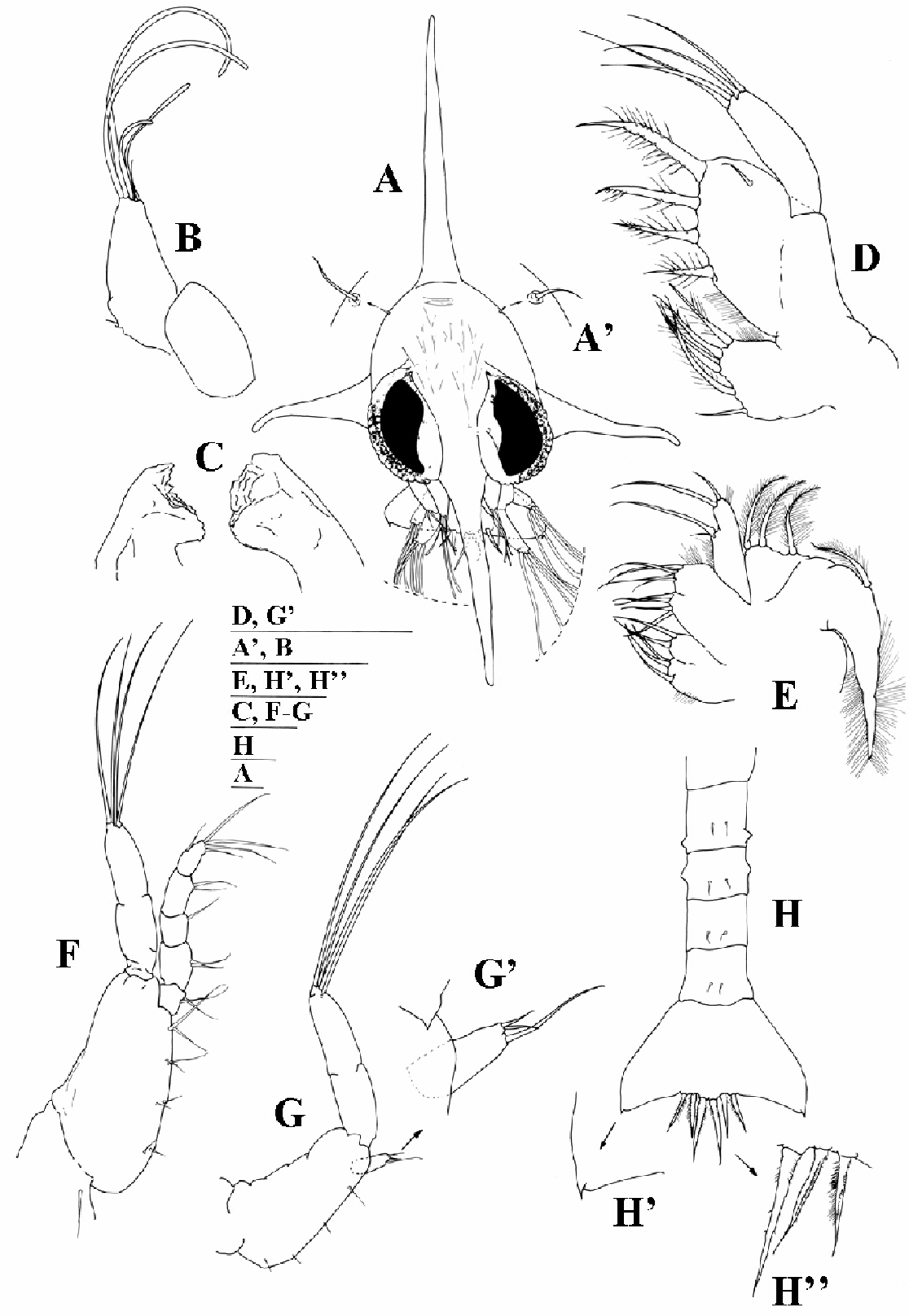

Fig. 1- *Ilia nucleus.* First zoea: **A**, general aspect, frontal view; **A'**, detail of setae on carapace; **B**, antennule and antenna; **C**, mandibles; **D**, maxillule; **E**, maxilla; **F**, first maxilliped; **G**, second maxilliped; **G'**, detail of second maxilliped endopod; **H**, dorsal view of abdomen and telson; **H'**, detail of furcal spine; **H''**, detail of furcal setae. Scale bars: 0.1 mm.

Exopod unsegmented, bearing 4 long plumose natatory setae terminally.

Second maxilliped (Figs. 1G, G'): coxa without setae; basis with 4 medial setae arranged 1+1+1+1 on the inner side; endopod unsegmented with 3 (one subterminal and two terminal) setae. Exopod unsegmented, bearing 4 long plumose natatory setae terminally.

Third maxilliped and pereiopods: absent.

Abdomen (Fig. 1H): 5 somites. A pair of dorsolateral processes on the posterior margin of somite 2, and another pair in the median portion of somite 3. Somites 2-5 with a pair of pappose dorso-marginal setae each.

Pleopods: absent.

Telson (Figs. 1H, H', H''): subtriangular, posterio-external angles each with one small furcal spine and with 6 setae on posterior margin, being the central ones the longest.

Second zoea (Figure 2)

Dimensions: RDL= 2.440± 0.160 mm; CL= 0.818± 0.055 mm; CW= 0.608± 0.059 mm; CWls= 1.598± 0.188 mm.

Carapace (Fig. 2A): dorsal spine as long as rostral spine and lateral spines unchanged. Eyes stalked.

Antennule (Fig. 2B): unchanged besides size.

Antenna (Fig. 2B): unsegmented rounded bud.

Mandible (Fig. 2C): unchanged besides size.

Maxillule (Fig. 2D): coxal endite with 7 setae (1 simple and 6 papposerrate setae); basial endite with 8 strong (7 papposerrate and 1 serrulate) and 1 simple seta. Exopod pappose seta present on outer margin.

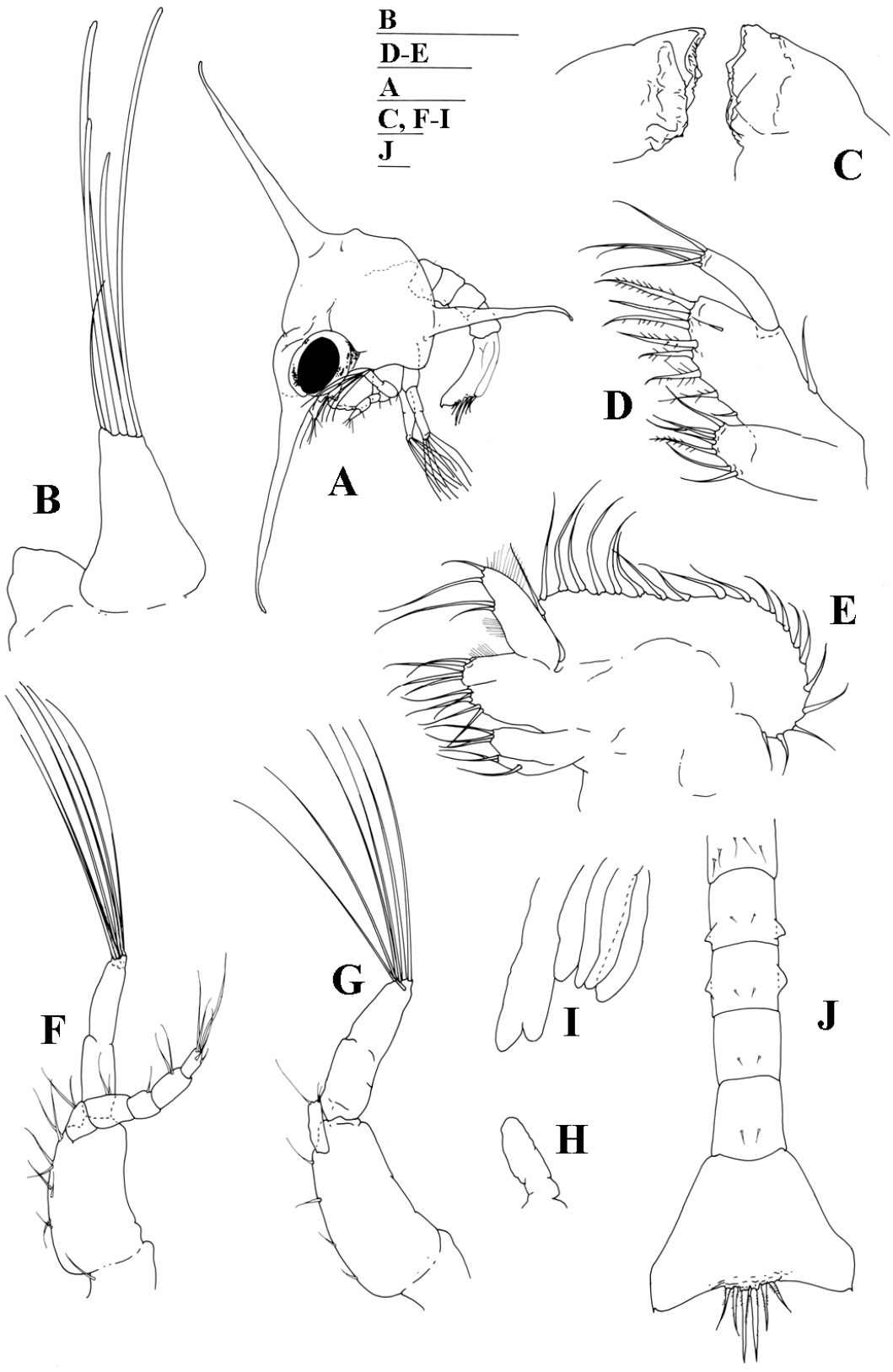

Fig. 2- *Ilia nucleus*. Second zoea: **A**, general aspect, lateral view; **B**, antennule and antenna; **C**, mandibles; **D**, maxillule; **E**, maxilla; **F**, first maxilliped; **G**, second maxilliped; **H**, third maxilliped; **I**, pereiopods; **J**, dorsal view of abdomen and telson. Scale bars: 0.5 mm (**A**); 0.1 mm (**B-J**).

Maxilla (Fig. 2E): coxal endite bilobed with 3+3 sparsely papposerrate setae, basial endite bilobed with 5+4 sparsely papposerrate setae. Scaphognathite with 20-21 marginal plumose setae.

First maxilliped (Fig. 2F): exopod unsegmented, bearing 6 long plumose natatory setae terminally.

Second maxilliped (Fig. 2G): exopod unsegmented, bearing 6 long plumose natatory setae terminally.

Third maxilliped (Fig. 2H): present as bud.

Pereiopods (Fig. 2I): present as undifferentiated buds.

Abdomen (Fig. 2J): first somite with 4-5 pappose setae as illustrated.

Pleopods: absent.

Telson (Fig. 2J): unchanged.

Third zoea (Figure 3)

Dimensions: RDL= 3.154± 0.092 mm; CL= 1.081± 0.025 mm; CW= 0.842± 0.035 mm; CWls= 2.131± 0.080 mm.

Carapace (Fig. 3A): rostral spine measuring approximately two thirds of dorsal spine length; lateral spines almost as long as rostrum.

Antennule (Figs. 3B, B'): endopod present as a small bud; exopod with 6 aesthetascs arranged 1 subterminal and 5 terminal, and 1 simple seta terminally.

Antenna (Fig. 3B): endopod bud present.

Mandible (Fig. 3C): unchanged besides size.

Maxillule (Fig. 3D): coxal endite with 6-7 setae (1 simple and 5-6 papposerrate setae); basial endite with 9-11 strong (7-9 papposerrate and 2 serrulate) and 1 simple seta.

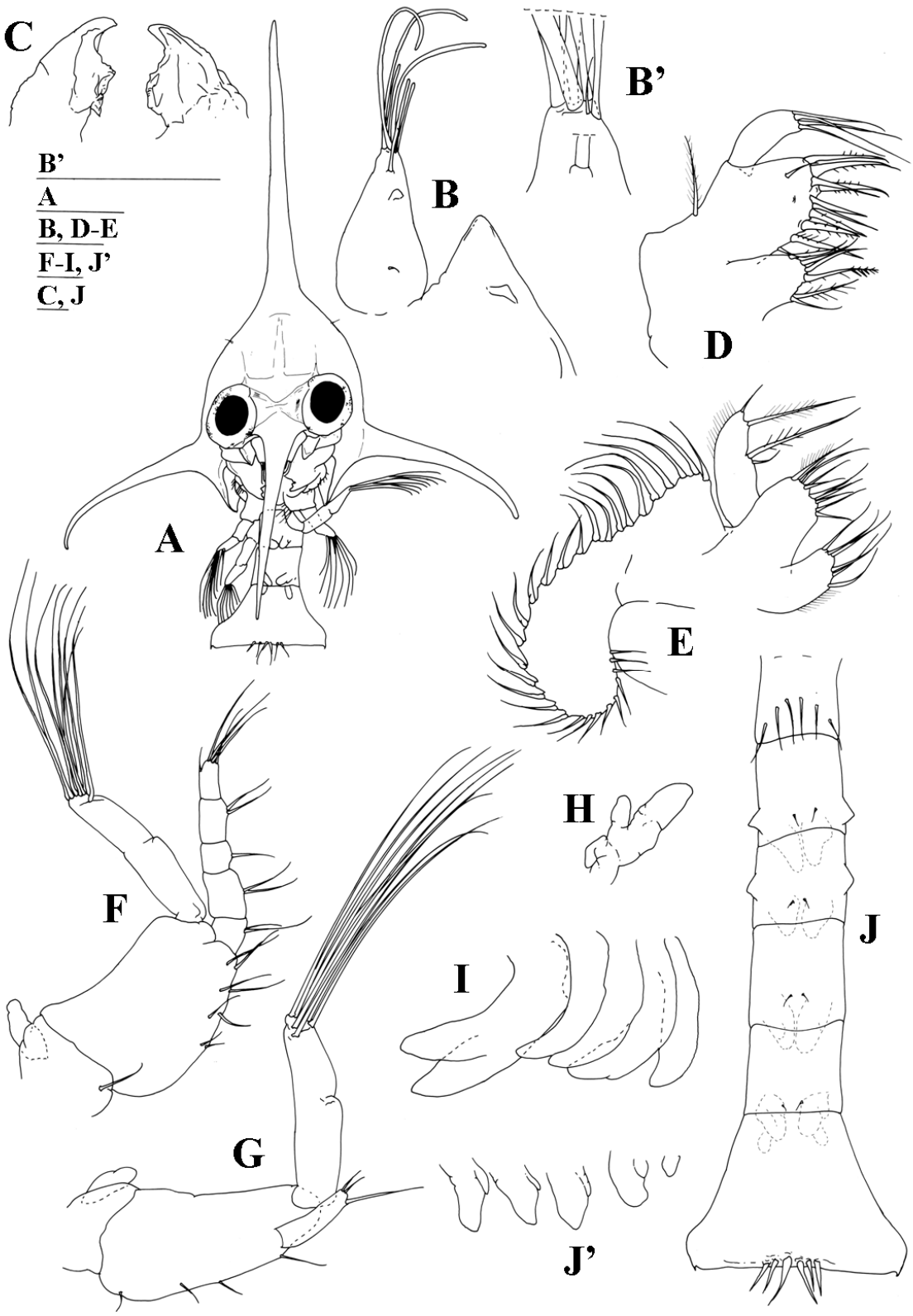

Fig. 3- *Ilia nucleus.* Third zoea: **A**, general aspect, frontal view; **B**, antennule and antenna; **B'**, detail of terminal aesthetascs and seta; **C**, mandibles; **D**, maxillule; **E**, maxilla; **F**, first maxilliped; **G**, second maxilliped; **H**, third maxilliped; **I**, pereiopods; **J**, dorsal view of abdomen and telson; **J'**, pleopods. Scale bars: 0.1 mm.

Maxilla (Fig. 3E): basial endite bilobed with 5+4-5 sparsely papposerrate setae. Scaphognathite with 30-33 marginal plumose setae.

First maxilliped (Fig. 3F): exopod unsegmented, bearing 8 long plumose natatory setae terminally.

Second maxilliped (Fig. 3G): exopod unsegmented, bearing 8 long plumose natatory setae terminally.

Third maxilliped (Fig. 3H): biramous unsegmented bud. Epipodite present as a very small bud.

Pereiopods (Fig. 3I): elongated. Chelipeds bilobed and pereiopods unsegmented.

Abdomen (Fig. 3J): first somite with 6 dorsal pappose setae; somites 2-5 with a pair of posterodorsal setae each.

Pleopods (Fig. 3J'): second to fifth pleopods present as small biramous buds; sixth pleopod very small, hidden at the base of the telson.

Telson (Fig. 3J): unchanged besides size.

Fourth zoea (Figure 4)

Dimensions: RDL= 3.589± 0.182 mm; CL= 1.243± 0.035 mm; CW= 1.065± 0.042 mm; CWls= 2.647± 0.185 mm.

Antennule (Fig. 4B): endopod bud enlarged; exopod with 10 aesthetascs arranged 2 + 4 subterminal and 4 terminal and 1 simple seta terminally.

Antenna (Fig. 4B): enlarged in size.

Mandible (Fig. 4C): palp bud present.

Carapace (Fig. 4A): unchanged besides size.

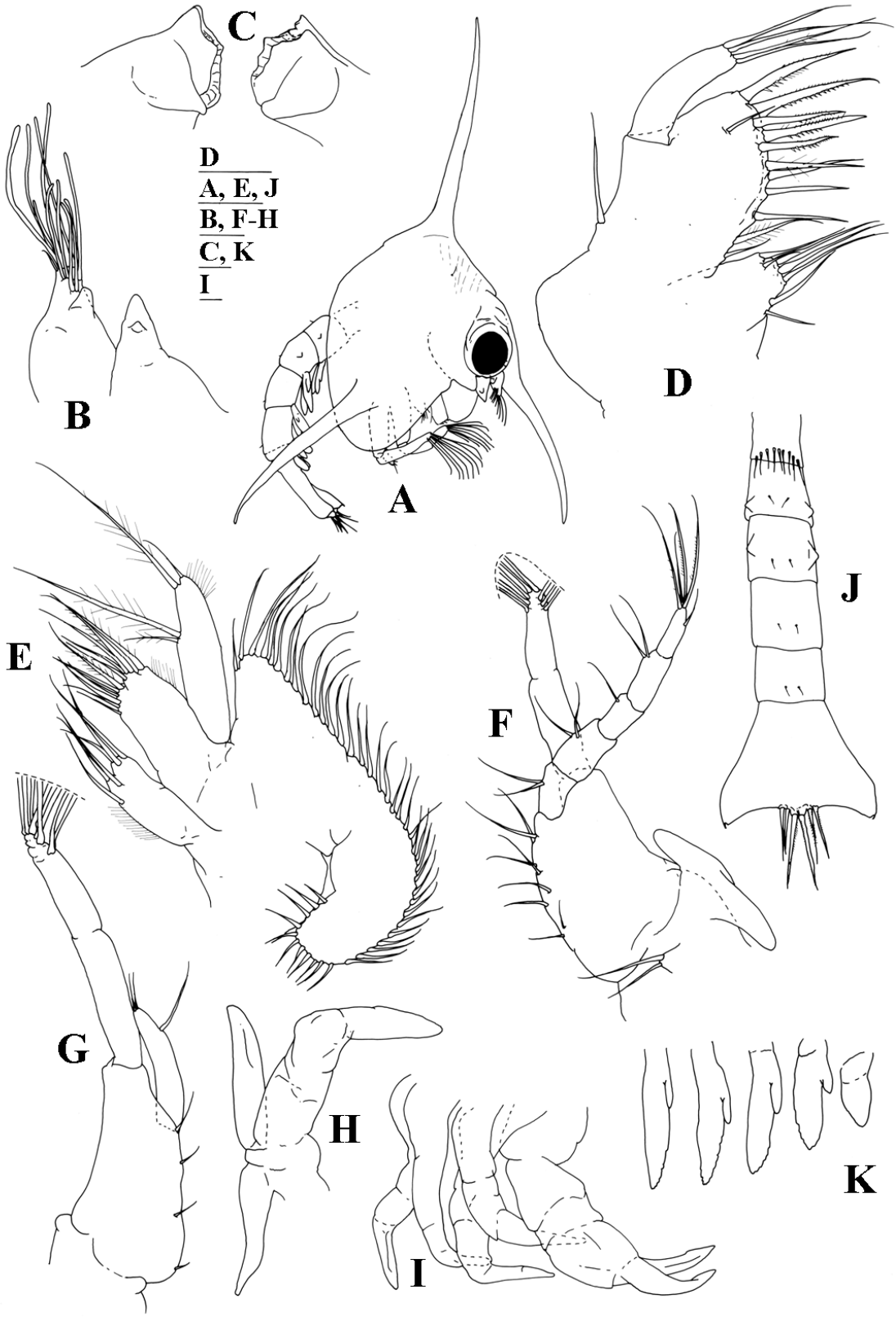

Fig. 4- *Ilia nucleus*. Fourth zoea: **A**, general aspect, lateral view; **B**, antennule and antenna; **C**, mandibles; **D**, maxillule; **E**, maxilla; **F**, first maxilliped; **G**, second maxilliped; **H**, third maxilliped; **I**, pereiopods; **J**, dorsal view of abdomen and telson; **K**, pleopods. Scale bars: 0.5 mm (**A**, **J**); 0.1 mm (**B**-**I**, **K**).

Maxillule (Fig. 4D): coxal endite with 7-8 setae (1 simple and 6-7 papposerrate setae); basial endite with 11-12 strong (8-9 papposerrate and 3 serrulate) and 1 simple seta.

Maxilla (Fig. 4E): basial endite with 6-5+6-5 sparsely papposerrate setae. Scaphognathite with 40-46 marginal plumose setae. Microtrichia as figured.

First maxilliped (Fig. 4F): coxa with 2 setae. Exopod unsegmented, bearing 8 long plumose natatory setae terminally.

Second maxilliped (Fig. 4G): exopod unsegmented, bearing 8 long plumose natatory setae terminally.

Third maxilliped (Fig. 4H): lobes of epipod, endopod and exopod enlarged.

Pereiopods (Fig. 4I): chelipeds and pereiopods 2-5 more developed and feebly segmented.

Abdomen (Fig. 4J): first somite with 9 dorsal pappose setae.

Pleopods (Fig. 4K): pleopod buds more developed in somites 2-5. Sixth pleopod bud uniramous.

Telson (Fig. 4J): unchanged besides size.

Megalopa (Figures 5, 6)

Dimensions: TL= 2.781± 0.077 mm; CL= 1.754± 0.057 mm; CW= 1.369± 0.075 mm.

Carapace (Figs. 5A, 5A', 5A''): rectangular, convex on lateral margins. Rostrum directed obliquely downward, very small; frontal region broad. Submedian lobes hardly developed; hepatic region swollen; a pair of epibranchial- mesobranchial carinae; a cardiac swelling and an intestinal tubercle developed. Setae distributed as illustrated.

Antennule (Fig. 5B): peduncle 3-segmented, basal segment with 4, middle segment with 1 and distal segment with 2-3 setae respectively. Endopod margin with 1 subterminal

and 4 terminal (arranged 1+3) simple setae. Exopod 5-segmented with 1 seta, 1 seta+ 6 aesthetascs, 1 seta+ 7 aesthetascs, 4 aesthetascs, and 1 subterminal aesthetasc + 1 terminal seta.

Antenna (Fig. 5C): peduncle unsegmented with two simple setae and with the shortened exopod process; flagellum 4-segmented, segments first to fourth progressing proximally to distally, each with 0, 0, 0, 3 terminal (one shorter and two longer) simple setae.

Mandibles (Fig. 5D): asymmetric, with a broad plate-shaped incisor process with an acute inner margin; palp 2-segmented, first segment with 1 simple seta, distal segment with 13-14 papposerrate setae.

Maxillule (Fig. 5E): coxal endite with 8-10 papposerrate setae; basial endite with 20-24 papposerrate setae. Endopod unsegmented without any seta.

Maxilla (Fig. 5F): coxal endite with one seta; basial endite with 5+4-5 setae. Endopod unsegmented without any seta. Scaphognathite with 75-82 marginal plumose setae and blade with 2-3 simple setae.

First maxilliped (Fig. 5G): coxa with 7-9 setae; basis with 27-30 setae. Endopod unsegmented with 5 setae. Exopod 2-segmented with 2 plumose setae on proximal segment, and 2 setae on distal segment. Epipod with 4-5 setae.

Second maxilliped (Fig. 5H): coxa with 1-2 setae; basis with 1-3 setae. Endopod 5-segmented with 1, 0, 2, 5-6 and 6-8 papposerrate setae, respectively. Exopod 2-segmented, first segment without setae, distal segment with 2 plumose setae.

Third maxilliped (Fig. 5I): arthrobranch and podobranch gill buds present; coxa with 10-12 setae; basis not clearly differentiated with 2 setae. Endopod 5-segmented, with 28-31, 8-10, 5-6, 8-10 and 10-12 setae, respectively. Exopod reduced to a single segment with 12 or 14 setae. Epipod elongated with 7-8 setae.

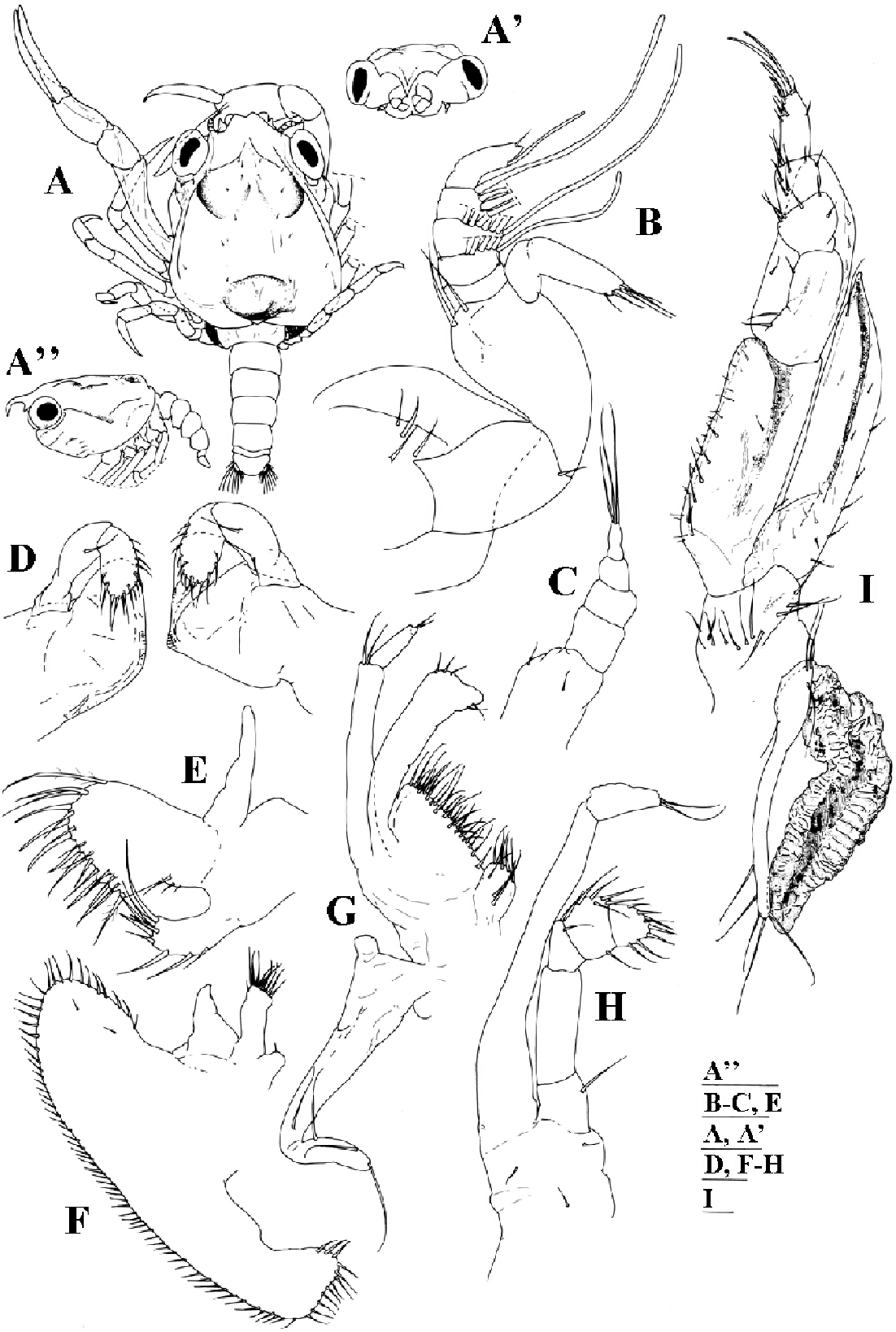

Fig. 5- *Ilia nucleus*. Megalopa: **A**, general aspect, dorsal view; **A'**, detail of frontal view of the rostrum; **A''**, general aspect, lateral view; **B**, antennule; **C**, antenna; **D**, mandibles; **E**, maxillule; **F**, maxilla; **G**, first maxilliped; **H**, second maxilliped; **I**, third maxilliped. Scale bars: 1.0 mm (**A''**); 0.1 mm (**A-I**).

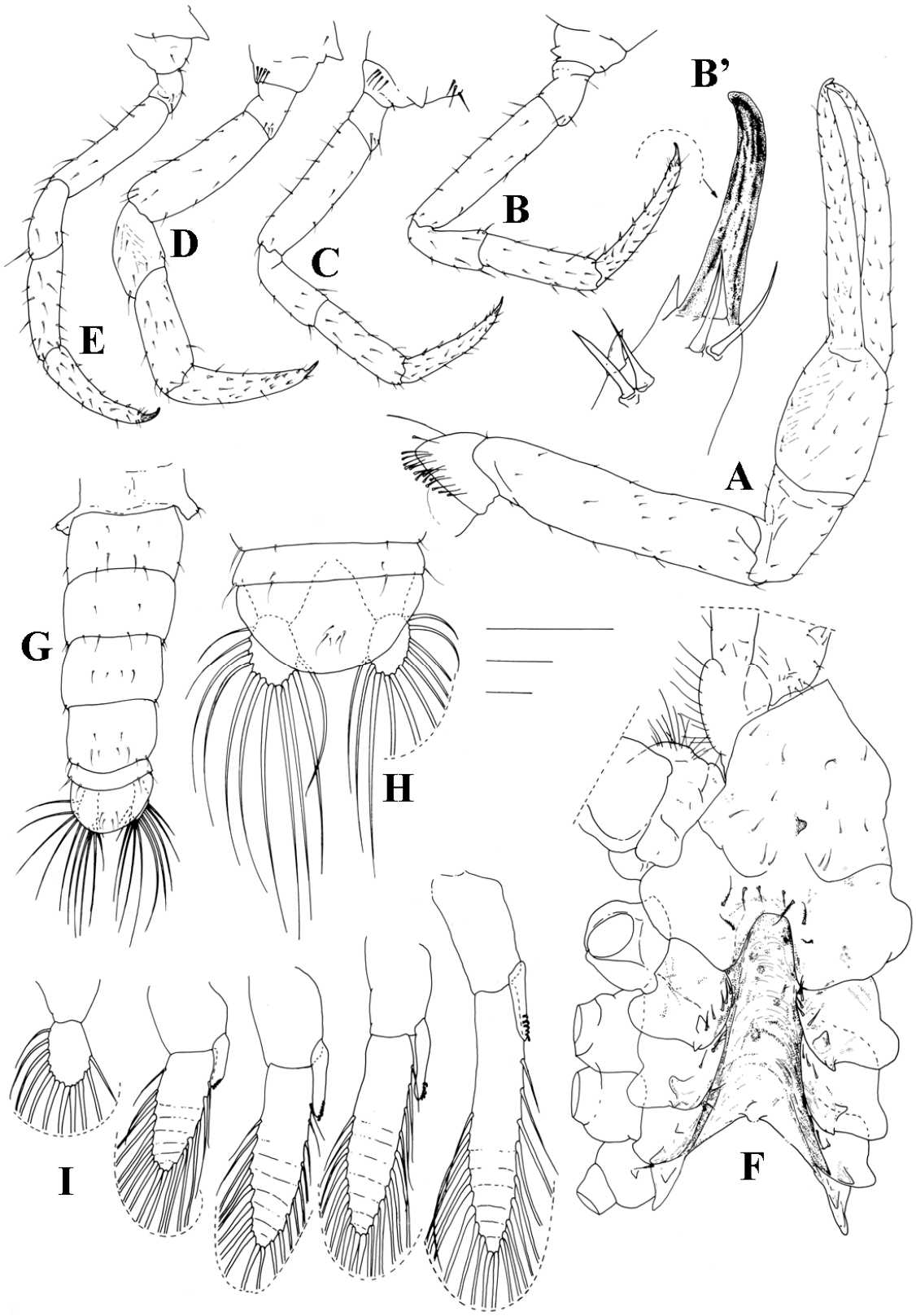

Fig. 6- *Ilia nucleus*. Megalopa: **A**, first pereiopod; **B**, second pereiopod; **B'**, detail of dactylus of second pereiopod; **C**, third pereiopod; **D**, fourth pereiopod; **E**, fifth pereiopod; **F**, sternum; **G**, dorsal view of abdomen and telson; **H**, telson and uropods; **I**, pleopods. Scale bars: 0.5 mm (**A-E**, **G**); 0.1 mm (**B'**, **F**, **H-I**).

Pereiopods (Figs. 6A-E): all segments well differentiated, with setae as figured. Chelipeds equal, with a slender dactylus longer than the palm (D/ P= 0.54- 0.68). Pereiopods 2-5 long, with sharp dactyls.

Sternum (Fig. 6F): maxillipeds and cheliped sternites fused with 10-11 pairs of setae and one medial small process; sternites of pereiopods 2-5 with 5-6, 3-5, 2 and 1 setae and 2 broad protuberance each which progressively become smaller anteriorly.

Abdomen (Figs. 5A, 5A'', 6G): six somites, broader than long, with well developed tergites. Somites 1-6 proximally to distally with 4, 10, 10, 10, 10 and 8 simple setae dorsally and laterally distributed.

Pleopods (Figs. 6H, I): biramous, well developed decreasing in size from 1st to 4th. Endopod unsegmented with 5-6, 4-6, 4-6 and 4-5 distal cincinuli on outer margin respectively. Exopod unsegmented with 19-20, 19-20, 18-19 and 16-18 marginal plumose natatory setae respectively. Uropods lacking endopod, 2-segmented, proximal segment (protopod) without setae, and distal segment (exopod) with 11-12 marginal plumose natatory setae.

Telson (Figs. 6G, H): broad, rounded posterior margin, with 2 pairs of simple setae on dorsal surface.

6.5- Discussion

The morphological features of the zoeal stages of *Ilia nucleus* fit in the characters proposed by Rice (1980) for the family Leucosiidae: 4 zoeal stages; a globose carapace that may have dorsal, lateral and rostral spines, as in genera *Persephona*, *Arcania*, *Myra* and *Ilia*, or all spines absent as in the *Ebalia* species, or almost all the variations between these for the other genera; antenna reduced to a bud without setae; maxillule endopod unsegmented bearing 4

terminal setae and maxilla endopod with 4 setae; first maxilliped endopod with 2, 2, 1, 2 and 5 setae; second maxilliped basis with 4 setae and endopod unsegmented with 3 setae; abdomen with 5 somites in all stages, somites 2 and 3 with small dorso-lateral knobs, and pleopods appearing in the third stage; subtriangular telson with posterio-external angles each with one small furcal spine. Correspondingly, the larval stages of *I. nucleus* described in the present study are very similar to the described by previous authors (Cano 1891, Boraschi 1921, Bourdillon-Casanova 1960 and Heegard 1963). The features observed coincide well with Heegard's description (1963), showing only minor differences. However some features pointed out by Heegard, such as a bifurcated antenna and the lack of one seta on the endopod of the maxilla (the author describes 2+1 setae instead of 2+2), differ from those described in the present work. This fact can be explained by differences in specimen preparation or by the precision of the optical observations.

In Northeastern Atlantic and Mediterranean Sea it has been considered that the family Leucosiidae is represented by subfamilies Iliinae, Leucosiinae, and Ebaliinae (see Zariquiey-Alvarez 1968), with *Ilia nucleus* included in the subfamily Iliinae. However, based on adult morphology, Ng et al. (2008) recently proposed a new classification of Leucosiidae family. The family Leucosiidae, is represented by subfamilies Cryptocneminae, Ebaliinae and Leucosiinae (until now no larvae have been described for the first subfamily). The authors have discussed the general consensus that Leucosiidae has 4 or 5 subfamilies: Cryptocneminae, Leucosiinae, Ebaliinae, Philyrinae and perhaps Iliinae (being the last the only one with a wholly Atlantic distribution). However considering that Ebalinae, Philyrinae and Iliinae were not well defined, they have decided to include all genera from these three groups in the subfamily Ebaliinae due to the seniority of this name until a complete generic reappraisal could be conducted.

Nevertheless, Ko (2000) considered *I. nucleus* within subfamily Philyrinae and separated this subfamily in 4 different groups, taking as distinctive characters the presence of dorsal, lateral and rostral spines of the carapace, the number of setae on the endopod of maxilla, and the number of spines on posterolateral telson margin. The 4 groups considered by the author were formed by *Persephona*, *Arcania* and *Myra* genera in the first group, the single genus *Ilia* in the second group, and the species included in the *Philyra* genus were separated in the third and fourth groups. The assignment of the genus *Ilia* to the Ko's second group, was based on Heegard's (1963) description, that considered the presence of 2+1 setae on the endopod of maxilla. However, present study demonstrates that *I. nucleus* larvae have 2+2 setae on the endopod of the maxilla, character that would include this species in the first group. In fact, *I. nucleus* zoeal stages are very similar to those defined by Ko (2000) for the first group of Philyrinae, having all carapace spines; 4 setae on maxillule endopod, 4 and 5-6 setae on maxillule basial and coxal endites, respectively; 2+2 setae on maxilla endopod; and one spine on posterolateral telson margin. This first group of larvae was considered by Ko (2000) the most ancestral group in Leucosiidae family, and providing the same group of characters *I. nucleus* would also be classified as an ancestral species in the family.

Also, the megalopa shows the typical characteristics of a leucosiid crab according to Quintana (1986): a smooth carapace with dorsal regions slightly swollen, a rudimentary rostrum, and 2 broad protuberances on sternites 2-4. Even though the significance of antennular armature seems to be unclear, Quintana (1986) stated that Leucosiidae megalopae could be divided in three subfamilies (Ebaliinae, Philyrinae and Leucosiinae) regarding the segmentation and armature of outer flagellum of antennule, a consistent character at the subfamily level. The megalopa of *I. nucleus*, presents a 5-segmented antennular outer flagellum, aesthetascs in the terminal segment in a subterminal position, and the higher number of aesthetascs observed in

the family (a total of 18), which according to Quintana (1986) place it in the Ebaliinae, and which also agrees with Ng et al. (2008) recent classification. Taking into account the number of aesthetascs, *I. nucleus* is more closely related with the *Arcania*, *Myra* and *Philyra* group, identified as Philyrinae by Quintana (1986), since these exhibit 16-17 aesthetascs in the antennular flagellum. These results are also coherent with Ng et al. (2008), once that Ebaliinae subfamily was synonymised with the Philyrinae. We can state that the megalopa of Ebaliinae presents a 3- to 5-segmented antennular flagellum, the aesthetascs on distal segment can be absent or present, and the total number of aesthetascs varies among 5-18.

Larval morphological characters are useful for species to infer phylogenetic relationships. Hence, Quintana (1986) assumed that characters exhibited by megalopae belonging to *Ebalia* and *Nucia* were plesiomorphic, being family Ebaliinae the most primitive leucosiid group, and consequently the *Leucosia* species (subfamily Leucosiinae) should be the apomorphic group. Despite that, the inferences about the phylogenetic position considering the megalopal stage will also define *I. nucleus* as the most primitive among the Ebaliinae. As Ng et al. (2008) affirmed, the actual subfamily Ebaliinae is a very heterogeneous group and considering the larval characters, it includes the most ancestral species in the Leucosiidae and also the most derived ones.

Finally, *I. nucleus* shows eight natatory setae on exopod of the maxillipeds in third and fourth zoeal stages. Nevertheless, the typical sequence of the number of natatory plumose setae on exopods of maxillipeds in Brachyura larval series with four zoeal stages is: four setae in first zoea, six in second, eight in third and ten in fourth. As in *I. nucleus*, Jorgensen (1925) described analogous results for *Ebalia sp.*, presenting the sequence of zoea II to IV with 6 natatory plumose setae on maxilliped exopods. Afterwards, Lebour (1928) described *E.*

tuberosa with six natatory plumose setae on maxilliped exopods in the second and third zoeae, and eight in fourth zoea. Lebour (1928) concluded that eight setae on maxilliped exopods in the fourth zoeal stage was a particular feature of *Ebalia*, and Salman (1982) concluded the same when studying the larval development of this genus. Other authors, e.g. Krishnan and Kannupandi (1990) described *Philyra globosa* as having 4, 6 and 6 plumose natatory setae on maxilliped exopods in first to third zoeae; Ko (2000 and 2001) described *P. platychira* and *P. kanekoi* respectively with 4, 6 and 6 setae through zoeal stages; and Ghory and Siddiqui (2008) described *Leucosia* aff. *biannulata* and *Philyra* aff. *platychira* as having 6 setae on maxilliped exopods in third zoeal stage. However, Negreiros-Fransozo et al. (1989) described the normal sequence (4, 6, 8 and 10 setae) for *Persephona mediterranea* zoeal stages. Consequently in leucosiid crab zoeal stages a first zoea always have 4 plumose natatory setae on maxilliped exopods, a second zoea always have 6, a third zoea can have 6 or 8, and a fourth zoeal stage would present 8 or 10 plumose natatory exopod setae. As a result, we suggest that for the correct identification of a zoeal stage of a leucosiid besides counting the number of setae on maxilliped exopods, the antennule development as well as the pereiopods development should be used as additional characters.

Taking into account that Leucosiinae and Ebaliinae are the only subfamilies with their larvae known, and identifying the existent heterogeneity in Ebaliinae, we are of the opinion that the present larval morphology conclusions do not give many clarifications to the current Code, and maybe a complete generic reappraisal is still needed. More larval descriptions of Leucosiidae genera and species are needed. Present work describes the 16th leucosiid megalopae, and in the last 20 years Negreiros-Fransozo et al. (1989) and Ko (2000) were the only publications that presented the complete larval development of this Family species. As Rice (1980) and Quintana (1986) stated, the development of the Leucosiidae is still very

poorly known and the information of their zoeal and megalopal morphology would be significantly modified as further information becomes available.

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CHAPTER 7

General Discussion and Conclusions

This thesis has provided detailed information about the horizontal and vertical distribution patterns of decapod crustacean larvae in the Portuguese upwelling ecosystem. In the first two chapters, the four specific objectives related with the larval ecology were fulfilled.

Present work showed that the diel vertical migration behaviour previously described by dos Santos et al. (2008) is consistent through the larval development, of almost all the studied taxa, and demonstrated that the larval retention strategy hypothesized by the authors exists. The decapod larvae were distributed along meridionally elongated patches concordant with their origins: the inner shelf species were sampled very close to the shore, the shelf species were distributed in the middle shelf, and the slope species appeared over the continental shelf break. Thus, one of the most important conclusions of present study is that the self recruitment for parental populations is a probable scenario for the decapods larvae in the sampling area, even under upwelling conditions. The presence of the complete larval series for most of the studied species in the same area, many times in the same station for the several larval forms studied support our supposition.

It is also demonstrated that the caridean shrimps, the anomuran and the brachyuran crabs had a similar larval distribution in the water column, responding in a similar way to the environment. The ontogenetic vertical migration in the water column when observable showed the first zoeal stage in a more superficial position relatively to the second zoea, and the last stage was always more deeply distributed. The megalopa had an average depth of distribution similar to the one recorded for the last zoea, but it was many times concentrated in the neuston layer during the night, position that can reflect a transport mechanism strategy. During the sampling the hydrological conditions were complex, and the arrival of the less saline water lens to the fixed point could be the responsible for the less evident ontogenetic

vertical migration behaviour, once that a probable larval rearrangement as a response to the hydrological changes could have happened.

It is demonstrated that the vertical migration movements', ontogenetical and diel, permit to decapod larvae to regulate their transport, and even considering the larval stages separately, the larval retention over the shelf was the result of a constant response to the environmental conditions, response that is most of the times reflected as the vertical larval position. This seems to be true for the three studied larval forms: the caridean shrimps, the hermit crabs and the crabs.

The last specific objective was related with the larval morphology of those three selected larval forms. The morphological aims of present thesis were also fulfilled in the third to sixth chapters.

Concerning the caridean shrimps larval development, in Chapter 4 we studied two closely related trans-isthmian *Lysmata* species, *L. galapagensis* and *L. moorei*. Both had a homogeneous development, and even in the presence of incomplete larval series, we believe that both will complete their development with a maximum of nine zoeal stages. Both species hatch with the first and fifth pereiopods as biramous and uniramous buds, respectively, similarly to *L. seticaudata* (Calado et al. 2004). As present thesis demonstrates, the study of larval *Lysmata* will certainly be of valuable help for researchers addressing phylogeny subjects, once that analyzing the available descriptions for the genus is possible to hypothesize at least two scenarios for the developmental pattern for these species: the first one similar to *L. galapagensis*, *L. moorei* and *L. seticaudata* where the larvae hatch with the first and fifth pereiopods as buds, that will complete their development with 8 or 9 zoeae, and the second one, longer, with the larvae hatching without buds. The first group of species belongs

to the "Cosmopolitan Clade"/ "*Lysmata* Clade" (Baeza et al. 2009 and Fiedler et al. submitted), so we suppose that the presence of the first and fifth pereiopod buds in the newly hatched larvae is a character shared by all the species within this clade. The presence of a small protuberance in the dorsal region of the third pleomere in *L. galapagensis* second zoeal stage, which in later zoea differentiates to a well defined anteriorly curved spine makes it easily distinguishable from all *Lysmata* larvae studied. This species late zoea is very similar to the material described by Gopalakrishnan & Laurs (1971), so, we believe that the larvae described as *E. corniger* for the Eastern Tropical Pacific are in fact *L. galapagensis*. Gurney (1937) Species A. V, *Eretmocaris corniger,* from the Atlantic Ocean, presents the same general form of *L. galapagensis*. Regardless of the close phylogenetic relationship suggested by the molecular studies of Baeza et al. (2009) and Fiedler et al. (submitted) between the Eastern Pacific *L. galapagensis* and the Western Atlantic *L. moorei*, the most evident larval character shared by both species is the presence of a similar rostrum, therefore we conclude that there is an unknown *Lysmata* species, probably phylogenetically more close to *L. galapagensis* than to *L. moorei* in the Eastern Atlantic. Having in mind that *L. galapagensis* occurs in the eastern Pacific and the most similar larvae known so far are those of *Eretmocaris corniger* collected in the eastern Atlantic, some pertinent questions were raised. Due to the occurrences of these three species, we believe that we are in the presence of an unknown species complex.

For the hermit crabs larval development we decided to describe the larval stages of *Clibanarius* species. We made a redescription according to modern standards of *C. erythropus* and a description of *C. aequabilis*. The larval stages of the two northeastern Atlantic *Clibanarius* species, were not easily differentiated. An exhaustive comparison of the morphological larval characters of the species described herein and those described by other

authors lead us to the conclusion that the genus is very homogeneous. Regardless of the general larval morphology homogeneity in *Clibanarius*, there is some variation among congeneric species, particularly in the telson formula and in the morphology of the telson processes beyond the second larval stage, as well as in the number of zoeal stages. We suggest that as a generic character of *Clibanarius* zoeal stages the transformation of the plumose fourth telson process into a fused spine (either well developed or reduced) from the second to the third zoea.

Finally, the complete larval development of the crab *Ilia nucleus* was studied. The morphological features of the four zoeal stages and the megalopa of this species fit in the characters proposed by Rice (1980) for the zoeal stages and by Quintana (1986) for the megalopa of the family Leucosiidae. The larval stages described in the present study are very similar to those described by previous authors. Ng et al. (2008) recently proposed a new classification of Leucosiidae family, with only three subfamilies the Cryptocneminae, Ebaliinae and Leucosiinae, and affirmed that the actual subfamily Ebaliinae is a very heterogeneous group. Considering the larval characters studied we verified that the Ebaliinae include the most ancestral species in the Leucosiidae and also the most derived ones, so we must conclude that this subfamily is a heterogeneous group. We also verified that for the correct identification of a zoeal stage of a leucosiid besides the number of setae on maxillipeds exopods, the development of the antennule and pereiopods must be used. Due to the lack of knowledge about this family larval development, our conclusions were not capable of clarify present code, so we believe that all the assumptions made about the leucosiid zoeal and megalopal morphology would be significantly modified as further information becomes available.

The morphological aims of present thesis demonstrate the importance of taxonomy in the study of decapods crustacean larvae. The species described and the questions raised in the previous chapters can create new working hypothesis, such as the study of the adults' phylogeny, the phylogeography, or the aquaculture.

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