Universitat d'Alacant
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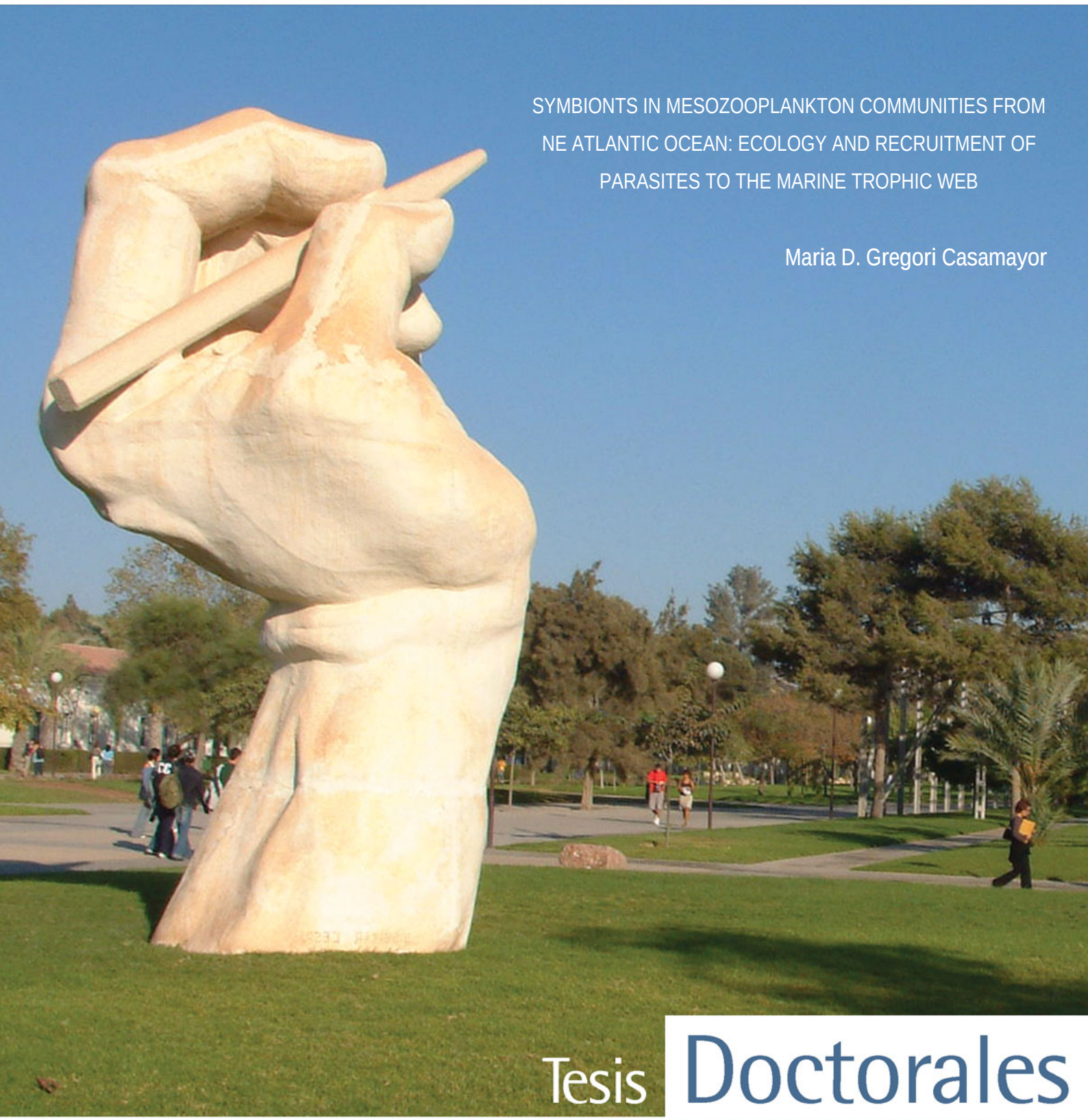
SYMBIONTS IN MESOZOOPLANKTON COMMUNITIES FROM NE ATLANTIC OCEAN: ECOLOGY AND RECRUITMENT OF PARASITES TO THE MARINE TROPHIC WEB

Maria D. Gregori Casamayor



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Dpt. de Ciències del Mar i Biologia Aplicada
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SIMBIONTS EN LES COMUNITATS MESOZOOPLACTÒNIQUES DEL ATLÀNTIC NORD-EST: ECOLOGIA I RECLUTAMENT DE PARÀSITS A LA XARXA TRÒFICA MARINA

SIMBIONTES EN LAS COMUNIDADES MESOZOOPLANKTONICAS DEL ATLÁNTICO NORESTE: ECOLOGÍA Y RECLUTAMIENTO DE PARÁSITOS A LA RED TROFICA MARINA

Memòria presentada per a optar al grau de Doctora en la Universitat d'Alacant per

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A tota aquella persona que m'ha acompanyat en aquest viatge que sàpiga que estaré per sempre agraïda, per l'enriquiment personal i professional que m'han aportat totes i cadascuna d'elles.

Gràcies a tots els que heu format part en la meua vida, als que la formen i als que la formaran.

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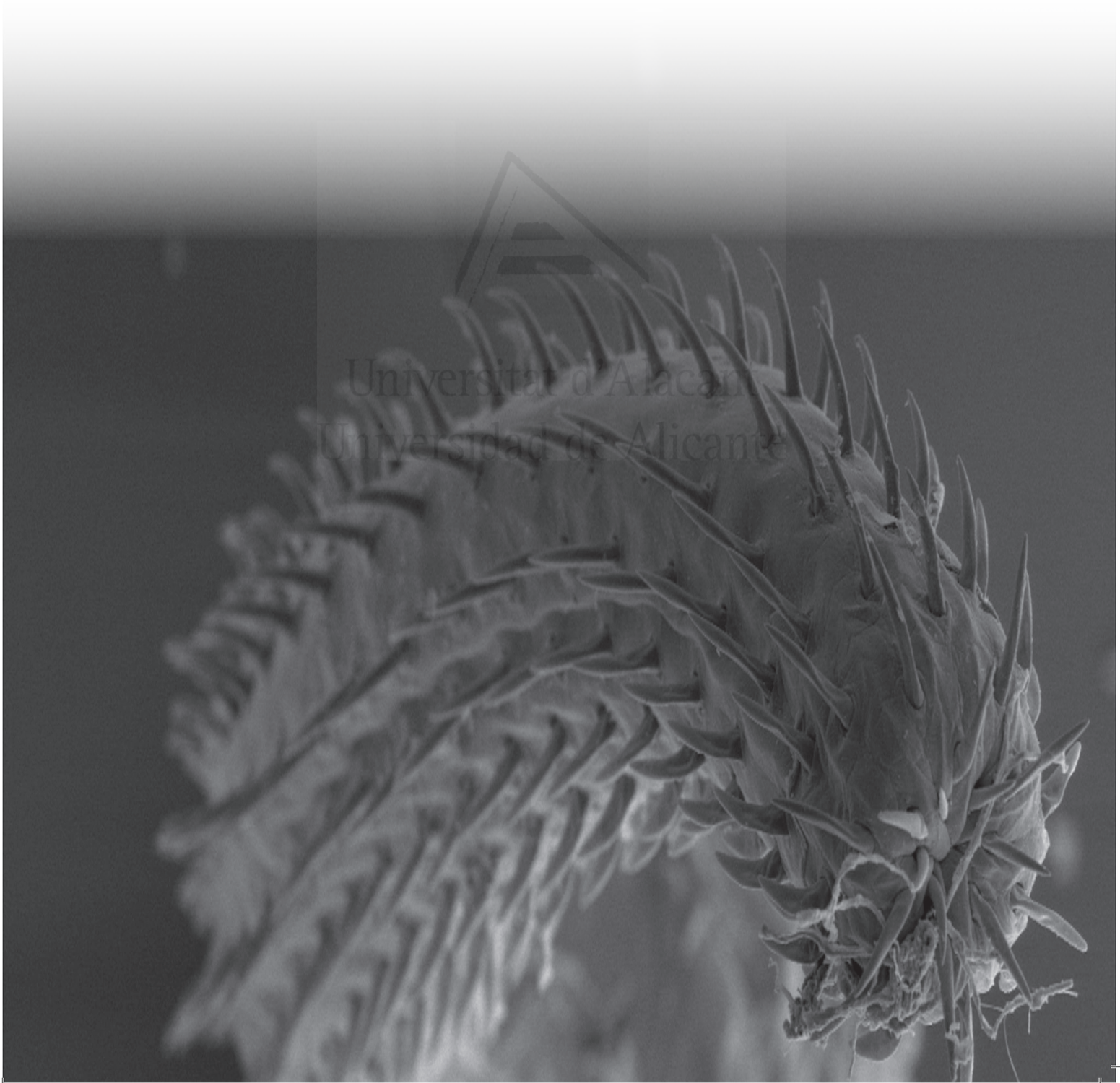
Gracias a todos los que habéis formado parte en mi vida, a los que la forman y los que la formarán.

Contents

Resum General



RESUM GENERAL

Els paràsits marins i els epibionts, són importants com a components dels ecosistemes marins, però molt sovint són descuidats a nivell mesozooplanctònic en el regne pelàgic. Això és així per a la dificultat que comporta la seva recerca en els petits animals que el componen en un regne tridimensional i molt diluït. El seu estudi implica gran quantitat de recursos materials i personals, així com el desenvolupament d'un treball multidisciplinari en què els taxonoms, genètics, estadístics i oceanògrafs treballin de forma conjunta.

La parasitologia estudia una ampla gamma de relacions simbiòtiques i és per això que depenent de l'investigador es poden trobar diverses interpretacions d'aquest concepte . Des d'un punt de vista ecològic el parasitisme s'entén com una associació estreta entre dos organismes, un dels quals (el paràsit) depèn de l'altre (l'hoste). L'ecologia parasitària (ecoparasitologia) és basa en els mateixos principis que l'ecologia d'organismes de vida lliure, però amb una major complexitat ja que, el propi sistema hoste-paràsit està sotmès a dues pressions de selecció. D'una banda el microambient, que és l'interior del cos de l'hoste i el macroambient (el medi ambient extern al sistema) que afectaria a les dues espècies (directa i indirectament). Les relacions simbiòtiques són tan estretes i fràgils que qualsevol canvi tant en el microambient com en el macroambient pot desembocar en un tipus de relació o un altre. És per això que en ecoparasitologia s'han de tenir en compte tant l'ecologia de l'hoste, que és l'hàbitat del paràsit, com el cicle de vida del paràsit. Depenent del paràsit en qüestió diverses etapes del seu cicle vital és donen en diferents hàbitats o hostes pel que el seu estudi és complica una mica més. Per la seva pròpia naturalesa, tant paràsits com epibionts marins són essencials en l'ecosistema, ja que són capaços d'afectar de diferents formes les poblacions i comunitats d'hostes que al seu torn són el seu propi ecosistema.

En els sistemes epibiont-basibiont, on l'epibiont és l'organisme de vida sèssil que viu sobre el substrat viu, basibiont s'han descrit avantatges i desavantatges que afecten a tots dos components del sistema. En aquest sentit, tant els paràsits com els epibionts marins causen una àmplia varietat d'efectes sobre els seus amfitrions, per exemple, afecten a la conducta de l'hoste,

disminueixen la seua condició corporal, redueixen la seua fecunditat i fins i tot poden causar la mort o incrementar la seua mortalitat. Recíprocament, l'hoste o basibiont pot causar la mort del paràsit o epibiont, disminuir el seu reclutament, etc.

De forma general, la fauna de paràsits és més diversa en als ecosistemes marins especialment en els peixos i els depredadors superiors (top predators). Açò és causat principalment pel fet que les xarxes tròfiques són més extenses al medi marí on un gran nombre d'hostes són utilitzats per a la transmissió de paràsits. Aquesta utilització de múltiples hostes es considera com una característica del medi marí, on els paràsits amb cicles de vida complexos són capaços d'utilitzar hostes paratènics amb la finalitat d'explotar noves vies d'infecció a través de les xarxes tròfiques. A més, els paràsits tenen la capacitat de "transformar" als seus hostes en bioacumuladors en diferents nivells tròfics, zooplàncton, peixos, etc. que després transmeten paquets de paràsits als hostes definitius. En aquest immens macroambient els paràsits i els epibionts mostren una baixa especificitat cap als hostes invertebrats o vertebrats que actuen com a intermediaris o paratènics. Aquesta baixa especificitat és el resultat de l'adaptació a un mitjà extremadament diluït on trobar a l'hoste definitiu pot ser complicat i extremadament difícil.

El zooplàncton al medi pelàgic marí posseeix una alta diversitat tant d'espècies com dels grups taxonòmics superiors que són capaços de connectar la xarxa tròfica microbiana amb la resta de la cadena tròfica marina al ser capaces d'alimentar-se de microzooplàncton i paràsits. En aquest sentit, els sistemes d'aflorament costaner mantenen una alta productivitat que es transmet a través de la cadena tròfica. Mentre que els oceans (mar obert) en general són deserts de vida, els sistemes d'aflorament són formiguers de vida on les comunitats mesozooplanctòniques exerceixen un paper crucial en la transmissió de paràsits a través de la cadena tròfica. Les comunitats mesozooplanctòniques malgrat viure en un ambient en constant canvi, espacial i temporal són capaços de mantenir-se estables. En aquest advectiu macroambient, els paràsits que utilitzen el zooplàncton com a hostes intermediaris han hagut d'adaptar-se a aquestes variacions. Algunes d'aquestes adaptacions són la seua baixa especificitat cap a l'hoste zooplanctònic intermediari, la seua transmissió a través de les relacions de

depredador-presa, i tenir diferents estadis de desenvolupament en cadascun d'aqueixos intermediaris mentre arriba fins al seu hoste definitiu. Per tant, en aquest hàbitat extremadament fragmentat, diluït i en tridimensional, els paràsits han desenvolupat cicles de vida complexos, que han implicat canvis ontogènics que, al seu torn, han donat lloc a metamorfosi i transferència d'hàbitats i nínxols. Molts Platelmints paràsits poden patir més d'una metamorfosi i diverses mudes al llarg d'un sol cicle de vida. Aquest cicle de vida pot incloure tant estadis de vida lliure com parasitaris, on dues o més fases parasitaries es produeixen en invertebrats i vertebrats. Aquests patrons en la història de vida permeten als paràsits augmentar la seua probabilitat de ser ingerits per l'hoste adequat final al mateix temps que maximitza la durada i la disponibilitat del conjunt infeccións dins del zooplàncton. Totes aquestes estratègies en conjunt permeten que les larves infectives es canalitzen cap als hostes definitius, incrementant la probabilitat de completar el seu cicle vital. Encara que la transmissió de paràsits a través de la cadena tròfica és la més estudiada no és l'única. Exemples de paràsits que es transmeten de forma activa, nadant per a cercar a l'hoste adequat penetrant-hi activament en ell, inclou oncomiracidis de monogeneus, miracidis i cercaries de trematodes, copepodits de copèpodes paràsits, etc.

Les taxes d'infecció (prevalença) en zooplàncton marí amb paràsits helmints són extremadament baixes. Els copèpodes malgrat ser els animals més abundants exhibeixen unes taxes, en general, de menys d'un animal infectat per cada 1.000, encara que poden ser molt més baixes. En eufausids per exemple aquesta taxa es redueix encara més, només un animal infectat per cada 100.000. Quetògnats i Ctenòfors són els animals més intensament infectats ja que cada individu pot portar diversos paràsits dins. Aquesta, la baixa prevalença, sens dubte és una altra de les característiques dels paràsits en el zooplàncton.

Pel que fa als epibionts protozous marins la superfície dels cossos calcificats dels crustacis decàpodes sembla ser un hàbitat adequat per a ells. Així, molts protozous ciliats es troben sovint com epibionts sobre crustacis decàpodes marins. Aquesta associació simbiòtica por estar composta per diferents organismes com protistes, algues, bacteris, hidrozous, percebes, rotífers, etc. Els epibiontes per tant, són aquells que durant el seu estadi de vida sèssil es fixen a un substrat viu. Per

contra el substrat viu és conegut com basibiont. Aquest tipus de relació, en els sistemes marins, encara segueix sent poc coneguda sobretot en relació amb les conseqüències, avantatges i desavantatges que té per a tots dos components de la relació. Igual que en el parasitisme qualsevol organisme marí és susceptible de ser "epifitat" o parasitat.

Des d'un punt de vista evolutiu l'especificitat entre epibionts i basibionts crustacis implica una àmplia gamma d'adaptacions morfològiques i fisiològiques. Així l'estudi ecològic d'aquesta relació té les mateixes connotacions que la ecoparasitologia ja que, la relació epibiont-basibiont en ella mateixa posseeix funcions ecològiques i és veu doblement afectada tant pel macro com pel microambient.

Aquesta tesi té com a objectiu general millorar el coneixement sobre els paràsits i els epibionts marins a nivell mesozooplanctònic en l'aflorament estacional en el NE de l'Oceà Atlàntic a través de mostrejos nocturns realitzats en l'estiu i en la tardor de 2008. En aquest sentit, es pretén proporcionar dades valuoses sobre la transmissió de paràsits en aquesta àrea geogràfica, descriure el paper del mesozooplàncton en les relacions epibionte-basibionte i hoste-paràsit. Aportar dades descriptives de la població i comunitats mesozooplanctòniques, així com de les infracomunitats parasitàries. Descriure la distribució espacial i especificitat d'epibionts entre basibionts i hoste-paràsit proporcionant dades descriptives i cicles de vida. Es presenten també seqüències d'ADN útils i noves de les larves de paràsits i epibionts relacionats amb la morfologia i la taxonomia especifiques de cada animal. Finalment, s'han avaluat els coneixements adquirits per a intentar comprendre millor la influència de l'oceanografia sobre el reclutament de macroparàsits. En aquest sentit, l'aflorament estacional que té lloc en el NE de la península Ibèrica crea un ambient advectiu on el mesozooplàncton s'enfronta a un transport cap al oceà que és forçat pel propi sistema. Malgrat açò el mesozooplàncton té la capacitat de canviar la seua posició verticalment per a evitar ser exportat a mar obert, per la qual cosa les comunitats mesozooplanctòniques tenen la capacitat de mantenir la seua integritat durant tota la temporada d'aflorament.

Com a objectius específics destaquem:

Identificació de larves de paràsits de acantocèfals, nematodes i trematodes usant taxonomia clàssica i mètodes moleculars.

Identificació de paràsits protozous i noves espècies d'hostes.

Descriptores de la població mesozooplanctònica.

El cicle de vida del acantocèfal *Bolbosoma balaenae*.

Avaluació del reclutament de paràsits influenciada per diferents escenaris oceanogràfics.

Descripció d'un nou sistema epibionte-basibionte Suctoria-Copepoda proporcionant una nova espècie de epibiont Suctoria per a la ciència.

Dades ecològiques sobre l'especificitat de paràsits i epibionts.

I dades parsito-demografiques a nivell infrapoblació.

Per tot açò, aquest treball s'ha realitzat des d'un punt de vista multidisciplinari, on taxonomistes, genetistes, oceanògrafs i estadístics han contribuït a fer-lo possible. S'han aplicat diferents metodologies acoblades entre si, com per exemple la taxonomia clàssica i la genètica moderna per a la identificació de les espècies, i també models lineals generalitzats (GLM) juntament amb l'oceanografia biològica convencional. A més el treball s'emmarca dins d'una de les més notables hipòtesi proposada per Pascual et al. (2007). Aquesta suggereix que el reclutament del paràsits a les comunitats d'hostes podria estar condicionat directament per l'oceanografia. Així, les condicions de major estabilitat en les masses d'aigua millorarien el reclutament de paràsits, especialment per als paràsits heteroxens amb cicles de vida complexos i diversos hosts. Aquesta hipòtesi va ser llançada en observar a escala global que els sistemes d'afloramento la parasito fauna s'empobreix mentre que els esdeveniments enfonsament propicien unes condicions òptimes per al reclutament. Com a exemple utilitzaren *Anisakis* spp. Per primera vegada en aquest estudi es pogueren identificar diferents

sistemes epibiont-basibiont i hoste-paràsit en mostres de mesozooplàncton en l'aflorament estacional en el nord-est de l'Oceà Atlàntic relacionats amb els esdeveniments oceanogràfics locals.

Dels coneixements adquirits al capítol 3 destaca la troballa d'una nova relació epibionte Suctoria-Copepoda on una nova espècie de Suctorian ha estat identificada com *Pelagacineta hebensis* que es va trobar ancorada a la superfície del copèpod *Paraeuchaeta hebes*. A més, es va analitzar l'abundància i distribució dels epibionts en la superfície dels copèpodes, tenint en compte el sexe del crustaci, cosa que va revelar certa preferència per les femelles i certa preferència pel punt d'unió diferent entre tots dos sexes. Addicionalment, les seqüències d'ADN obtingudes per primera vegada per a aquest epibiont van contribuir a ampliar el coneixement en la filogènia de Phyllopharingea que segueix sent bastant confusa.

En la segona part del capítol, (3.II) el protozou *Ellobiopsis chattoni* es va trobar penetrant la superfície del cos de diferents espècies de copèpodes: *Calanus helgolandicus, Calanoides carinatus, Centropages chierchiae, Acartia clausii,* i *Temora longicornis*. Entre les espècies de copèpodes estudiats *Paraeuchaeta hebes* va ser l'únic que no es va trobar parasitat per aquest protozou. Fins on sabem aquesta és la primera vegada que *Ellobiopsis chattoni* s'ha trobat en *Calanoides carinatus, Centropages chierchiae i Metridia lucens* en aigües de l'Atlàntic nord-est estenent el rang d'hostes d'aquest paràsit. Les prevalences com calia esperar van ser baixes. Els nivells d'infecció van mostrar ser homogenis entre les comunitats estudiades. Certa especificitat va ser detectada ja que, *C. helgolandicus* va mantenir la prevalença més alta. Les femelles van ser la fracció de la població que més es va veure més afectada per *Ellobiopsis*. Finalment, els punts d'ancoratge preferits per *E. chattoni* van ser els apèndix bucals.

Sobre la base dels resultats obtinguts al capítol 4 els cistacants dels acantocèfals *Bolbosoma balaenae* i *Rhadinorhunchus* sp. van ser trobats encapsulats en el cefalotòrax del eufausid *Nyctiphanes couchii* en l'àrea d'estudi. Les seues prevalences van ser molt baixes i les intensitats d'1. Es van utilitzar anàlisis morfològiques i filogenètiques per a identificar els animals a nivell d'espècie i per a situar-los filogenèticament dins de Polymorphidae. Les

seqüències d'ADN obtingudes, per primera vegada per a aquests cistacants es van dipositades en la seua corresponent base de dades. Finalment, la filiació taxonòmica dels paràsits i l'ecologia tròfica de la zona de mostreig suggeriren que *N. couchii* és l'hoste intermediari per a *B. balenae* i *Rhadinorhunchus* sp. D'altra banda, el cicle de vida de *B. Balaenae* es va proposar assumint que *Balaenoptera physalus* i *B. acutorostrata* són els seus hostes definitius. Finalment, les diferents prevalences trobades en la infrapoblació de *Rhadinorhynchus* sp. a través de les comunitats de mesozooplàncton tant a l'estiu com a la tardor, van revelar que el reclutament d'aquests paràsits es veu afectat per les diferents condicions oceanogràfiques.

Com a resultat dels coneixements adquirits al capítol 5 es va diagnosticar l'eufausid *N. couchii* i a un misidaci com a hostes intermediaris de les larves (L_3) del complex *Anisakis simplex* en la comunitat de mesozooplàncton estudiada. Les larves van ser identificades molecularment utilitzant l'espaiador intern transcrit (ITS). Per primera vegada es va identificar *Anisakis pegreffii* com a paràsit del krill. L'existència de larves L_3 d'Anisakids en diversos organismes del mesozooplàncton suggereixen que les rutes de transmissió de *A. simplex* i *A. pegreffii* són més àmplies del que s'esperava. D'altra banda, els resultats van demostrar que aquestes dues espècies de Anisakids no són específics per als seus hostes intermediaris. Finalment, els nostres resultats llancen llum sobre la influència de l'oceanografia en el reclutament del complex *A. simplex*, sent diferent sota condiciones d'aflorament o d'enfonsament.

En la segona part d'aquest capítol (5.II) es va detectar un nou segon hoste intermediari, *Muggiaea* sp. per a metacercàries d*'Opechona bacillaris* (Trematoda). *O. bacillaris* és un dels endoparàsits més importants de peixos amb cicles de vida complexos que utilitza una àmplia gamma d'animals gelatinosos com meduses, ctenòfors i quetògnats com a hostes intermediaris secundaris. La seua transmissió als peixos es produeix a través d'interaccions depredador-presa, on els peixos ingereixen meduses, sent aquest un fenomen generalitzat. Aquesta és la primera aproximació realitzada per a entendre la importància de les condicions oceanogràfiques en el reclutament d'aquests paràsits en les comunitats de mesozooplàncton. Les prevalences trobades a través de les comunitats estan

d'acord amb el caràcter costaner i estuarí de la infecció registrada en trematodes. Finalment, la variació de la prevalença s'acobla amb les diferents condicions oceanogràfiques i el comportament dels sifonòfors.

Arran de les troballes del capítol 6 podem afirmar que molt de treball queda encara per realitzar sobre tot quant a identificació de sistemes hoste-paràsit i epibiont-basibiont en les comunitats de mesozooplankton. En aquest capítol es va abordar l'estudi de diversos sistemes pertanyents a zones amb diferents condicions oceanogràfiques dins dels sistemes d'aflorament. Parasito fauna procedent de sistemes d'aflorament estacional com el del Nord-Oest de la península Ibèrica i la parasito fauna procedent del sistema d'aflorament perenne africà.

Com a anàlisi final aquesta tesi va més enllà revelant que existeixen importants interaccions entre els paràsits i les malalties d'origen marines amb la salut del medi ambient i dels éssers humans. En el context del canvi climàtic diversos estressors d'origen antropogènic poden exacerbar els seus efectes a causa de l'augment de la temperatura, que directa o indirectament, afectarà a les activitats recreatives econòmiques i a la salut humana. Aquesta tesi ha ajudat a detectar que les diferents condicions oceanogràfiques afecten directament al reclutament dels paràsits en la base de la cadena alimentària (mesozooplàncton). Per tant, en situacions d'estabilitat de les masses d'aigua i de baixa intensitat d'aflorament la colonització dels paràsits en les xarxes alimentàries tendirà a augmentar. Aquest augment de la càrrega parasitaria en aquesta sensible baula pot tenir conseqüències devastadores, ja que els seus impactes es poden transmetre a tota la comunitat i l'ecosistema en un efecte trencada (a dalt - a baix). Aquest efecte ha estat àmpliament demostrat en multitud de treballs destacant als paràsits i als epibionts com a reguladors de les poblacions d'hostes influint en la composició, estructura i funció de les comunitats biològiques.

Existeix evidència suficient que suggereix que els paràsits i la transmissió de malalties, juntament amb la virulència augmentarà amb l'escalfament global. Altes intensitats de paràsits (des de microparàsits a macroparàsits) poden afectar als mercats, on les espècies fortament parasitades són rebutjades pels

consumidors, amb la conseqüent pèrdua de confiança. També pot afectar a les autoritats sanitàries ja que, han de retirar partides completes del mercat amb el conseqüent cost econòmic. D'altra banda, si el producte afectat arriba al consumidor els costos sanitaris augmenten, ja que s'han de combatre malalties o al·lèrgies procedents de partides contaminades.

La introducció d'espècies exòtiques o la propagació d'espècies tropicals cap als sistemes de clima temperat poden portar nous paràsits i agents patògens per als quals les poblacions natives no estan preparades, provocant l'entrada de noves malalties i brots episòdics. La pèrdua de sincronia en les poblacions de depredadors i preses a causa de l'augment de temperatura pot causar la interrupció dels cicles de vida dels paràsits que portaria a una reducció de l'abundància de paràsits, d'una banda obligant-los a cercar noves estratègies per a sobreviure, com la colonització d'espècies que no estan preparades per a la invasió, i d'altra banda per l'alteració que la pèrdua d'un component regulador de la població té sobretot l'ecosistema. Amb aquesta pèrdua de components en les poblacions les estratègies evolutives es veuen involucrades, ja que l'eliminació sistemàtica dels paràsits adults causa biaixos en les seues poblacions, avançant la maduresa i reproducció de juvenils i la propagació de les larves.

L'estrès causat per l'augment de la temperatura és un altre efecte que es suma als causats per parasitisme o la malaltia, ja que pot augmentar la virulència dels patògens o paràsits, i fins i tot afavorir la seua transmissió. Així, els animals parasitats moren més fàcilment que els no infectats. A més, la susceptibilitat de l'hoste a l'adquisició de malalties o paràsits augmenta fent-los més vulnerables. Canvis en el comportament de l'hoste, com per exemple majors taxes d'ingestió d'una determinada presa clau en la transmissió, podrien accelerar l'adquisició de paràsits o malalties. Temperatures elevades, més càlides, provoquen també l'estratificació de la columna d'aigua reduint el cabal i el volum d'aigua. En aquest context, la concentració d'oxigen per sota de la termoclina es redueix. La hipòxia juntament amb les altes temperatures de la superfície forcen als peixos a concentrar-se (en altes densitats) en bandes més estretes de les condicions tolerables on la transmissió i els brots de malalties es transmeten amb molta més facilitat. A part d'aquesta facilitat de transmissió, susceptibilitat per part de l'hoste,

etc. la migració vertical del mesozooplànkton i d'organismes bentopelàgics es veu afectada, per la qual cosa la connexió entre diferents vies d'infecció es veuen seccionades.

Els canvis biològics i ambientals que ací es resumeixen tindran conseqüències econòmiques per a la subsistència de la pesca (comercial i esportiva), juntament amb les activitats humanes associades. D'altra banda, també es repercutirà en la salut pública ja que, algunes malalties i infeccions procedents de peix contaminat pot ser transmesa als éssers humans . No solament aquestes malalties poden ser transmeses als humans des dels peixos, mol·luscs, etc. també poden ser transmeses per l'aigua on ens banyem o la que consumim així es descriuen diverses malalties com Anisakiosi, picor del nadador, la esquistosomiasis, les malalties diarreiques, criptosporidiosis, giardiasis, etc. Aquests brots de malalties són extremadament cars per a la pesca, l'aqüicultura, les indústries relacionades i la salut pública.

Malgrat la nostra capacitat per a detectar els vincles entre la variabilitat climàtica i els brots de malalties infeccioses marines han millorat, els efectes sobre la majoria de les interacciones hoste-patogen són encara poc conegudes. En peixos, les malalties i el parasitisme s'han estudiat àmpliament, així com els agents causants. No obstant això, la dinàmica de transmissió segueix sent una mica desconeguda en l'entorn salvatge. En aquest sentit, la comunitat mesozooplanctònica juga un paper important, ja que els seus components serveixen com a hostes intermediaris dels paràsits amb cicles de vida complexos que són capaces d'explotar les vies de la xarxa alimentària. Els seus components també són l'objectiu (hostes) tant per a microparàsits (protozous) com macroparàsits (p. i. cucs helmints) i també dels epibionts. Cadascun amb els seus respectius papers en la població, la comunitat i l'ecosistema. Tant la comunitat mesozooplanctònica com la parasitària es consideren bons indicadors del canvi climàtic al medi marí per diverses raons pel que el seu estudi i seguiment aconseguiria augmentar el nostre coneixement en aspectes bàsics per al disseny d'estratègies que permeten valorar de forma efectiva els riscos de determinats canvis ambientals, permetent-nos adoptar les mesures pal·liatives pertinents.

En aquesta tesi s'ha demostrat que efectivament el mesozooplàncton és clau en la transmissió de paràsits com acantocèfals, nematodes, trematodes, protozous... en el NE Atlàntic. Diferents escenaris oceanogràfics s'han relacionat amb el reclutament parasitari. A més tècniques moleculars han estat aplicades conjuntament amb la taxonomia clàssica demostrant ser eines útils en la detecció de larves de paràsits. Aquesta tesi també ha contribuït a destacar la importància del mesozooplàncton en la diversitat composició i supervivència d'epibionts, alguns dels quals han estat descrits i identificats per primera vegada.

Per tant, es reafirma que el treball multidisciplinari és necessari per a fer front a aquesta enorme tasca. És evident que hi ha un risc significatiu per als ecosistemes, l'economia i la salut humana, que són conduïts pel canvi climàtic que actua sinèrgicament amb altres factors d'estrès ambientals.

Per tant, s'han de prendre mesures de gestió per a eliminar o minimitzar aquests riscos, la qual cosa garanteix que s'aconsegueixen en conjunt els beneficis econòmics, socials i ecològics per a moltes activitats marines. És clar que amb el creixement esperat de la població humana, la pressió sobre els ecosistemes marins augmente (aqüicultura, el turisme, la pesca, etc.). En el futur, qualsevol política racional per a fer front a la pèrdua de biodiversitat, brots de malalties d'origen marí, etc. han de centrar-se primer en la prevenció, encara que seguides de fortes mesures de gestió per a eliminar, o almenys reduir qualsevol dany ecològic que poguera succeir. Aquestes mesures han de ser rendibles i integrar els beneficis de la protecció del medi als beneficis econòmics. Per són necessaris programes de seguiment per a millorar la comprensió, l'alerta primerenca i el tractament de malalties i parasitosis en els sistemes marins sota els efectes del canvi climàtic. Per poder dur a terme aquestes tasques, es requereix el desenvolupament de mètodes innovadors que permeten una detecció ràpida de vectors i hostes intermediaris al mesozooplàncton quan s'analitzen grans quantitats d'invertebrats com els representats en el mesozooplàncton. Aquests mètodes han de ser precisos, fiables i efectius el que permetrà al seu torn fer un diagnòstic de l'estat de l'art. En aquest sentit l'aplicació de tècniques immunològiques o moleculars, tals com ELISA o NGS (seqüenciació de nova generació) podria produir molt ràpidament una gran quantitat d'informació quantitativa i qualitativa. Tota aquesta informació ajudaria

a dissenyar estratègies de gestió més eficaces per a mitigar l'impacte de les malalties i parasitisme desenvolupant estratègies específiques i adaptatives per fer front a les malalties. Aquestes estratègies han de basar-se en coneixements científics sòlids, que incloguen el seguiment i la supervisió a llarg termini dels efectes del canvi climàtic i dels organismes afectats. En aquest sentit, el treball experimental cobra un paper fonamental per a posar a prova els efectes dels factors d'estrès climàtic en les interacciones hoste-patogen. Amb tot açò, bones eines de suport, assessorament i informació són imprescindibles.

Amb aquest treball s'han aconseguit identificar moltes qüestions bàsiques en el coneixement parasitari al mesozooplàncton en el medi pelàgic, que és necessari respondre per poder fer front als desafiaments plantejats pel canvi climàtic. La identificació dels sistemes hoste-paràsit és fonamental per poder saber què hi ha, on i quants. Estudis de dinàmica de poblacions parasitàries, les seues comunitats, georeferenciació oceanogràfica, les vies de transmissió, ecologia de la transmissió, canvis en el reclutament causats per canvis ambientals, etc. estan pendents de ser resolts. La seua resolució progressiva ajudarà a millorar l'avaluació de riscos.

Després de l'informe presentat per les Nacions Unides Rio +20 i al 7PM_Horizon 2020 algunes estratègies podrien adoptar-se de forma immediata per a reduir el risc de malalties relacionades amb el canvi climàtic i el declivi de la biodiversitat al medi marí amb la finalitat de garantir el subministrament i la sostenibilitat de l'alimentació saludable per a les creixents poblacions humanes. Algunes d'aquestes mesures podrien ser:

- Integració de les conseqüències relacionades amb el clima en la gestió basada en els ecosistemes o el disseny de AMP, així com en l'ordenació pesquera. Aquí seria recomanable la integració dels sistemes paràsit-hoste per a una correcta pràctica d'aquestes eines de gestió.

- Millorar la gestió de les aigües residuals. Alguns factors estressants que podrien millorar-se amb aquesta mesura són la contaminació costanera, la pèrdua

d'hàbitat (eutrofització) i l'arribada dels agents patògens als humans evitant moltes al·lèrgies i malalties de la pell que estan associats.

• Evitar la sobreexplotació dels recursos reduint la pèrdua de biodiversitat i variabilitat genètica necessàries per a la supervivència en un món canviant.

• Identificar els factors immunològics que proporcionen resistència a les malalties.

• Compartir les millors pràctiques i els coneixements adquirits.

Algunes mesures, com la no eliminació dels descarts en alta mar (UE la política pesquera de 2014 (CE) n º 2371/2002) ja s'estan aplicant en el sector pesquer. Amb això es pretén garantir la salut animal i del medi ambient marí, i per tant la restauració de la confiança del consumidor, assegurant bones pràctiques, així com la qualitat dels aliments.

Al 7PM_Horizon 2020 diversos objectius han estat també delineats de cara a garantir la sostenibilitat, l'explotació i gestió dels recursos aquàtics vius que permeten maximitzar els beneficis socials i econòmics dels oceans i els mars d'Europa. En aquest sentit, van proposar optimitzar la contribució de la pesca sostenible, marc en el qual s'inclou el projecte PARASITE. Aquesta eina de gestió contribuirà a la millora dels mariscs, enfortiment de la competitivitat de la indústria europea del mar i la millora de la política europea en seguretat alimentària. A més, aquest projecte ha inclòs el desenvolupament, el disseny i la implementació del BIOBANCO una eina objectiu de la qual és posar al servei de la comunitat científica mostres de paràsits obtingudes dels productes pesquers. En aquest mateix sentit, la xarxa de PARNET es presenta com un servei en la vigilància tecnològica per a la gestió de la cartera d'I + D + I en parasitosis als recursos explotats marins renovables.

En el seu conjunt, un estudi complet dels paràsits a través del seu cicle de vida i la seua ubicació en els ecosistemes oceànics està limitat per interessos econòmics i polítics d'una banda i pel costós de temps d'inspecció i identificació dels estadis larvaris dins de cadascuna de les espècies que componen el mesozooplàncton. Per tant , queda molt treball científic per fer. En aquest sentit

treballs orientats a descobrir com funciona la retenció de les larves parasitaries en un ambient advectiu com el de l'aflorament estacional que ocorre en aigües galegues (NE Oceà Atlàntic) és necessari.

Els resultats d'aquesta tesi suggereixen i apunten, en una escala geogràfica local com la Ria de Vigo i plataforma adjacent, que les diferents condicions oceanogràfiques determinen el reclutament dels paràsits en la base de la cadena alimentària (mesozooplàncton) seguint el patró descrit en (Pascual et al. 2007). Si, segons el predit pel canvi climàtic, l'estabilitat i estratificació de les masses d'aigua augmenta i la intensitat de l'aflorament disminueix, cal esperar un augment de les larves de paràsits en els hostes intermediaris amb conseqüències devastadores que poden ser transmeses a través de tot l'ecosistema, com per exemple el col·lapse dels recursos pesquers a causa de la falta de subministrament d'aliment, mort massiva per parasitosi del recurs pesquer, propagació de malalties, etc. Sent extensible a totes les activitats relacionades com a aqüicultura, turisme, etc.

Arribats a aquest punt hem de tenir en compte que les espècies de paràsits tenen una àmplia gamma d'estils de vida i estratègies, així com una àmplia varietat de diferents hostes intermediaris i definitius. Pel que impacten en els ecosistemes de forma diferent, per això el seu estudi ha d'estar enfocat, dissenyat, desenvolupat i abordat específicament per a cadascun dels paràsits en tota la seua extensió. Però per a açò i com s'ha anomenat anteriorment, és essencial el desenvolupament de mètodes ens permeten detectar i comptabilitzar tantes formes com siga possible en un curt periode de temps per a abordar cadascuna de les estratègies necessàries per al seu estudi.

Aquesta tesi presenta la primera aproximació a totes aquestes qüestions, on els resultats es fan evidents a la llum de les nombroses hipòtesi que la consecució del projecte de tesi han plantejat, sobretot en relació amb dos aspectes de gran rellevància científica i soci-econòmica dom són:

• El coneixement sobre les maneres de colonització de les formes infectants parasitàries a la cadena tròfica en els ecosistemes marins, especialment en les primeres etapes de desenvolupament en les comunitats de mesozooplancton.

• I l'existència de la relació que existeix entre l'oceanografia de les masses d'aigua i el reclutament dels paràsits zoonòtics o característiques patogèniques, que encaixen en el repte social de l'Horitzó 2020 "Canvi Climàtic i Seguretat Alimentària".

Algunes de les conclusions més rellevants d'aquesta tesi s'engloben en dos grans apartats, d'una banda l'estudi faunístic i per un altre l'estudi ecològic. Així aquesta tesi ha aportat:

1. L'extensiva recerca faunística en les comunitats mesozooplanctòniques de les aigües del NE Atlàntic, proporcionant una forta evidència de l'establiment de prop d'uns 17 sistemes simbionts que representen un grup d'alta la diversitat de paràsits i epibionts amb baixes prevalences en poblacions les de mesozooplàncton. Els sistemes hoste-epibiont/paràsit identificats comprenen:

1.1. Una nova espècie epibiont *Pelagacineta hebensis* sp. n., que es troba en individus adults del copèpode *Paraeuchaeta hebes*. És la primera vegada que aquest copèpode es registra com a basibiont per a aquesta espècie de protozou epibiont. El nou epibiont es descriu tant morfològicament com molecular, la qual cosa contribueix a ampliar les seqüències genètiques disponibles per a la Classe Phyllopharyngea. D'altra banda, també es proporciona el diagnòstic taxonòmic d'aquest nou epibiont descobert.

1.2. Tres noves espècies de copèpodes hostes del protozou paràsit *Ellobiopsis chattoni*. Aquest es troba en formes adultes dels copèpodes *Calanoides carinatus, Centropages chierchiae* i *Metridia lucens* el que estén la gamma d'hostes per a aquest paràsit. Les prevalences entre copèpodes van ser baixes mostrant diferents susceptibilitats a la infecció d'*Ellobiopsis*. D'altra banda, *E. Chattoni* va mostrar certa predilecció per *Calanus helgolandicus*.

1.3. Per primera vegada el acantocèfal *Bolbosoma balaenae* es va trobar parasitant l'eufausid *Nyctiphanes couchii*, que juga el paper d'hoste intermediari. D'altra banda, es va proposar el cicle de vida *B. balaenae*

assumint que *Balaenoptera physalus* és l'hoste definitiu. Aquests mamífers marins serien els responsables del subministrament de la infra-població infectiva per als eufausids.

1.4. Per primera vegada el acantocèfal *Rhadinorhynchus* sp. s'ha identificat infectant *Nyctiphanes couchii*. És probable que aquest eufausid actue mitjançant les interaccions depredador-presa com a hoste intermediari. Tant l'estudi morfològic com el filogenètic, juntament amb la informació epidemiològica disponible sobre *R. pristis* en peixos de les famílies Scombridae i Xiphiidae de les costes de Portugal, les Illes de Madeira i l'Oceà Atlàntic Nord, suggereixen que els cistacants ací descrits pertanyen probablement a *R. pristis*. Malgrat tot, fortes discrepàncies entre la filogenia que ací es presenta i "l'estat de l'art". Per tant, es recomana una revisió exhaustiva tant de les espècies com de la família Rhadinorhynchidae.

1.5. Quant als Nematodes paràsits, la recerca faunística va proporcionar el primer registre d'*Anisakis pegreffii* infectant *N. couchii*, aquest actuaria mitjançant les interaccions depredador-presa, com a hoste intermediari en les aigües costaneres del Nord-Oest peninsular. A més, aquestes espècies germanes d'*Anisakis* (*A. simplex* i *A. pegrefii*) comparteixen el mateix hoste intermediari en una distribució simpàtrica. El misidaci infectat indica que *Anisakis* spp. és generalista a nivell de mesozooplàncton i que és capaç d'utilitzar diferents hostes per a creuar hàbitats (bentònic-pelàgic) ampliant les seues vies de reclutament amb la finalitat de trobar el seu hoste definitiu (mamífer marí).

1.6. Aquesta tesi ha proporcionat el primer registre del trematode *Opechona bacillaris* sensu stricto en el Sifonòfor *Muggiaea* sp. En les aigües costaneres del NE Atlàntic (NO Península Ibèrica). *Muggiaea* sp. probablement actue, mitjançant interaccions depredador-presa com a segon hoste intermediari.

2. La recerca ecològica en el reclutament de paràsits a nivell mesozooplanctònic realitzat en les aigües temperades del NE Atlàntic ha

proporcionat importants troballes sobre les dimensions dels nínxols dels sistemes de simbionts identificats. El més rellevant és el baix grau de saturació dels molts nínxols potencialment disponibles per als paràsits en les comunitats de zooplancton. A més:

2.1. Els paràsits van mostrar diferents graus d'especificitat pels hostes: des dels que infecten un sol taxó hoste fins als generalistes amb una àmplia gamma d'hostes entre les comunitats de mesozooplàncton. D'altra banda, en els primers (els que mostren especificitat) també mostraren una marcada preferència per determinats microhàbitats sobre o en el cos de l'hoste. L'especificitat a nivell mesozooplanctònic representa una solució de compromís entre els mecanismes que garanteixen la dispersió (ampliació del rang d'hostes) i l'agregació (distribució en l'espai).

2.2. Diferents condicions oceanogràfiques (macrohàbitat) surgència-enfonsament influencien el reclutament (colonització) de larves infectives. En els sistemes de surgència les faunes de paràsits amb cicles de vida complexos amb múltiples hostes intermediaris s'empobreixen mentre que, els esdeveniments d'enfonsament amb masses d'aigua més estables propicien les condicions òptimes per a l'èxit del reclutament parasitari. La hipòtesi que l'estabilitat de les masses d'aigua augmenta el reclutament parasitari va ser proposada en una escala global de Pascual et al. (2007). Els resultats obtinguts en aquesta tesi suggereixen i recolzen un efecte similar, on els processos oceanogràfics estables (enfonsament) afavoreixen la colonització dels paràsits al mesozooplàncton a escala local a la Ria de Vigo.

3. Encara que només una petita proporció dels nínxols estan plens (parasitològicament parlant), les comunitats mesozooplanctòniques juguen un paper crucial en el cicle de vida tant de macroparàsits com microparàsits tròficament transmesos amb el conegut problema de salut pública i econòmic que açò comporta. Aquests resultats reforcen el prometedor futur de la vigilància i control de paràsits en el zooplàncton en els estudis d'avaluació de riscos integrats en la pesca, l'aqüicultura i la gestió de la seguretat alimentària dels productes pesquers dins del Marc Nacional i de l'Horitzó 2020.

Resumen General

RESUMEN GENERAL

Los parásitos marinos y los epibiontes, son importantes como componentes de los ecosistemas marinos, pero muy a menudo son descuidados a nivel mesozooplanctónico en el reino pelágico. Esto se debe principalmente a la dificultad que conlleva su búsqueda en los pequeños animales que lo componen en un reino tridimensional y muy diluido. Su estudio implica gran cantidad de recursos materiales y personales, así como el desarrollo de un trabajo multidisciplinar en el que los taxonomistas, genéticos, los estadísticos y oceanógrafos trabajen de forma conjunta.

La parasitología estudia una amplia gama de relaciones simbióticas y es por ello que dependiendo del investigador se pueden encontrar diversas interpretaciones de este concepto. Desde un punto de vista ecológico el parasitismo se entiende como una asociación estrecha entre dos organismos, uno de los cuales (el parásito) depende de la otro (el hospedador). La ecología parasitaria (ecoparasitología) se basa en los mismos principios que la Ecología de organismos de vida libre, pero presenta una mayor complejidad ya que el propio sistema hospedador-parásito está sometido a dos presiones de selección. Por un lado el microambiente, que sería el interior del cuerpo del hospedador y el macroambiente (el medio ambiente externo al sistema) que afectaría a ambas especies (directa e indirectamente). Las relaciones simbióticas son tan estrechas y frágiles que cualquier cambio tanto en el microambiente como en el macroambiente puede desembocar en un tipo de relación u otro. Es por ello que en ecoparasitología tenemos que tener en cuenta tanto la ecología del hospedador que a su vez es el hábitat del parásito, como el ciclo de vida del parásito. Dependiendo del parásito en cuestión diversas etapas de su ciclo vital se dan en diferentes hábitats (hospedadores) por lo que su estudio se complica un poco más. Por su propia naturaleza, tanto parásitos como epibiontes marinos son esenciales en el ecosistema, ya que son capaces de afectar de diferentes formas a las poblaciones y comunidades hospedadoras que a su vez son su propio ecosistema.

En los sistemas epibionte-basibionte, donde el epibionte es el organismo de vida sésil que vive sobre el substrato vivo, basibionte se han descrito ventajas y

desventajas que afectan a ambos componentes del sistema. En este sentido, tanto los parásitos como los epibiontes marinos causan una amplia variedad de efectos sobre sus anfitriones, por ejemplo, afectan a la conducta del hospedador, disminuyen su condición corporal, reducen su fecundidad e incluso pueden causar la muerte o incrementar su mortalidad. Recíprocamente, el hospedador o basibionte puede causar la muerte del parásito o epibionte, disminuir su reclutamiento, etc.

En general, la fauna de parásitos es más diversa en los ecosistemas marinos especialmente en los peces y los depredadores superiores (top predators). Esto es debido principalmente a que las redes tróficas son más extensas en el medio marino donde un gran número de hospedadores son utilizados para la transmisión de parásitos. Esto se considera como una característica en medio marino, donde los parásitos con ciclos de vida complejos son capaces de utilizar hospedadores paraténicos para explotar nuevas vías de infección a través de las redes tróficas. Además, los parásitos tienen la capacidad de "transformar" a sus hospedadores en bioacumuladores en distintos niveles tróficos, zooplancton y peces que luego transmiten paquetes de parásitos a los hospedadores definitivos. En este inmenso macroambiente los parásitos y los epibiontes muestran una baja especificidad hacia los hospedadores invertebrados o vertebrados que actúan como intermediarios o paraténicos. Esta baja especificidad es resultado de la adaptación a un medio extremadamente diluido donde encontrar al hospedador definitivo puede ser complicado y extremadamente difícil.

El zooplancton en el medio ambiente pelágico marino posee una alta diversidad tanto de especies como de los grupos taxonómicos superiores que son capaces de conectar la red trófica microbiana con el resto de la cadena trófica marina al ser capaces de alimentarse de microzooplancton y parásitos. En este sentido, los sistemas de afloramiento costero mantienen una alta productividad que se transmite a través de la cadena trófica. Mientras que los océanos en general son desiertos de vida, los sistemas de afloramiento son hervideros de vida donde las comunidades mesozooplanctónicas desempeñan un papel crucial en la transmisión de parásitos a través de la cadena trófica. Las Comunidades mesozooplanctónicas a pesar de vivir en un ambiente en constante cambio,

espacial y temporal son capaces de mantenerse estables. En este advectivo macroambiente, los parásitos que utilizan el zooplancton como hospedadores intermediarios han tenido que adaptarse a estas variaciones. Algunas de estas adaptaciones son su baja especificidad hacia el hospedador zooplanctónico intermediario, su transmisión a través de las relaciones de depredador-presa, y tener diferentes estadios de desarrollo en cada uno de esos intermediarios mientras llega hasta su hospedador definitivo. Por lo tanto, en este hábitat extremadamente parcheado, diluido y en tridimensional, los parásitos han desarrollado ciclos de vida complejos, que han implicado cambios ontogenéticos que, a su vez, han dado lugar a metamorfosis y transferencia de hábitats y nichos. Muchos Platelmintos parásitos pueden sufrir más de una metamorfosis y varias mudas a lo largo de un solo ciclo de vida. Este ciclo de vida puede incluir tanto estadios de vida libre como parasitarios, donde se producen dos o más fases parasitarias en invertebrados y vertebrados. Estos patrones en la historia de vida permiten a los parásitos aumentar su probabilidad de ser ingerido por el hospedador final adecuado al mismo tiempo que maximiza la duración y la disponibilidad del conjunto infeccioso dentro del zooplancton. Todas estas estrategias en conjunto permiten que las larvas infectivas se canalicen hacia los huéspedes definitivos, lo que incrementa la probabilidad de completar su ciclo vital. Aunque la transmisión de parásitos a través de la cadena trófica es la más estudiada no es la única. Ejemplos de parásitos que se transmiten de forma activa, nadando para buscar al hospedador adecuado penetrando activamente en él, incluye oncomiracidias de monogeneos, miracidios y cercarias de trematodos, copepoditos de copépodos parásitos etc.

Las tasas de infección (prevalencia) en zooplancton marino con parásitos helmintos son extremadamente bajos. Los copépodos a pesar de ser los animales más abundantes exhiben unas tasas de parasitismo, por lo general, de menos de un animal infectado por cada 1.000, aunque pueden ser aun mucho más bajas. En eufausiáceos por ejemplo esta tasa se reduce todavía más, sólo un animal infectado por cada 100.000. Quetognatos y Ctenóforos son los animales más fuertemente infectados ya que cada individuo puede llevar a varios parásitos dentro. Esta sin duda es otra de las características de los parásitos en el zooplancton.

Por lo que respecta a los protozoos epibiontes marinos la superficie de los cuerpos calcificados de los crustáceos decápodos parece ser un hábitat adecuado. Así, muchos protozoos ciliados se encuentran a menudo como epibiontes sobre crustáceos decápodos marinos. Esta asociación simbiótica se compone de diferentes organismos como protistas, algas, bacterias, hidrozoos, percebes, rotíferos, etc. Los epibiontes por tanto, son aquellos que durante su estadio de vida sésil se fijan a un sustrato vivo. Por el contrario el sustrato vivo es conocido como basibionte. Este tipo de relación, en los sistemas marinos, aún sigue siendo poco conocida sobre todo en lo relacionado con las consecuencias, ventajas y desventajas que tiene para ambos componentes de la relación. Al igual que en el parasitismo cualquier organismo marino es susceptible de ser "epifitado" o parasitado.

Desde un punto de vista evolutivo la especificidad entre epibiontes y basibiontes crustáceos implica una amplia gama de adaptaciones morfológicas y fisiológicas. Así el estudio ecológico de esta relación tiene las mismas connotaciones que la ecoparasitología ya que la relación epibionte-basibionte en sí misma posee funciones ecológicas y es afectada doblemente tanto por el macro como por el microambiente.

Esta tesis tiene como objetivo general mejorar el conocimiento sobre los parásitos y los epibiontes marinos a nivel mesozooplanctónico en el afloramiento estacional en el NE Océano Atlántico a través de muestreos nocturnos realizados en el verano y en el otoño de 2008. En este sentido pretendemos proporcionar datos valiosos sobre la transmisión de parásitos en este área geográfica, describir el papel del mesozooplancton en las relaciones epibionte-basibionte y hospedador-parásito. Aportar datos descriptivos de la población y comunidades mesozooplanctonicas, así como de las infracomunidades parasitarias. Describir la distribución espacial y especificidad de epibiontes entre basibiontes y hospedador-parásito proporcionando datos descriptivos y ciclos de vida. Hemos pretendido presentar secuencias de ADN útiles y novedosas de las larvas de parásitos y epibiontes encontrados. Por último, se han evaluado los conocimientos adquiridos para intentar comprender mejor la influencia de la oceanografía sobre el reclutamiento de macroparásitos. En este sentido, el afloramiento estacional que

se desarrolla en el NE de la península Ibérica crea un ambiente advectivo donde el mesozooplancton se enfrenta a un transporte hacia el oceáno que es forzado por el propio sistema. A pesar de ello el mesozooplancton tiene la capacidad de cambiar su posición verticalmente para evitar ser exportado a mar abierto, por lo que las comunidades mesozooplanctonicas son capaces de mantener su integridad durante toda la temporada de afloramiento.

Como objetivos específicos porporcionamos:

Identificación de larvas de parásitos de acantocéfalos, nematodos y trematodos usando taxonomía clasica y métodos moleculares.

Identificación de parásitos protozoos y nuevas especies de hospedadores.

Descriptores de población de parásitos a nivel mesozooplanctonico.

El ciclo de vida del acantocéfalo *Bolbosoma balaenae*.

Evaluación de la contratación parásitos influenciada por diferentes escenarios oceanográficos.

Descripción de un nuevo sistema epibionte-basibionte Suctoria-Copepoda proporcionando una nueva especie de epibionte Suctoria para la ciencia.

Datos ecológicos sobre la especificidad de parásitos y epibiontes.

Y datos parsito-demograficos a nivel infrapoblación

Por todo ello, este trabajo ha sido realizado desde un punto de vista multidisciplinario, donde taxonomistas, genetistas, oceanógrafos y estadísticos han contribuido a hacerlo posible. Se han aplicado diferentes metodologías acopladas entre sí, como por ejemplo la taxonomía clásica y la genética moderna para la identificación de las especies, y también modelos lineales generalizados (GLM) junto con la oceanografía biológica convencional. Además el trabajo se enmarca dentro de una de las más notables hipótesis propuesta por Pascual et al. (2007). Esta sugiere que el reclutamiento de los parásitos a las comunidades hospedadoras

podría estar condicionada directamente por la oceanografía. Así, las condiciones de mayor estabilidad en las masas de agua mejorarían el reclutamiento de parásitos, especialmente para los parásitos heteroxenos con ciclos de vida complejos y diversos hospedadores. Observaron a escala global que los sistemas de afloramiento la parasito fauna se empobrece mientras que los eventos hundimiento propician unas condiciones óptimas para el reclutamiento exitoso utilizando como ejemplo *Anisakis* spp. Por primera vez en este estudio se pudieron identificar diferentes sistemas epibionte-basibionte y hospedador-parásito en muestras mesozooplancton en el afloramiento estacional en el noreste del Océano Atlántico relacionados con los eventos oceanográficos locales.

De los conocimientos adquiridos en el capítulo 3 destaca el hallazgo de una nueva relación epibionte Suctoria-Copepoda donde una nueva especie de Suctorian ha sido identificada como *Pelagacineta hebensis* que se encontró anclada a la superficie del copépodo *Paraeuchaeta hebes*. Además, se analizó la abundancia y distribución de los epibiontes en la superficie de los copépodos, teniendo en cuenta el sexo del crustáceo, revelando cierta preferencia por las hembras y cierta preferencia por el punto de unión que resultó ser diferente entre ambos sexos. Adicionalmente, las secuencias de ADN obtenidas por primera vez para este epibionte contribuyeron a ampliar el conocimiento en la filogenia de Phyllopharingea que sigue siendo bastante confunsa.

En la segunda parte del capítulo, (3.II) el protozoo *Ellobiopsis chattoni* se encontró penetrando la superficie del cuerpo de diferentes especies de copépodos: *Calanus helgolandicus*, *Calanoides carinatus*, *Centropages chierchiae*, *Acartia clausii*, y *Temora longicornis*. Entre ellos *P. hebes* fue el único que no se encontró parasitado por este protozoo. Hasta donde sabemos esta es la primera vez que *Ellobiopsis chattoni* se ha encontrado en *Calanoides carinatus*, *Centropages chierchiae* y *Metridia lucens* en aguas del Atlántico noreste extendiendo el rango de hospedadores de este parásito. Las prevalencias como cabía esperar fueron bajas. Los niveles de infección mostraron ser homogéneo entre las comunidades estudiadas. Cierta especificidad fue detectada ya que, *C. helgolandicus* mantuvo la prevalencia más alta. Las hembras fueron la fracción de la población que más se

vio afectada por *Ellobiopsis*. Finalmente, los puntos de anclaje preferidos de *E. chattoni* fueron los apéndices de la bucales.

Sobre la base de los resultados obtenidos en el capítulo 4 los cistacantos del acantocéfalo *Bolbosoma balaenae* (Gmelin, 1790) y *Rhadinorhunchus* sp. fueron encontrados encapsulados en el cefalotórax del eufáusido *Nyctiphanes couchii* (Bell, 1853) en el área de estudio. Sus prevalencias fueron muy bajas y las intensidades fueron de 1. Se utilizaron análisis morfológicos y filogenéticos para en primer lugar identificar los animales a nivel de especie y en segundo lugar para situarlos filogenéticamente dentro de Polymorphidae. Las secuencias de ADN obtenidas, por primera vez para estos cistacantos se depositaron en su correspondiente base de datos. Finalmente, la filiación taxonómica de los parásitos y la ecología trófica de la zona de muestreo sugirieron que *N. couchii* es el hospedador intermediario para *B. balenae* y *Rhadinorhunchus* sp. Por otro lado, el ciclo de vida de *B. Balaenae* se propuso asumiendo que *Balaenoptera physalus* (Linnaeus, 1758) y *B. acutorostrata* (Lacepède, 1804) son sus hospedadores definitivos. Por último, las diferentes prevalencias encontradas en la infrapoblación de *Rhadinorhynchus* sp. a través de las comunidades de mesozooplancton tanto en verano como en otoño, revelaron que el reclutamiento de estos parásitos se ve afectado por las diferentes condiciones oceanográficas.

Como resultado de los conocimientos adquiridos en el capítulo 5 se diagnosticó al eufausiáceo *N. couchii* y a un misidaceo como hospedadores intermediarios de las larvas (L_3) del complejo *Anisakis simplex* en la comunidad de mesozooplancton estudiada. Las larvas fueron identificadas molecularmente utilizando el espaciador interno transcrito (ITS). Por primera vez se identificó *Anisakis pegreffii* como parásito del krill. La existencia de larvas L_3 de Anisakidos en diversos organismos del mesozooplancton sugirió que las rutas de transmisión de *A. simplex* y *A. pegreffii* son más amplias de lo que se esperaba. Por otra parte, los resultados demostraron que estas dos especies de Anisakidos no son específicos para sus hospedadores intermediarios. Por último, nuestros resultados arrojan luz sobre la influencia de la oceanografía en el reclutamiento del complejo *A. simplex*, siendo diferente bajo condiciones afloramiento o hundimiento.

En la segunda parte de este capítulo (5.II) se detectó un nuevo segundo hospedador intermediario, *Muggiaea* sp. para metacercarias de *Opechona bacillaris* (Trematoda). *O. bacillaris* es uno de los endoparásitos más importantes de peces con ciclos de vida complejos que utiliza una amplia gama de animales gelatinosos como medusas, ctenóforos y quetognatos como hospedadores intermediarios secundarios. Su transmisión a los peces se produce a través de interacciones depredador-presa, donde los peces ingieren medusas, siendo este un fenómeno generalizado. Esta es la primera aproximación realizada para entender la importancia de las condiciones oceanográficas en el reclutamiento de estos parásitos en las comunidades de mesozooplancton. Las prevalencias encontradas a través de las comunidades están de acuerdo con el carácter costero y estuarino de la infección registrada en trematodos. Finalmente, la variación de la prevalencia se acopla con las diferentes condiciones oceanográficas y el comportamiento de los sifonóforos.

A raíz de los hallazgos encontrados en el capítulo 6 podemos afirmar que mucho trabajo queda aun por realizar en cuanto a identificación de sistemas hospedador-parásito y epibionte-basibionte en las comunidades de Mesozooplankton. En este capítulo se abordó el estudio de diversos sistemas pertenecientes a zonas con diferentes condiciones oceanográficas dentro de los sistemas de afloramiento. Parasito fauna procedente de sistemas de afloramiento estacional como el del Noroeste de la península Ibérica y la parasito fauna procedente del sistema de afloramiento perenne africano.

Como análisis final esta tesis va más allá revelando que existen importantes interacciones entre los parásitos y las enfermedades marinas con la salud del medio ambiente y de los seres humanos. En el contexto del cambio climático varios estresores de origen antropogénico pueden exacerbar sus efectos debido al aumento de la temperatura, que directa o indirectamente, afectará a las actividades recreativas económicas y a la salud humana. Esta tesis ha ayudado a detectar que las diferentes condiciones oceanográficas afectan directamente al reclutamiento de los parásitos en la base de la cadena alimenticia (mesozooplancton). Por lo tanto, en situaciones de estabilidad de las masas de agua y de baja intensidad de afloramiento la colonización de los parásitos en las

redes alimentarias tenderá a aumentar. Este aumento de la carga parasitaria en este sensible eslabón puede tener consecuencias devastadoras, ya que sus impactos se pueden transmitir a toda la comunidad y el ecosistema en un efecto cascada (arriba - abajo). Este efecto ha sido ampliamente demostrado en multitud de trabajos destacando a los parásitos y los epibiontes como reguladores de las poblaciones hospedadoras influyendo en la composición, estructura y función de las comunidades biológicas.

Existe bastante evidencia que sugiere que los parásitos y la transmisión de enfermedades, junto con la virulencia aumentará con el calentamiento global. Altas intensidades de parásitos (desde microparásitos a macroparásitos) pueden afectar a los mercados, donde las especies fuertemente parasitadas son rechazadas por los consumidores, con la consiguiente pérdida de confianza. También puede afectar a las autoridades sanitarias ya que, tienen que retirar partidas completas del mercado con el consiguiente costo económico. Por otro lado, si el producto afectado llega al consumidor los costes sanitarios aumentan ya que se han de combatir enfermedades o alergias procedentes de partidas contaminadas.

La introducción de especies exóticas o la propagación de especies tropicales hacia los sistemas de clima templado pueden traer nuevos parásitos y agentes patógenos para los cuales las poblaciones nativas no están preparadas, provocando la entrada de nuevas enfermedades y brotes episódicos. La pérdida de sincronía en las poblaciones de depredadores y presas debido al aumento de temperatura puede causar la interrupción de los ciclos de vida del parásito que llevaría a una reducción de la abundancia de parásitos, por un lado obligándolos a buscar nuevas estrategias para sobrevivir, como la colonización de especies que no están preparadas para la invasión, y por otro lado por la alteración que la pérdida de un componente regulador de la población tiene sobre todo el ecosistema. Con ésta pérdida de componentes en las poblaciones las estrategias evolutivas se ven involucradas, ya que la eliminación sistemática de los parásitos adultos causa sesgos en sus poblaciones, adelantando la madurez y reproducción de juveniles y la propagación de las larvas.

El estrés causado por el aumento de la temperatura es otro efecto que se suma a los causados por parasitismo o la enfermedad, ya que puede aumentar la virulencia de los patógenos o parásitos, e incluso favorecer su transmisión. Así, los animales parasitados mueren más fácilmente que los no infectados. Además, la susceptibilidad del hospedador frente a la adquisición de enfermedades o parásitos aumenta haciéndolos más vulnerables. Cambios en el comportamiento del hospedador, como por ejemplo mayores tasas de ingestión de una determinada presa clave en la transmisión, podrían acelerar la adquisición de parásitos o enfermedades. Temperaturas elevadas más cálidas provocan también la estratificación de la columna de agua reduciendo el caudal y el volumen de agua. En este contexto, la concentración de oxígeno por debajo de la termoclina se reduce. La hipoxia junto con las altas temperaturas de la superficie fuerzan a los peces a concentrarse (en altas densidades) en bandas más estrechas de las condiciones tolerables donde la transmisión y los brotes de enfermedades se transmiten con mucha más facilidad. A parte de esta facilidad de transmisión, susceptibilidad por parte del hospedador, etc. la migración vertical del mesozooplancton y de organismos bento-pelágicos se ve afectada, por lo que la conexión entre diferentes vías de infección se ven cortadas.

Los cambios biológicos y ambientales que aquí se resumen tendrán consecuencias económicas para la subsistencia de la pesca (comercial y deportiva), junto con las actividades humanas asociadas. Por otra parte, también se repercutirá en la salud pública ya que, algunas enfermedades e infecciones procedentes de pescado contaminado puede ser transmitida a los seres humanos . No sólo estas enfermedades pueden ser transmitidas a los humanos por los peces, moluscos, etc. también pueden ser transmitidas por el agua donde nos bañamos o la que consumimos así se describe Anisakiasis, picazón del nadador, la esquistosomiasis, las enfermedades diarreicas, criptosporidiosis, giardiasis, etc. Estos brotes de enfermedades son extremadamente caros para la pesca, la acuicultura, las industrias relacionadas y la salud pública.

Aunque nuestra capacidad para detectar los vínculos entre la variabilidad climática y los brotes de enfermedades infecciosas marinas ha mejorado, los efectos sobre la mayoría de las interacciones hospedador-patógeno son todavía

poco conocidas. En peces, las enfermedades y el parasitismo se han estudiado enormemente, así como los agentes causantes. Sin embargo, la dinámica de transmisión sigue siendo un poco desconocida en el entorno salvaje. En este sentido, la comunidad mesozooplanctónica juega un papel importante, ya que sus componentes sirven como huéspedes intermediarios de los parásitos con ciclos de vida complejos que son capaces de explotar las vías de la red alimentaria. Sus componentes también son el objetivo (hospedadores) tanto para microparásitos (protozoos) como macroparásitos (p. e. gusanos helmintos) y también de los epibiontes. Cada uno con su respectivo papel en la población, la comunidad y ecosistema. Tanto la comunidad mesozooplanctónica como la parasitaria se consideran buenos indicadores del cambio climático en el medio marino por diversas razones por lo que su estudio y seguimiento conseguiría aumentar nuestro conocimiento en aspectos básicos para el diseño de estrategias que permitan valorar de forma efectiva los riesgos de determinados cambios ambientales, permitiéndonos adoptar las medidas paliativas pertinentes.

En esta tesis se ha demostrado que efectivamente el mesozooplancton es clave en la transmisión de parásitos como acantocéfalos, nematodos, trematodos, protozoos… en el NE Atlántico. Diferentes escenarios oceanográficos se han relacionado con el reclutamiento parasitario. Además técnicas moleculares han sido aplicadas conjuntamente con la taxonomía clásica demostrando ser herramientas útiles en la detección de larvas de parásitas. Esta tesis también ha contribuido a destacar la importancia del mesozooplancton en la diversidad composición y supervivencia de epibiontes, algunos de los cuales han sido descritos e identificados por primera vez.

Por lo tanto, se reafirma que el trabajo multidisciplinar es necesario para hacer frente a esta enorme tarea. Es evidente que hay un riesgo significativo para los ecosistemas, la economía y la salud humana, que son conducidos por el cambio climático que actúa sinérgicamente con otros factores de estrés ambientales. Por lo tanto, se deben tomar medidas de gestión para eliminar o minimizar estos riesgos, lo que garantiza que se alcanzan en conjunto los beneficios económicos, sociales y ecológicos para muchas actividades marinas.

Está claro que con el crecimiento esperado de la población humana, la presión sobre los ecosistemas marinos aumente (acuicultura, el turismo, la pesca, etc.). En el futuro, cualquier política racional para hacer frente a la pérdida de biodiversidad, brotes de enfermedades de origen marino, etc. deben centrarse primero en la prevención, aunque seguidas de fuertes medidas de gestión para eliminar, o al menos reducir cualquier daño ecológico que pudiera suceder. Estas medidas deben ser rentables e integrar los beneficios de la protección del medio a los beneficios económicos. Por ello, se necesitan programas de seguimiento para mejorar la comprensión, la alerta temprana y el tratamiento de enfermedades y parasitosis en los sistemas marinos bajo los efectos del cambio climático.

Para estas tareas, se requiere el desarrollo de métodos innovadores que permitan una detección rápida de vectores y hospedadores intermediarios en el mesozooplancton cuando se analizan grandes cantidades de invertebrados como los representados en el mesozooplancton. Estos métodos deben ser precisos, fiables y efectivos lo que permitirá a su vez hacer un diagnóstico del estado del arte. En este sentido la aplicación de técnicas inmunológicas o moleculares, tales como ELISA o NGS (secuenciación de nueva generación) podría producir muy rápidamente una gran cantidad de información cuantitativa y cualitativa.

Toda esta información ayudaría a diseñar estrategias de gestión más eficaces para mitigar el impacto de las enfermedades y parasitismo desarrollando estrategias específicas y adaptativas frente a enfermedades. Estas estrategias deben basarse en conocimientos científicos sólidos, que incluyan el monitoreo y la supervisión a largo plazo de los efectos del cambio climático y de los organismos afectados. En este sentido, el trabajo experimental cobra un papel fundamental para poner a prueba los efectos de los factores de estrés climáticos en las interacciones hospedador-patógeno. Con todo ello, buenas herramientas de apoyo, asesoramiento e información son imprescindibles.

Con este trabajo se han logrado identificar muchas cuestiones básicas en el conocimiento parasitario en el mesozooplancton en el medio pelágico, que son necesarias responder para poder hacer frente a los desafíos planteados por el cambio climático. La identificación de los sistemas hospedador-parásito es fundamental para saber qué hay, dónde y cuánto. Estudios de dinámica de las poblaciones parasitarias, las comunidades, georeferenciación oceanográfica, las

vías de transmisión, ecología de la transmisión, cambios en el reclutamiento causados por cambios ambientales, etc. están pendientes de ser resueltos. Su resolución progresiva ayudará a mejorar la evaluación de riesgos.

Tras el informe presentado por las Naciones Unidas Rio +20 y en el 2020 7PM_Horizon algunas estrategias podrían adoptarse de forma inmediata para reducir el riesgo de enfermedades relacionadas con el cambio climático y el declive de la biodiversidad en el medio marino con el fin de garantizar el suministro y la sostenibilidad de la alimentación saludable para las crecientes poblaciones humanas.

Algunas de estas medidas podrían ser:

- Integración de las consecuencias relacionadas con el clima en la gestión basada en los ecosistemas o el diseño de AMP, así como en la ordenación pesquera. Los sistemas parásito-hospedador deben ser incluidos también para una correcta práctica de estas herramientas de gestión.
- Mejorar la gestión de las aguas residuales. Algunos factores estresantes que podrían mejorarse con esta medida son la contaminación costera, la pérdida de hábitat (eutrofización) y la llegada de los agentes patógenos humanos evitando muchas alergias y enfermedades de la piel que están asociados.
- Evitar la sobreexplotación de los recursos reduciendo la pérdida de biodiversidad y variabilidad genética necesaria para la supervivencia en un mundo cambiante.
- Identificar los factores que proporcionan resistencia a las enfermedades inmunológicas.
- Compartir las mejores prácticas y los conocimientos adquiridos.

Algunas medidas, como la no eliminación de los descartes en alta mar (UE la política pesquera de 2014 (CE) n º 2371/2002) ya se están aplicando en el sector pesquero. Con ello se pretende garantizar la salud animal y el medio ambiente marino, y por lo tanto la restauración de la confianza del consumidor, asegurando buenas prácticas, así como la calidad de los alimentos.

En 7PM_Horizon 2020 varios objetivos han sido también delineados de cara a garantizar la sostenibilidad, la explotación y gestión de los recursos acuáticos

vivos que permitan maximizar los beneficios sociales y económicos de los océanos y los mares de Europa. En este sentido, propusieron optimizar la contribución de la pesca sostenible, marco en el que se incluye el proyecto PARASITE. Esta herramienta de gestión contribuirá a la mejora de los mariscos, fortalecimiento de la competitividad de la industria europea del mar, y la mejora de la política europea de seguridad alimentaria. Además, este proyecto ha incluido el desarrollo, el diseño y la implementación del BIOBANCO una herramienta cuyo objetivo es poner al servicio muestras de parásitos obtenidos de los productos pesqueros a la comunidad científica. En este mismo sentido, la red de PARNET se presenta como un servicio en la vigilancia tecnológica para la gestión de la cartera de I + D + I en parasitosis en los recursos marinos explotados renovables.

En su conjunto, un estudio completo de los parásitos a través de su ciclo de vida y su ubicación en los ecosistemas oceánicos está limitado por intereses económicos y políticos por un lado y por el costoso de tiempo de inspección e identificación de los estadios larvarios dentro de cada una de las especies que componen el mesozooplancton. Por lo tanto , queda mucho trabajo científico por hacer. En este sentido trabajos orientados a desvelar cómo funciona la retención de las larvas parasitarias en un ambiente advectivo como el afloramiento estacional que ocurre en aguas gallegas (NE Océano Atlántico) es necesario. Los resultados de esta tesis sugieren y apuntan, en una escala geográfica local como la Ría de Vigo y plataforma adyacente, que las diferentes condiciones oceanográficas determinan el reclutamiento de los parásitos en la base de la cadena alimenticia (mesozooplancton) siguiendo el patrón descrito en (Pascual et al. 2007). Si, según lo predicho por el cambio climático, la estabilidad y estratificación de las masas de agua aumenta y la intensidad del afloramiento disminuye, cabe esperar un aumento de las larvas de parásitos en los hospedadores intermediarios con consecuencias devastadoras que pueden ser transmitidas a través de todo el ecosistema, como por ejemplo el colapso de los recursos pesqueros debido a la falta de suministro de alimento, muerte masiva por parasitosis del recurso pesquero, propagación de enfermedades, etc. Siendo extensible a todas las actividades relacionadas como acuicultura, turismo, etc.

Llegados a este punto tenemos que tener en cuenta que las especies de parásitos tienen una amplia gama de estilos de vida y estrategias, así como una amplia variedad de diferentes hospedadores intermediarios y definitivos. Por lo que impactan en los ecosistemas de forma distinta, por ello su estudio debe estar enfocado, diseñado, desarrollado y abordado específicamente para ese parásito en toda su extensión. Pero para ello y como se mencionó anteriormente, es esencial el desarrollo de métodos nos permitan detectar y contabilizar tantas formas como sea posible en un corto tiempo para abordar cada una de las estrategias necesarias para su estudio

Esta tesis presenta la primera aproximación a todas estas preguntas, donde los resultados se hacen evidentes a la luz de las numerosas hipótesis que la consecución del proyecto de tesis ha planteado, sobre todo en relación con dos aspectos de gran relevancia científica y socio-económica:

- El conocimiento acerca de los modos de colonización de las formas infectantes parasitarias a la cadena alimenticia en los ecosistemas marinos, especialmente en las primeras etapas de desarrollo en las comunidades mesozooplancton.
- Y la existencia de la relación que existe entre la oceanografía de las masas de agua y el reclutamiento de los parásitos zoonóticos o características patogénicas, que encajan en el reto social de Horizonte 2020 "Cambio Climático y Seguridad Alimentaria".

Algunas de las conclusiones más relevantes de esta tesis se engloban en dos grandes apartados, por un lado el estudio faunístico y por otro el estudio ecológico. Así esta tesis ha aportado:

1. La extensiva investigación faunística de las comunidades mesozooplanctónicas de las aguas del NE Atlántico, ha proporcionado una fuerte evidencia del establecimiento de cerca de unos 17 sistemas simbiontes que representan un grupo de alta la diversidad de parásitos y epibiontes con bajas prevalencias en poblaciones de mesozooplancton. Los sistemas hospedador-epibionte/parásito identificados comprenden:

1.1. Una nueva especie epibionte ***Pelagacineta hebensis* sp. n.**, que se encuentra en individuos adultos del copépodo *Paraeuchaeta hebes*. Es la primera vez que este copépodos se registra como basibionte para esta especie de protozoo epibionte. El nuevo epibionte se describe tanto morfológicas como molecularmente, lo que contribuye a ampliar las secuencias genéticas disponibles para la Clase Phyllopharyngea. Por otra parte, también se proporciona el diagnóstico taxonómico de esta nuevo epibionte descubierto.

1.2. Tres nuevas especies de **copépodos hospedadores del protozoo parásito *Ellobiopsis chattoni***. Éste encuentra en formas adultas de los copépodos ***Calanoides carinatus*, *Centropages chierchiae* y *Metridia lucens*** lo que extiende la gama de huéspedes para este parásito. Las prevalencias entre copépodos fueron bajas mostrando diferentes susceptibilidades a la infección de *Ellobiopsis*. Por otra parte, *E. Chattoni* mostró cierta predilección por el hospedador *Calanus helgolandicus*.

1.3. Por primera vez el acantocéfalo ***Bolbosoma balaenae*** se encontró parasitando el eufausiáceo *Nyctiphanes couchii*, que puede desempeñar el papel de hospedador intermediario. Por otra parte, se propuso el ciclo de vida *B. balaenae* asumiendo que *Balaenopter*a *physalus* es el hospedador definitivo. Estos mamíferos marinos serían los responsables del suministro de la infra-población infectiva para los eufausiáceos.

1.4. Por primera vez el acantocéfalo ***Rhadinorhynchus* sp**. se ha identificado infectando *Nyctiphanes couchii*. Es probable que este eufáusido actúe a través de las interacciones depredador-presa como huésped intermediario. Tanto el estudio morfológico como filogenético, junto con la información epidemiológica disponible sobre *R. pristis* en peces de las familias Scombridae y Xiphiidae de las costas de Portugal, las Islas de Madeira y el Océano Atlántico Norte, sugieren que los cistacantos aquí descritos pertenecen probablemente a *R. pristis*. Sin embargo, se encontraron discrepancias entre la filogenia que aquí se presenta y el "estado del

arte". Por lo tanto, se recomienda una revisión exhaustiva tanto de las especies como de la familia Rhadinorhynchidae.

1.5. En cuanto a los Nematodos parásitos, la investigación faunística proporcionó el primer registro de ***Anisakis pegreffii*** infectando *N. couchii*, éste actuaría a través de las interacciones depredador-presa, como hospedador intermediario en las aguas costeras del noroeste peninsular. Además, estas especies hermanas de *Anisakis* (*A. simplex* y *A. pegrefii*) comparten el mismo hospedador intermediario en una distribución simpátrica. El misidáceo infectado indica que *Anisakis* spp. es generalista a nivel de mesozooplancton y que es capaz de utilizar diferentes hospedadores para cruzar hábitats (bentónico-pelágico) ampliando sus vías de reclutamiento con el fin de encontrar su hospedador definitivo (mamífero marino).

1.6. Esta tesis ha proporcionado el primer registro del trematodo *Opechona bacillaris sensu stricto* en el Sifonóforo *Muggiaea* sp. En las aguas costeras del NE Atlántico (NO Península Ibérica). *Muggiaea* sp. probablemente actúe, a través de interacciones depredador-presa como segundo hospedador intermediario.

2. La investigación ecológica en el reclutamiento de parásitos a nivel mesozooplanctónico realizado en las aguas templadas del NE Atlántico ha proporcionado importantes hallazgos sobre las dimensiones de los nichos de los sistemas de simbiontes identificados. El más relevante es el bajo grado de saturación de los muchos nichos potencialmente disponibles para los parásitos en las comunidades de zooplancton. Además:

2.1. Los parásitos mostraron diferentes grados de especificidad por los hospedadores: desde los que infectan un solo taxón hospedador hasta los generalistas con una amplia gama de hospedadores entre las comunidades de mesozooplancton. Por otra parte, en los primeros (los que muestran especificidad) también muestrearon una incluso marcada preferencia por determinados microhábitats sobre o en el cuerpo del hospedador. La especificidad a nivel mesozooplanctónico representa una solución de compromiso

entren los mecanismos que garanticen la dispersión (ampliación del rango de hospedadores) y la agregación (distribución en el espacio).

2.2. Diferentes condiciones oceanográficas (macrohábitat) surgencia-hundimiento influencian el reclutamiento (colonización) de larvas infectivas. En los sistemas de surgencia las faunas de parásitos con ciclos de vida complejos con múltiples hospedadores intermediarios se empobrecen mientras que, los eventos de hundimiento con masas de agua más estables propician las condiciones óptimas para el éxito del reclutamiento parasitario. La hipótesis de que la estabilidad de las masas de agua aumenta el reclutamiento parásito fue propuesta en una escala global de Pascual et al. (2007). Los resultados obtenidos en esta tesis sugieren y apoyan un efecto similar, donde los procesos oceanográficos estables (hundimiento) favorecen la colonización de los parásitos al mesozooplancton a escala local en la Ría de Vigo.

3. Aunque sólo una pequeña proporción de los nichos están llenos (parasitológicamente hablando), las comunidades mesozooplanctónicas juegan un papel crucial en el ciclo de vida tanto de macroparásitos como microparásitos tróficamente transmitidos con el conocido problema de salud pública y económico que ello conlleva. Estos resultados refuerzan el prometedor futuro de la vigilancia y control de parásitos en el zooplancton en los estudios de evaluación de riesgos integrados en la pesca, la acuicultura y la gestión de la seguridad alimentaria de los productos pesqueros dentro de los marcos Nacionales y del Horizonte 2020.

General Abstract

General Abstract

Marine parasites and epibionts as component of the marine ecosystems are important, but very often neglected at the mesozooplankton level in the pelagic realm. This is mainly due to the difficulty in finding them into little components of the mesozooplankton in a three-dimensional and very dilute realm. Their study implies lot of material and personal resources as well as to develop a multidisciplinary work in which taxonomists, genetics, statisticians and oceanographs work together. Both, marine parasites and marine epibionts are essential into the ecosystem because they are able to impact in different ways the host populations and communities, being those hosts their own ecosystem. From their origin due to their very nature, parasites are able to divert the energy in each of the trophic level where they are placed. Thus marine parasites and marine epibionts cause a variety of effects on their hosts, for example influencing behaviour, lower body condition, fecundity reduction and even mortality.

Therefore, this thesis aimed to improve the knowledge about both, marine parasites and epibionts at the mesozooplankton level in the seasonal upwelling of NE Atlantic Ocean through nocturnal surveys undertaken on summer and autumn of 2008.

This work has been made from an interdisciplinary point of view, where taxonomists, genetics and statisticians contributed to make it possible. It has been treated to use different methodologies coupled each other, as for example classical taxonomy and modern genetic for species identification as well as generalized linear model (GLM) and conventional biological oceanography.

Thus, for the first time it could be identified different systems: epibiont-basibiont and host-parasite from mesozooplankton samples.

From the knowledge acquired in chapter 3 highlights the finding of a new epibiont relationship Suctoria-Copepoda where a new species of Suctoria was identified as *Pelagacineta hebensis* which was found attached to the surface of *Paraeuchaeta hebes* copepod. Moreover, the abundance and distribution of the

epibiont on the copepod surface was analysed, taking into account the sex of the crustacean, revealing some preference for females and also a different attachment point in both sexes. Furthermore, the DNA sequences obtained for the first time for this epibiont contributed to enlarge the knowledge of the Phyllopharingea phylogeny that remains quite confuse.

In addition to that, (chapter 3.II) *Ellobiopsis chattoni* was found penetrating the body surface of different copepod species: *Calanus helgolandicus*, *Calanoides carinatus*, *Centropages chierchiae*, *Acartia clausii*, *Paraeuchaeta hebes* and *Temora longicornis*. Among them *P. hebes* always were found free for this parasite whereas in the other studied species were found. Here we would like to emphasize that as far as we know this is the first time that *Ellobiopsis chattoni* has been found on *Calanoides carinatus*, *Centropages chierchiae* and *Metridia lucens* in NE Atlantic waters extending the host range for *E. chattoni*. Prevalences as expected were low. The infection levels showed to be homogeneous among the communities studied. Specificity detection was present since *C. helgolandicus* showed the highest prevalence Females are the population fraction that were more affected by *Ellobiopsis* parasite. Finally, The preferred attachment points of *E. chattoni* were the mouth appendages.

On the basis of the results obtained in chapter 4 Cystacanths of the acanthocephalan *Bolbosoma balaenae* and *Rhadinorhunchus* sp. were found encapsulated in the cephalothorax of the euphausiid *Nyctiphanes couchii* from the same studied area. Their prevalences were very low and the intensities were 1. Morphological and phylogenetic analyses were used to identify species and their position on the Polymorphidae phylogeny respectively. DNA sequences were obtained for the first time for these cystacanths and deposited on their corresponding database. Taxonomic affiliation of parasites and trophic ecology in the sampling area suggested that *N. couchii* is the intermediate host for *B. balenae and Rhadinorhynchus* sp. On the other hand, a life cycle was proposed for *B. balaenae* where *Balaenoptera physalus* and *B. Acutorostrata* are its definitive hosts. Finally, different prevalences of *Rhadinorhynchus* sp. through mesozooplankton communities in summer and autumn revealed that the recruitment of parasites might be affected by the oceanography.

As a result of the knowledge acquired in chapter 5 we diagnosed the euphausiid *N. couchii* and an unidentified mysid as intermediate hosts for third-stage larvae (L_3) of the *Anisakis simplex* complex in the mesozooplanktonic community. Parasite larvae were molecularly identified using the internal transcribed spacer (ITS) region. *Anisakis pegreffii* were identified for the first time infecting this species of krill. The existence of parasites in a variety of mesozooplankton organisms suggested that the transmission routes of *A. simplex* and *A. pegreffii* are wider than expected. Moreover, the results demonstrated that these two *Anisakis* species are not specific for their intermediate hosts. Moreover, our results shed light on the influence of oceanography over the recruitment of *A. simplex* complex, being different under upwelling or downwelling conditions.

From the second part of this chapter (5.II), *Muggiaea* sp. was also identified for the first time as the second intermediate host for metacercariae of *Opechona bacillaris*. As Digenean *O. bacillaris* is one of the most important endoparasites of fish with complex life cycles that uses a wide range of gelationous animals like medusa, ctenophore and chaetognata as secondary intermediate hosts. Their transmission to fish occurs under predator-prey interactions since fish predation on jellyfish is a widespread phenomenon. This is the first approach to understand the importance of the oceanographic conditions in the recruitment of parasites into the mesozooplankton communities. Prevalences found through the communities agreed with the coastal and estuarine character of the infection registered in trematodes. Moreover, the prevalence variation is coupled with both different oceanographic conditions and Siphonophora behaviour.

On the basis of knowledge acquired in Chapter 6 we can say that much work still remains to be done to identify host-parasite and epibiont-basibionte systems in mesozooplankton communities. In this chapter summarized the study of Parasite Fauna from seasonal upwelling systems in the Northwest of the Iberian Peninsula and parasitefauna from the African perennial upwelling system..

Finally, we wanted to go beyond revealing through this thesis that there are important interactions between parasites and marine diseases with the environment and human's health. Within climate change context several

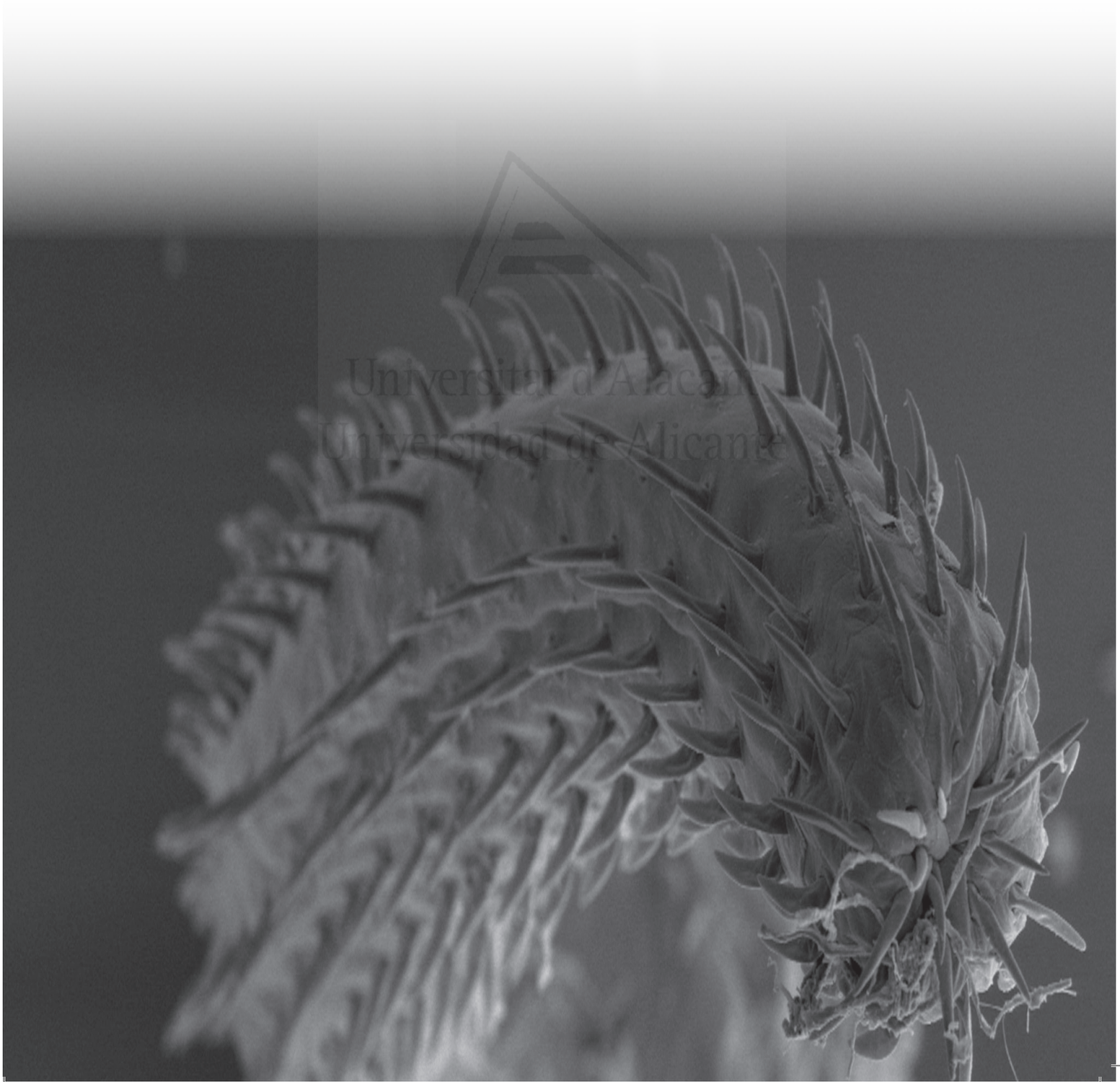
anthropogenic stressors may exacerbate its effects by the temperature raise that directly or indirectly, will affect economic, recreational activities and human health.

This thesis has helped to detect that different oceanographic conditions directly are affecting the recruitment of parasites in the base of the food chain (mesozooplankton). Thus, situations of water stability and low upwelling intensity will tend to increase colonization of parasites in food webs. These parasitic increases into this sensitive link can have devastating consequences since its impacts can be transmitted in a cascade effect (up - down). There is much evidence to suggest that parasite and disease transmission, and possibly virulence, will increase with global warming. High intensities of parasites (from microparasites to macroparasites) may indirectly affect, markets, where consumers reject heavily parasitized species.

In order to preserve environmental quality, the health of exploited natural resources, human health and economic activity resulting from the use of the sea and coastal areas, a better understanding of what happens in the environment is necessary. Hence, should be taken management measures to eliminate or minimize a wide range of risks, which ensures that economic, social, and ecological benefits from fisheries, aquaculture, truism, etc. are jointly achieved.

It is obvious that to take appropriate management measures, it is needed to carry out previous studies which allow us to understand the possible risks we face. This risk assessment will be more accurate and precise thus, the management response may be more easily understood and applied. Nevertheless, to get this understanding is necessary to adopt an interdisciplinary approach, so the involvement of scientists, business and the Government are imperative. Within this approach, knowledge and technology should be transferred and shared. As a consequence the long-term investment is essential.

Introduction



1. INTRODUCTION

1.I. BIOLOGICAL INTERACTIONS

1.I.1. Parasitism

Parasitology studies a wide range of symbiotic relationship. Thus, we can find as many parasitism definitions as existing researchers. From an ecological viewpoint parasitism is understood as a close association between two organisms, one of which (the parasite) depends on the other (the host)

Parasites are able to cause a deviation of some benefit from it with or without damaging the host. In many respects this relationships may change depending on the environmental conditions. For instance, a parasite may become a predator by killing its host, or mutualistic if the host receives some benefit. A commensal becomes parasites when it causes damage to the host. Thus From foresy to predation there is a range of relationship which are classed depending on the trophic linkages (Fig. 1.I.1) and the energy derived between simbionts (Bush et al. 2001). When in a relationship does not exist trophic interaction, this relation is named phoresy for example, when a butterfly obtains nectar from a flower becomes pollinator with pollen dusted from different flowers ensuring fertilization on the subsequent flowers. In commensalism the transfer of energy occurs only in one direction. Apparently, one partner obtains some benefit, deriving energy indirectly, and the other is not damaged or helped. The typical example is formed by the relationship between sharks and remoras. On the other hand when there is a di-

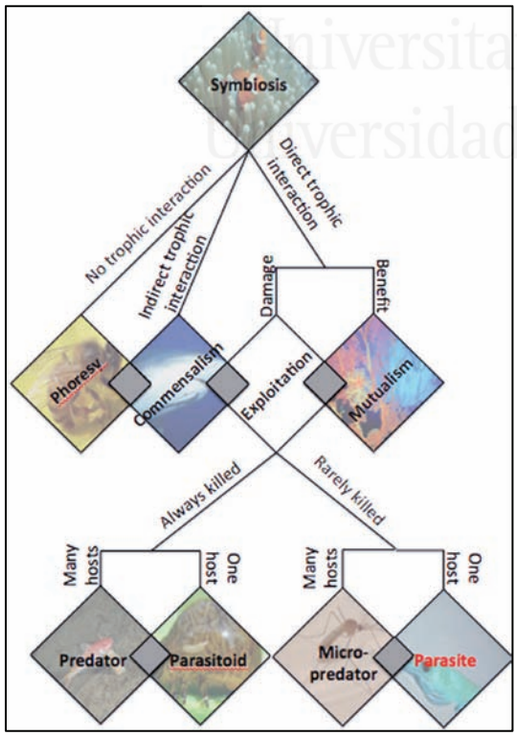


Fig. 1.I.1. Adapted from Bush et al. 2001 (Bush et al. 2001). Schematic vision of the parasitism position into symbiotic relationships.

rect transfer of energy between participants, in a biotic relationship, the interaction may be exploitative or mutualistic (Fig. 1.I.1). Whereas mutualistic relationship implies benefit and survival for both partners, exploitative ones imply a reciprocal benefit or damage. A mutualistic relationship occurs for example with the algae and coral reef-forming cnidarians.

The exploitative ones can be classed depending on the number of hosts attacked and the damage produced on the victim. Thus, while a micropredator is a symbiont that attacks more than one organism but does not kill them (i.e. hematophagous organisms such as mosquitoes) a predator is a symbiont that attack and always kill more than one organism. They become parasitoids if the aggressor only attacks and always kills only one host (female wasp that deposits her eggs into an insect). Larval parasitoid will kill its hosts after consuming it. At he end, if only one host is attacked but generally is not killed, the aggressor becomes a parasite.

The host-parasite system is subjected to two selective pressures, the microenvironment (the host) and macroenvironment or external environment, where the system is given. Thus, the host is capable of responding physiologically and immunologically to the parasite. Every change in the host can cause a dramatic change in their parasite fauna. Moreover, abiotic changes in the external environment can also indirectly affect parasites though the host. It is because the parasitism in itself is an ecological concept. It is because of that we have to consider both the host ecology in a parasite's life cycle and the host as a habitat for the parasite. In general the parasite fauna is more diverse in marine system than in freshwater, especially in fishes and top predators. This is mainly due to those larger food webs that exist in marine environment where a great number of hosts are used for transmission. This is considered as a feature in marine environment where parasites with complex life cycles are able to use paratenic hosts exploiting new food web pathways (Marcogliese 2002). In addition, parasites are able to accumulate extra trophic levels formed by large invertebrate (zooplankton) and fish predators which then transmit packets of parasites to definitive hosts (Marcogliese 2002, 2005a). Moreover, a low specificity for the invertebrate/vertebrate intermediate/paratenic hosts is also a common trait of marine parasites as a result of the adaptation to the dilution of this realm. In spite to that, how fishes or top predators are able to recruit large numbers of parasites

remains unknown, taking into account that prevalences among the invertebrate intermediate host are extremely low.

The parasitism study, whether from an ecological or physiological point of view, is an interesting exploration of these fascinating organisms and become even more intriguing when nothing is known about what are the best oceanographic conditions to promote their recruitment.

1.I.1.1. Kind of parasites, hosts and parasitic life cycles

Several types of parasites can be distinguished (Rohde 1993, Marcogliese 2005b). An obligate parasite cannot survive without their host (at least during certain stages of their life cycle) whereas a facultative can do it and their host becomes optional. An ectoparasite lives on their host surface whereas endoparasite lives inside the host's body. A permanent parasite is a parasite that establishes a long time relationship with their host while temporary parasites infects their host for a short period of time. Periodic parasites visit their hosts at interval time periods. Hyperparasites are parasites of parasites and may exist in several degrees.

Intraspecific parasitism is a union of two individuals of the same species as for example a male infecting female of its own species. Latent parasitism causes no obvious effects on the host, they remaining dormant.

Macroparasites are large and detected easily, like arthropods and most Platyhelminthes (anisakids, flatworms, tapeworm, thorny-headed worms, bopirids, etc.). On the other hand, microparasites are small as not easily detected, like protozoans and some tiny Platyhelminthes. Moreover, macroparasites do not proliferate in or on their host and also have longer generation times. They usually induce no or only weak immune responses depending on infection intensity and infections are normally prolonged through time, leading to morbidity than mortality. Microparasites are just the opposite. Moreover, we can distinguish different stages through life-cycle where the larval are parasitic during their larval stages and the adults are parasitic during their mature phase, either in or on the host.

Regarding hosts, we can describe different types as definitive (DH), intermediate host (IH) and paratenic or transport host (PH or TH). DH harbours

the sexually mature parasite whereas IH lodge the immature ones. PH is defined as a host in which no parasite development takes place but helps its dispersal. Finally, with regard to the parasite life cycle we can distinguish two general types, the direct (DLC) that involves only a single host and the indirect (ILC) that implicates several hosts.

1.I.1.2. Parasitism in zooplankton

It is known that in marine pelagic environment zooplankton posses a high diversity of species and a varied assortment of higher taxonomic groups. The primary production increases in upwelling areas as a consequence of wind-driven currents, which bring nutrient-rich deep water up into the photic zone (Bode et al. 2003). In this context mesozooplankton acts as a key component because they are able to connect the microbial food web to the rest of marine pelagic food chain by feeding on microzooplankton and parasites (Marcogliese 1995, 2002, Sherr & Sherr 2002, Calbet & Saiz 2005, Marcogliese 2005a).

In the oceans mesozooplankton are able to compound different community structures that play an important role in the transmission of parasites up the food chain. These communities contain several trophic levels that are exploited by the parasites in order to reach their definitive host through predator-prey interactions. Moreover, parasites in marine environment normally use as definitive hosts a wide range of vertebrates (fishes, marine mammals, reptilians and sea birds) especially in the case of the most representative worms as trematodes, cestodes, nematodes and acanthocephalans. Among mesozooplankton calanoids copepods predominate in marine environment followed by other crustaceans like euphausiids, cyclopoid copepods and hyperiid amphipods (Marcogliese 1995, 2002). Soft-bodied zooplankters like chaetognaths, coelenterates and ctenophores are also important. In the oceans, calanoid copepods are known hosts primarily for trematodes but also nematodes and cestodes (Dollfus 1960, 1963, Slankis & Shevchenko 1974, Køie 1975, 1979, 1989, 1993, Zander et al. 1993, Køie et al. 1995, Marcogliese 1995). The large predaceous zooplankters such as chaetognaths, coelenterates and ctenophores are generally infected with trematode larvae, such as the hemiurids, lepocreadids and accacoelids (Rebecq, 1965 in (Marcogliese 1995).

Trypanorhynchs and nematodes usually infect euphausiids (Slankis & Shevchenko 1974), but tetraphyllideans are often found in chaetognaths.

Zooplankton communities are not stable because they depend on their environment which is constantly changing from spatial to temporal scales. In this context, parasites that use zooplankton as intermediate hosts, has to adapt to these variations. Some of characteristics of this kind of parasites (that use zooplankton as intermediate hosts) are:

I. To possess low specificity for the zooplanktonic intermediate host
II. To possess the ability to transfer hosts through predatory interactions within the zooplankton assemblage en route to the final host.
III. To have the ability to bump into successive hosts in the life cycle where this encounter could be periodic or even infrequent owing to the patchy distribution and low population densities of the intermediate and definitive hosts.

Thus, in this extremely patchy, diluted and three-dimensional habitat, parasites have developed complex life cycles that have implied ontogenetic changes that in turn, have resulted in metamorphoses and habitat and niche transfers and even moults. Metazoan parasitic worms can suffer more than one metamorphoses and moults several times along only one life cycle. This life cycle may include both free-living and parasitic stages, where two or more parasitic phases occur in invertebrates and vertebrates. These life history patterns enable parasites to increase their opportunities of being ingested by a suitable final host, and at the same time maximize the duration and availability of the infective pool within the zooplankton. All these strategies together allow the infective larvae to be channelled towards the definitive hosts, increasing the probability of completing its life cycle.

From an ecological viewpoint both the parasite and free-living organisms distribution can fluctuate within the oceans depending on the substrate texture, depth and water stability (Marcogliese 2002, Pascual et al. 2007). Thus parasite transmission within habitats (free-living organisms) is also based on the spatial and temporal distribution of their invertebrate and vertebrate hosts (Marcogliese 2002). In the case of the trophic transmission, parasites are related to a particular

niche and host diet. In the marine realm ecological factors like host diet and habitat are controlling the host specificity nearly all of parasites fish or definitive host. In spite of the generalist character of parasites they are related to a particular type of hosts, functional groups or feeding guilds. Members of these groups are able to share life styles, diet preferences, depth ranges and a predilection for certain sediment type. As a result, the guilds member normally share a similar parasite fauna whose constituent species follow common transmission pathways (Marcogliese 2002). Concerning pelagic waters, parasite species richness declines from surface to depth and from in-shore to offshore zone. Euphausiids and chaetognaths show inshore-offshore differences in infections of both trematodes and nematodes (Marcogliese 1995). Variations with depth occur with Anisakis nematodes being most common in euphausiids between 100 and 200 m (Smith 1983). Another example of a parasite found in both, pelagic and benthic hosts is the anisakid nematode *Hystrothylacium aduncum* (Marcogliese et al. 1996).

Apart from this kind of transmission (through food web), other parasites are able to reach their definitive host via ingestion of, penetration and or attachment by, free-living stages (Marcogliese 1995, 2005a). Examples of parasites which are actively transmitted normally swim and search out a suitable host and penetrate it include monogenean oncomiracidia, trematode miracidia and cercariae and copepod copepodids. Parasite forms that are passively transmitted are usually ingested by the appropriate host in the life cycle for transmission to occur include cestode oncospheres, larval nematodes and acanthocephalan eggs (Marcogliese 2005a).

Rates of infections of marine zooplankton with helminths are extremely low. Copepods, which occur in a vast numbers in the oceans, exhibit a parasitism rates usually less than one infected animal per 1,000 though much lower rates have been observed. Euphausiids are normally infected at even lower rates still, only one infected animal in every 100,000. Chaetognaths, and ctenophores are more heavily infected and each individual may carry several parasites inside. This information is rarely calculated nevertheless the remarkably low rates are considered a feature at the zooplankton level (Marcogliese 1995, 2005a).

1.I.2. Epibiosis

Epibiosis is a facultative association that remains understudied in which an association is done between two organisms, the epibiont and the basibiont (Wahl 1989). Epibionts are organisms that, during their sessile phase life cycle, are fixed to the surface of a living substratum. Basibionts comprise some organisms that are able to carry and constitute a support for the epibiont (Threlkeld et al. 1993). Epibiosis entails a number of several aspects that are included at different levels. From an evolutionary viewpoint the specificity between epibionts and their crustacean basibionts involve a wide range of adaptation where both morphological and physiological adaptations are implied. On the other hand, epibionts form communities on the living substratum that are important at both bioconservation and biodiversity level. The ecology, in this case, entails all of the above mentioned levels, and the study of this relationship where basibiont and epibiont are describing, by itself, ecological functions (Wahl 1989).

Epibiosis is a widespread phenomenon in marine systems where wave turbulence has resulted in the evolution of different systems of attachment to hard, relatively stable surfaces provided by other living organisms that normally are heavier than the epibiont (Fernandez-Leborans 2003). Thus, Epibionts like sessile marine organisms, depend on the characteristics of the living substratum to which they adhere. In this sense, the structure, dynamics, physiology and ecology of the basibiont may reflect the colonization patterns of different epibiont species as well as the settlement and growth of protist communities. Consequently, through epibiosis study we can learn from some aspects of the basibiont biology. For instance (Fernandez-Leborans 2010), the density of epibionts and their distribution on various anatomical units of the basibiont may indicate the existence of a terminal moult, the size of the carapace when sexual matutiry is reached, seasonal differences in moults patterns between the two sexes, burying behaviour, etc.

Epibiosis is the evolutionary result of an interaction between environmental factors and benthic life form. This is a dynamic process and the benefits and disadvantages for the organisms involved vary depending on environmental conditions (Bush et al. 2001). As occurred with parasitosis, a

temporary colonization of epibionts can occur due to a diminution of basibiont defences. Moreover, the number of interactions between the basibiont and the biotic and abiotic components of the ecosystem may be modified by the epibiosis. As a symbiotic relationship, the epibiosis may evolve towards endoparasitism or endosymbiosis. As in parasitic relationship, the epibiotic has some advantages and disadvantages for both, the basibiont and the epibiont.

1.I.2.1. Epibiosis in zooplankton

The calcified surface of decapod crustaceans bodies appears to be an appropriate habitat for protozoan epibionts. Epibionts that belongs to peritrich, suctorian and chonotrich ciliates are often found as epibionts on marine decapod crustaceans (Fernandez-Leborans 2001, 2009, Fernandez-Leborans 2010). Planktonic crustaceans usually exhibit epibiotic relationships in aquatic environments, marine, stuarine and freshwater. These associations are composed of different epibiotic organisms such as protists, algae, bacteria, hydrozoans, barnacles, rotifers and so on. Epibiosis remains poorly understood in relation to its consequences, advantages and disadvantages for both components of the relationship particularly in marine systems. In this sense, all phyla containing aquatic free living organisms include species that can be basibionts. Their sizes vary from very large (such whales that carry cirripeds) to very small as the case of two ciliate protozoans where *Ephelota gemmipara* Hertwig, 1876 live on *E. gigantea* Nonle, 1929.

Some basibiont-epibiont characteristics has been summarized by Fernandez-Leborans (2010) where the basibiont have a life cycle longer than three months, they often belong to the epibenthic community, and have body surfaces that are physiologically inactive (carapaces or shells). Normally basibionts are larger than epibionts and can be either sessile or slow moving. On the other hand, epibionts should have a sessile phase in their life cycle and also the capacity for adhesion to the substrate. Moreover they should to have a short lifespan compared with their basibiont. Furthermore, epibonts should have facultative or obligate asexual reproduction, body flexibility and show trophic independence regarding the colonized substrate.

Epibionts may be classified as opportunistic when are they able to colonize diverse surfaces whereas they are specifics when are only able to settle on a specific basibiont surfaces. The last case is relatively scarce (Wahl & Mark 1999, Gregori et al. submitted). This phenomenon is due to multiple defence mechanisms of the basibiont (physical, chemical, mechanical, associational and so on). It is also is due to the dependence of the epibiont on the basibiont condition, and the evolutionary capacity of the specific epibiotic associations towards endoparasitism or endosymbiosis (Fernandez-Leborans 2010).

The root cause of the interactions diversity influenced by epibionts is not well known. There are few studies of predation on epibionts communities, hyperepibiosis, or both physical and biological factors related to the settlement of epibiont larvae. For example, it is known that the presence of epibionts decrease host reproduction. Moreover, there are increases the mortality of the basibiont because planktivorous fish consume colonized zooplankters more intensively than non-colonized individuals. Thus, epibionts play an important role in the zooplankton decline because they have the ability to increase mortality, both non predatory and predatory, and induce changes in both zooplankton size and species composition. To entirely understand the ecological significance of epibionts, elaborated data concerning these relationships under various biotic and abiotic states in water bodies of varying trophic status are required.

1.I.3. Oceanographic characterization: upwelling-downwelling system in NW peninsula Iberica

The Galician coast (NW Iberian Peninsula) is situated at the northern limit of one of the major upwelling areas in the NE Atlantic. This interesting oceanographic system is associated with the North Atlantic anticyclonic gyre and the presence of the Rias Baixas. This upwelling system splits into two regions, Iberian Peninsula coast and NW African coast. This coastal system from 10º N to 44º N plays an important role in the exploitation of fishery's resources, climate change and parasite recruitment. The upwelling event is associated with water fertilization that causes phytoplankton blooms which are able to increase the secondary production. This high secondary production is in turn able to maintain the commercially exploited animals fishery as well as the potential hosts for

parasite recruitment. The main engine of upwelling events is the Coriolis effect that deflects ocean currents to the right in the northern hemisphere in the same way as with the general circulation of winds. Ekman transport is another important factor that affects the upwelling waters because is the term given for the 90° net transport of the surface layer (the layer affected by wind) by wind forcing.

Apart from Coriolis effect and Ekman transport we have to take into account the following key points to understand how this particular system works: seasonal coastal wind cycles, water masses upwelled, inherent factors from estuarine environment like local winds, tides, rainfall, coastline form and bottom topography.

1.I.3.1. The seasonal coastal wind.

The major source of inter-annual variability in the North Atlantic atmospheric circulation is the North Atlantic Oscillation (NAO), defined as a large scale seesaw in atmospheric mass (Fig. 1.I.2) between the Azores subtropical high and the Iceland subpolar low (Rodriguez-Fonseca & de Castro 2002). In general over spring and summer the Azores High (or Azores anticyclone) is well established and strong whereas the Iceland Low is weak. The increase in temperature in Iberian Peninsula helps the formation of pressure gradient between the continent and Azores High causing Northeastern winds. Under these specific conditions the upwelling emerges. On the other hand, over autumn and winter the pressures between Azores High and Iceland Low change and also the wind's direction promoting downwelling events. This seasonal cycle only is able to explain over 10% of the variability in the winds regime. More of 70% is explained by a success of upwelling - relaxation events which vary from 1 - 3 weeks.

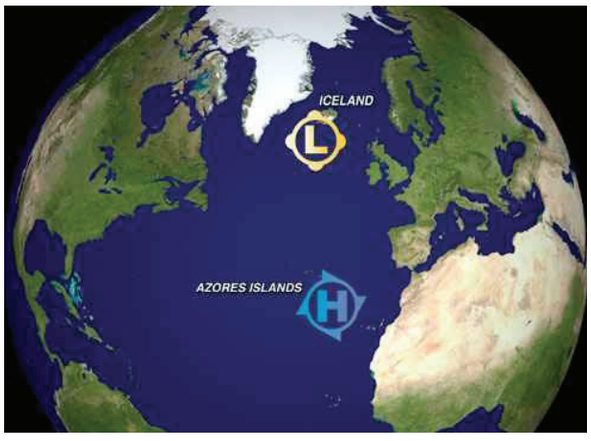


Fig. 1.I.2. General view of Azores High and Iceland Low located over Atlantic Ocean.

1.I.3.2. Water masses upwelled

The Gulf Stream is divided into Norh Atlantic current (CAN) and Azores Current (CA). Two major currents of the North Atlantic Ocean. The Iberian Current (IC) is derived from CAN and flows to the South (González-Garcés Santiso et al. 2011). The Iberian Poleward Current comes from CA and flows to the North (Fig. 1.I.3). These two currents transport different types of waters at different depths. The CI carries the Water of the subpolar North Atlantic Ocean ($NACWE_{sp}$) that has been formed at the North of 45º North in the wintry mixed layers (>400m). They are colder and less salty than the $NACWE_{st}$. On the other hand, the IPC carries the Water of the subtropical North Atlantic Ocean ($NACWE_{st}$) that has been formed at the South of 40º North in the wintry mixed layers (>150m) and is warm and salty (Huskin et al. 2003).

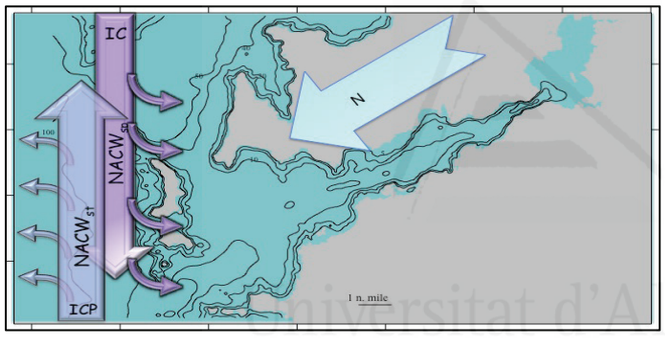


Fig. 1.I.3. Main currents transporting water masses in NW Iberian Peninsula during an Upwelling event. IC = Iberian Current; IPC = Iberian Poleward Current; N = North winds (Norhteastern winds); NACWst = Water of the North Atlantic Ocean subtropical; NACWsp = Water of the North Atlantic Ocean subpolar. Explanation of the upwelling system can be seen at: http://www.youtube.com/watch?v=j48Hu1BsSEQ

Upwelling event

Surface water shifted by wind

The coastal winds in the area display a marked seasonality that is related with the pattern of water circulation over the continental slope and shelf. From March - April to September - October, Northeastern winds are able to shift the water surface transported in the ICP, resulting in the movement of this water mass into the ocean, that is replaced by the much deeper water transported by IC. This fact promotes the upwelling event. The downwelling event is promoted when water surface is moved into the Ría by Southwestern winds during September - October to March - April.

Finally, the upwelling phenomena is able to export to the open ocean a lot of nutrients by means of filaments and eddies (Peliz & Fiúza 1999, Peliz et al. 2002).

Moreover, the hydrographic variability during the upwelling events is linked with alterations on bacteria, phytoplankton, and zooplankton biomasses delayed on the order of a day, days, and weeks respectively (Tenore et al. 1995).

1.I.3.3. The upwelling-downwelling events during 2008

The investigation carried by Roura et al. (2013) in Ría de Vigo during 2008, three well defined communities of mesozooplankton (coastal, frontal and oceanic) were identified according to downwelling-upwelling events. In this study surveys taken from 2nd to 11th of July (summer season) occurred under mainly downwelling conditions whereas upwelling conditions happened among surveys taken in autumn from 26th of September and 1st - 14th October. The oceanographic circumstances found in that study, i.e. downwelling in summer and upwelling in autumn, was diametrally opposed to the main seasonal pattern above described, where coastal Norhteastern winds marked a seasonal cycle, favouring from March-April to September-October the upwelling. As referred in Roura et al. (2013) the exceptional inverted event from summer of 2008 is related to the global warming ocean surface waters. In this sense species of Taliacea like salps were found in massive amounts in the oceanic community. These species jointly with the presence of *Temora sylifera* Dana, 1849 could act as bioindicators of warming waters and consequently of the climate change. Moreover, the salps presence could be used to detect downwelling conditions.

1.I.4. Parasite Ecology

In an ecological study the interactions existing among the organism studied and their environment must be described. As other free living organisms, parasites need to be also described in their own surrounding (Margolis et al. 1982, Bush et al. 1997).

Parasite population displays a patchy disposition because they are fragmented and separated spatially. Based on the life cycle, each developmental phase in the population, is fragmented in a variety of disrupted habitats (hosts). In this context, the habitat is the own host that is, the typical local environment in which parasites occur like tissues, organs or part of the body host in/on parasites

have been found (Bush et al. 1997, Bush et al. 2001). This characteristic aggregate spatial disposition promotes that few host individuals harbour many parasites while the rest of the hosts (most of them) carry with few or no parasites. Statistically, this disposition fits with the negative binomial frequency distribution (Bush et al. 1997, Poulin 1998). Several factors are able to promote this pattern such as host heterogeneity in susceptibility and exposure to become infected by the parasite, the parasite reproduction (inside/outside host), the varieties in the ability of the host killing their parasites, using both immunologic response or other mechanism (Bush et al. 1997, Poulin 1998).

For the conceptualization of the structure and dynamics parasite population several authors proposed a nested hierarchical classification scheme reviewed by (Bush et al. 1997). Accordingly, the infrapopulation includes all individuals of the same parasite species in one host at particular time, i.e. every *Nyctiphanes couchii* Bell, 1853 infected by black worms is considered an infrapopulation. In the example (Fig. 1.I.4) we observe 10 hosts but only 8 harbour infrapopulation: there are 6 infected hosts with black worms plus 4 with white worms, a total of 10 infrapopulation. Different infrapopulations are grouped into a component population (Fig. 1.I.5). This component population refers to all of the individuals of a specified life history phase at a particular place and time.

This term can usually be designated by reference to host taxa that harbour a specific parasite phase of interest even to the free-living stages of a species. Finally, a suprapopulation consider all of the parasites of a given species, in all stages off development, within a host in an ecosystem.

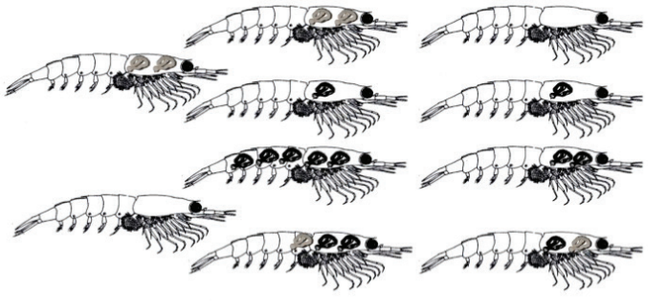


Fig. 1.I.4. Ten *Nyctiphanes couchii* infected with 2 different parasitic species. The black worm represents a particular species of parasite larva. White worm is the other parasite larva.

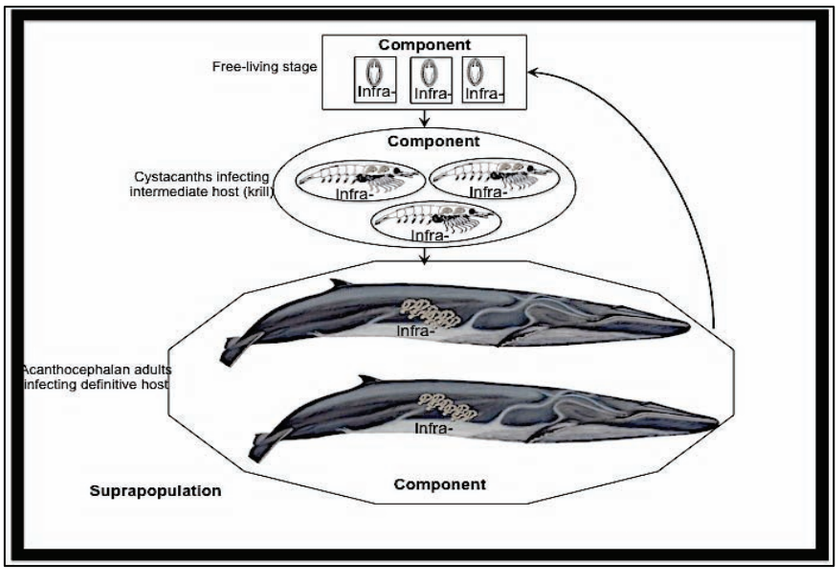


Fig. 1.I.5. A schematic representation for the hierarchical organization of parasite infra-, component and suparpopulation. This scheme could be virtually applied to any host-parasite system. The free-living stage is represented by Acanthocephalan eggs which in turn form a infrapopulation grouped in a rectangle that represents component population of several eggs. The second component population of cystacanths is formed by some infrapopulation of them into several intermediate hosts. The final hosts for Acanthocephalan adults form some infrapopulation grouped in a dodecahedron that represents a component population of Acanthocephalan adults. The large box circumscribing the entire figure.

In considering the suprapopulation as a nested hierarchy, the ideas of parasite and gene flow, recruitment and turnover, are also included.

At the level of parasite population some quantitative descriptors are used. The prevalence describes the presence or absence of parasites into a sample, splitting hosts into two categories, infected or non-infected. This particular term doesn't take into account when the hosts get the infection. Normally this term is expressed as a percentage and is calculated as the number of hosts infected with one or more individuals of specific parasites divided by the number of examined hosts for that particularly parasite. In (Fig. 1.I.4) ten host individuals are infected with none, 1 or 2 different parasite larvae. The prevalence of the black worm is 6/10 = 60% [26-88%] confidence interval (95%) while the prevalence of the white worm is 4/10 = 40% [12-74%] confidence interval (95%). As it can be seen to provide an accurate prevalence value it is necessary to include the 95% confidence limits for percentages.

The Intensity of infection is the number of individuals of a specific parasite in a single infected host. In (Fig. 4) six *N. couchii* are infected with black worms and

the intensities are 1, 1, 1, 2, 2, and 5. On the other hand, four hosts are infected with white worms and the intensities are 1, 1, 2, and 2. Mean intensity (±SE) is the intensity average thus in the first case would be 12/6 = 2.0±0.6. In the second one the mean intensity = 1.5±0.3.

The abundance (±SE) term is referred to the number of a particular parasite in or on a host regardless of whether or not the host is infected. Following the example at the Fig. 1.I.4 the abundance of the black worms is 0, 0, 0, 0, 1, 1, 1, 2, 2, and 5. The abundance of the white worms is 0, 0, 0, 0, 0, 0, 1, 1, 2 and 2. Thus the mean abundance for black worms are 12/10 = 1.2 ±0.5 whereas for the white ones are 6/10 = 0.6±0.3.

Regarding communities of parasites and following the revision made by Bush et al. (1997) these communities are also described in terms of nested hierarchy. Thus, all infrapopulations within the same host individual form an infracommunity. Each infracommunity is a subset of the species present in the component community. The latter defined as the set of infracommunities in a host population.

The community's descriptive study includes, the parasitic diversity using the analysis of taxonomic composition, species richness (number of species present) and evenness. This study can be conducted at either levels community component or infracommunity. Moreover, community studies have to include the population descriptors above defined.

Objectives

2. OBJECTIVES

2.I. GENERAL OBJECTIVES

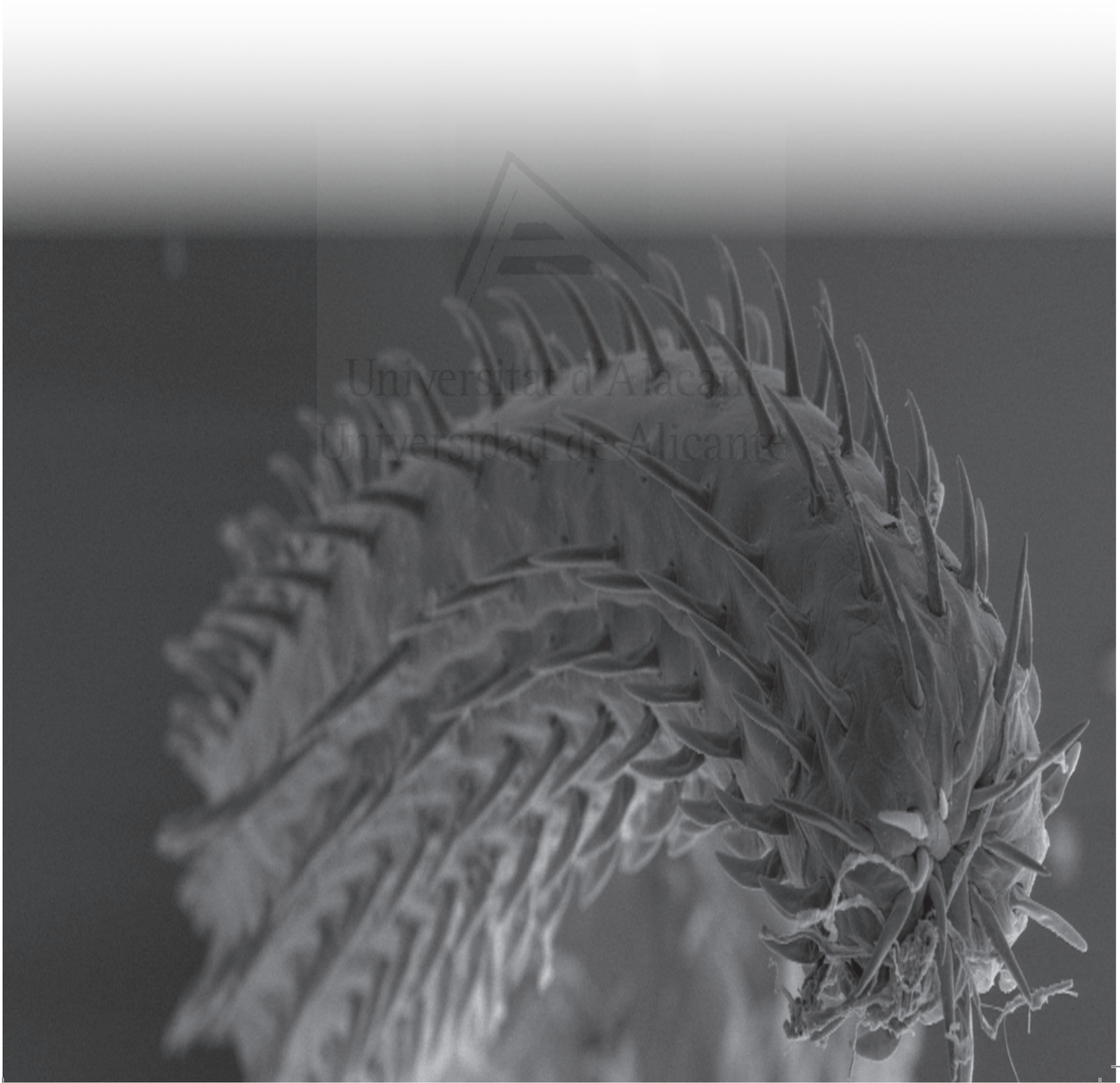
The first goal of this study is to provide valuable data regarding the role of mesozooplankton in the transmission of parasites in NE Atlantic Ocean, the role of mesozooplankton in the epibiosis relationships, descriptive data at infracommunity and infrapopulation parasite levels, spatial distribution and specificity of epibionts among basibionts, descriptive data for epibionts and the closed life cycle for parasites. Secondly, provide useful DNA data from parasite larvae identification and epibionts. Finally, to evaluate the knowledge acquired to shed light on the influence of oceanography over the recruitment of macroparasites, being different under upwelling or downwelling conditions.

2.II. SPECIFIC OBJECTIVES

Specifically, this is study is aimed at:

- Providing parasite larvae identification of Acanthocephalan, Nematodes and Trematodes using taxonomical and molecular methods, providing parasite population descriptors at the mesozooplankton level, closing the life cycle of the Acanthocephalan Bolbosoma balaenae.
- Evaluating parasite recruitment influenced by different oceanographic scenarios.
- Describing a new epibiotic relationship Suctoria-Copepoda, the description of a new Suctoria epibiont species, the specificity of the suctoria among copepods, their distribution on the copepoda surface and their link with the oceanography.

The Protozoa



3. THE PROTOZOA

3.I. DESCRIPTION OF A NEW EPIBIONTIC RELATIONSHIP (SUCTORIA - COPEPODA) IN NE ATLANTIC WATERS: FROM MORPHOLOGICAL TO PHYLOGENETIC ANALYSES

3.I.1. Abstract

Paraeuchaeta hebes is one of the most important carnivorous copepods in the coastal upwelling system off Galician waters (Ría de Vigo, NE Atlantic). A suctorian epibiont of the genus *Pelagacineta* was found attached to the surface of these copepods. The abundance and distribution on the copepod surface was analyzed, taking into account the sex of the crustacean, revealing some preference for females and also a different attachment point in both sexes. The morphological and molecular study allowed us to identify a new species of this Suctoria epibiont as *Pelagacineta hebensis*. A maximum-likelihood estimation (ML) tree inferred from the 18S rRNA gene revealed that this species belongs to the Phyllopharingea, showing a highly supported sister relationship with *Paracineta limbata.*

3.I.2. Introduction

Epibiotic associations are common in marine crustaceans. This facultative association which involves two organisms (the epibiont and the basibiont) is known as epibiosis (Wahl 1989). The term epibiont comprises organisms that, during the sessile phase of their life cycle, are fixed to the surface of a living substratum, while the basibiont carries and constitutes a support for the epibiont (Threlkeld et al. 1993). An important number of ciliates have been described as epibionts in many crustacean groups like amphipods, branchiopods, copepods, ostracods, mysids, euphausiids or decapods (Fernandez-Leborans et al. 1997, Fernandez-Leborans et al. 2002, Fernandez-Leborans 2009). Some of these crustaceans may constitute an important part of the zooplankton (Roura et al. 2013), which act as substrata for the epibionts and also as intermediate or final hosts of different parasite species (Chatton 1920, Amin 1985, Fernandez-Leborans et al. 2002, Skovgaard et al. 2005, Skovgaard & Saiz 2006, Skovgaard et al. 2007, Gómez et al. 2009, Gregori et al. 2012, Skovgaard et al. 2012, Gregori et al. 2013). Among ciliate species, suctorians have been described as epibionts of copepods

(Fernandez-Leborans 2009). These stalked ciliates do not penetrate the tegument of the copepod. However, the effects produced to the host are widely studied (Fernandez-Leborans 2010).

Copepods are by far, the most abundant organisms on earth, as well as a key link of marine food webs. As previously mentioned, the copepod surface seems to be a suitable habitat for many genera of Suctorian epibionts as *Acineta*, *Branchyosoma*, *Conchacineta*, *Cucumophrya*, *Choanophrya*, *Dentacineta*, *Dentacinetides*, *Ephelota*, *Lecanophrya*, *Lecanophryella*, *Loricodendron*, *Ophryodendron*, *Paracineta*, *Pelagacineta*, *Praethecacineta*, *Pseudocorynophrya*, *Rhabdophrya*, *Rhyncheta*, *Thecacineta*, *Tokophrya*, *Trematosoma* and *Trichophrya* have been described on Fernandez-Leborans (2009). Although Fernandez-Leborans (2009) extensively reviewed the species of copepod acting as basibionts, *Paraeuchaeta hebes* Giesbrecht, 1888 was not mentioned in their work. This copepod is one of the most important carnivorous found in the mesozooplanktonic communities of the Galician coastal upwelling system (Roura et al. 2013). Several specimens of *P. hebes* were found with an unknown ciliate colonizing their bodies.

Accordingly, the aim of this work was to carry a morphological study to identify the epibiont, accompanied with a detailed study of their location on the body of *P. hebes* to study if the epibiont display any preference for certain parts of the copepod Moreover, a molecular analysis was carried to confirm the phylogenetic position of the epibiont and supply additional molecular information for future studies on this assemblage.

3.I.3. Material and Methods

3.I.3.1. Biological sampling

The zooplankton samples were caught in the Ría de Vigo (NW Iberian Peninsula) on board of the RV *Mytilus* (Fig. 3.I.1). Ten surveys were undertaken at night, in the summer and autumn of 2008. Samples were collected by double oblique towing, using a 750 mm diameter bongo net equipped with 375 μm mesh. At a ship's speed of 2 knots, the bongo net was first lowered and stabilized near the bottom for a period of 15 min, then hauled to the surface at 0.5 ms^{-1}. The sample was fixed on board with 100% ethanol. Samples were later transferred to 70% ethanol in the laboratory and stored at -20ºC. Six species of the most abundant copepods were

analysed for epibionts within the samples collected in summer: *Acartia clausii* Giesbrecht, 1889, *Temora longicornis* Müller O.F. 1785, *Calanus helgolandicus* Claus, 1863, *Calanoides carinatus* Krøyer, 1849, *Centropages chierchiae* Giesbrecht, 1889 and *Paraeuchaeta hebes*.

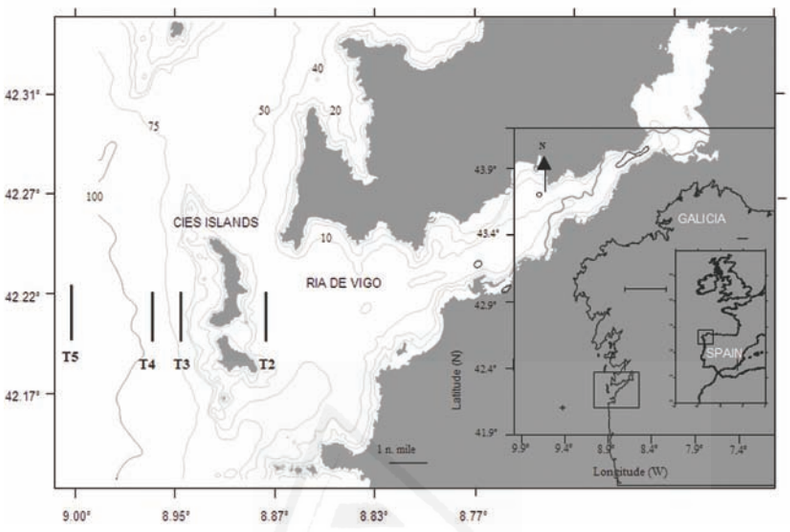


Fig. 3.I.1. Sampling area showing the transects where mesozooplankton samples were collected.

3.I.3.2. Collection and processing of epibionts

Basibionts (the six species of copepods above mentioned) were separately counted by sex and examined for epibionts using a stereomicroscope (20 x). When epibionts were detected, they were isolated and treated using the silver carbonate technique, according to the procedure described by Fernandez-Leborans and Castro de Zaldumbide (1986), and also with methyl green and neutral red. The distribution and number of epibionts on the anatomical parts of the basibionts was further analized. Sizes of epibionts were determined using an ocular micrometer. Light microscope images and morphometry of the epibionts were obtained using Image Analysis (KS300 Zeiss). Scanning electron microscopy (SEM) preparations in a Philips XL 30 were used to enhance the morphological examination. Voucher specimens were deposited at the Natural History Museum of London, UK, with the accession numbers NHM 2013.4.2.2, NHM 2013.4.2.3, NHM 2013.4.2.4, and NHM 2013.4.2.5.

3.I.3.3. Genomic DNA extraction and PCR amplification

Genomic DNA was isolated using Qiagen DNeasyTM Tissue Kit according to manufacturer's instructions. DNA quality and quantity was checked in a spectrophotometer Nanodrop® ND-1000 (Nanodrop technologies, Inc) and in 1% agarose gel. The primers 18SU467F (5'- ATC CAA GGA AGG CAG CAG GC-3') and 18SL1310R (5'- CTC CAC CAA CTA AGA ACG GC-3') (Suzuki et al. 2008) were employed to amplify a little fraction (521-788 bp) of the small subunit (18S) ribosomal RNA gene. PCR reactions were performed in a total volume of 25 µl containing 1 µl of genomic DNA (50-100 ng), 2.5µl 10x PCR buffer, 0.2 µl MgCl2, 0.5µl nucleotides (Roche Applied Science), 0.75µl primers and 0.625 U Taq DNA polymerase (Roche Applied Science). The cycling protocol for 18S rRNA gene was 2 min at 94 ºC, 35 cycles with 30 s at 94 ºC, 30 s at 55 ºC and 2 min at 72 ºC, followed by 7 min at 72 ºC. All PCRs were carried out in a TGradient thermocycler (Biometra) and a negative control (distilled water) was included for each set of PCR reactions.DNA sequencing and phylogenetic analysis

3.I.3.4. DNA sequencing and phylogenetic analysis

Positive PCR products were cleaned for sequencing using ExoSAP-IT© (USB corporation). Sequences were subject to BLASTn analyses against available sequences from GenBank through web servers of the National Center for Biotechnology Information (USA). All 18S rRNA sequences present in GenBank of the Class Phyllopharyngea were downloaded for phylogenetic analyses (n=17). Additionally, two sequences belonging to Nassophorea and Kariolelictea were used as outgroup, due to its close relation with the Phyllopharingea. Table 3.I.1 shows the species used for phylogenetic analyses and their accession numbers. These 18S rRNA sequences were first aligned using Clustal W implemented in Bioedit 7.0 (Hall 1999). GBlocks (Castresana 2000) were then used to identify and remove highly divergent regions and poorly aligned positions. Afterwards, a substitution model was selected under the Akaike information criterion (Akaike 1974) as implemented in jModeltest (Posada 2008). The GTR+I+G (Tavaré 1986) model was chosen to infer the evolutionary history by using the Maximum Likelihood (ML) method. The analysis involved 26 nucleotide sequences with a total of 364

conserved sites in the final dataset. Bootstrap probabilities with 1000 replications were calculated to assess reliability on each node of the ML tree. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011).

Table 3.I.1. Species and GenBank accession numbers of taxa used for 18S rDNA analyses.

Species	GenBank Accession Number
Paracineta limbata (Maupas, 1881) Collin, 1912	FJ865207
Acineta flava Kellicott, 1885	HM140400
Acineta tuberosa Ehrenberg, 1833	FJ865206
Acineta compressa Claparède and Lachmann, 1859	FJ865205
Acineta sp. Ehrenberg, 1833	AY332717
Ephelota mammillata Dons, 1918	EU600181
Ephelota gemmipara Hertwig, 1876	EU600180
Ephelota truncata Fraipont, 1878	EU600182
Ephelota sp. Kent, 1882	DQ834370
Ephelota sp.	AY331804
Ephelota sp.	AF326357
Tokophrya quadripartita Claparède and Lanchmann, 1859	AY102174
Tokophrya lemnarum Stein, 1859	AY332717
Tokophrya infusionum (Stein, 1859) Bütschli, 1889	JQ723984
Discophrya collini Root, 1914	L26446
Prodiscophrya sp. Kormos, 1935	AY331802
Heliophrya erhardi Saedeleer & Tellier, 1930	AY007445
Loxodes magnus Stokes, 1887	L31519
Orthodonella apohamatus Lin et al., 2004	DQ232761

3.I.4. Results

The suctorians observed on *Paraeuchaeta hebes* (Fig. 3.I.2 A, B) were identified as loricate ciliates. Their lorica was thecostyle type (prolongation of the stalk) and was surrounded, as much, a half lower body of the ciliate (Fig. 3.I.2 C). The funnel-shaped lorica was 84.60-108.00 µm long (Fig. 3.I.2 C), with a maximum width of 88.36-118.70 µm. Some young specimens presented a reduced lorica like a hat-shaped structure in contact with the rear end of the ciliated body. The lorica extended through the posterior part of the body in a narrow stalk (85.60-233.00

μm), which finished on an oval basal disk (Fig. 3.I.2 D). Longitudinal striations were clearly observed covering the stalk surface (Fig. 3.I.2 E). The body of the suctor was ovoid (Fig. 3.I.2 F) with a length of 60.16-97.60 μm and 50.76-70.83 μm in width (Table 3.I.2). Numerous tentacles sticking out through the different parts of the surface of the suctor body thus they were not in contact with the lorica (Fig. 3.I.2 G). There were 54-142 similar capitate tentacles that were highly contractile (Fig. 3.I.2 H). The macronucleus (Ma) was located centrally in the body and it was oval, sometimes transversely elongated (31.20-40.36 μm long, 23.20-32.84 μm width). Near the Ma was a small and dense spherical micronucleus (Fig. 3.I.2 I). Some specimens showed buds in their body (Fig. 3.I.3 A). The budding is endogemmic, with a unique bud (monogemmic) or with more than one (polygemmic) (Fig. 3.I.3 B-C). These buds will develop into asymmetric and elongated swarmers with a long between 17.40-20.80 μm and a width between 7.20-8.80 μm (Fig. 3.I.3 D).

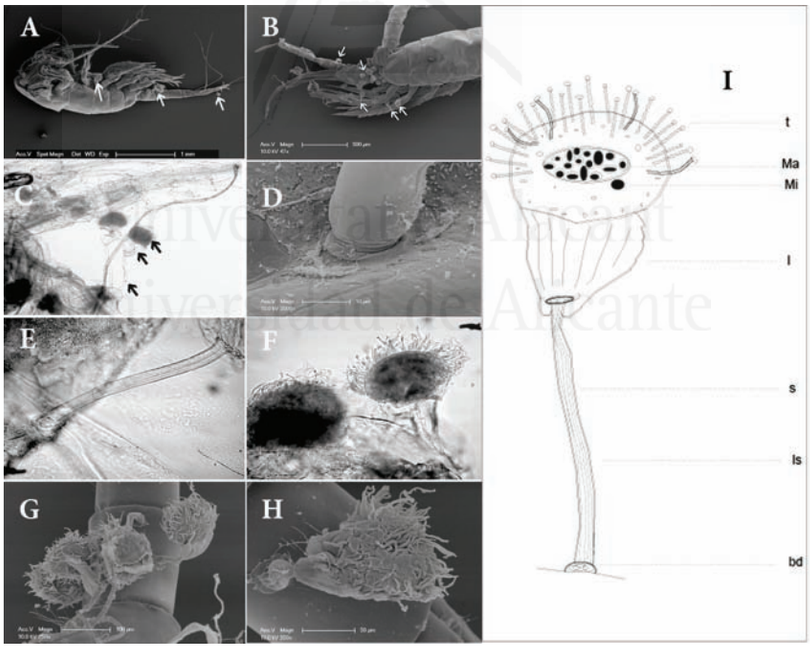


Fig. 3.I.2. Light and SEM micrograph of (A) Pelagacineta hebensis attached to the female of Paraeuchaeta hebes. (B) P. hebensis attached to P. hebes male. (C) Two specimens attached to the surface of the genital segment of the female basibiont. Lorica thecostyle type. (D) Oval basal disk on the attachment point surrounded with epibiont bacteria. (E) Longitudinal striations on the stalk. (F) Ovoid suctorian body. (G) Numerous tentacles sticking out through the different parts of the surface of the suctorian body thus they were not in contact with the lorica. (H) Similar capitate tentacles that were highly contractile. (I) Schematic P.hebensis where is shown tentacles (t), macronucleus (Ma), micronucleus (Mi), lorica (l), stalk (s), striated stalk (ls) and basald disk (bd).

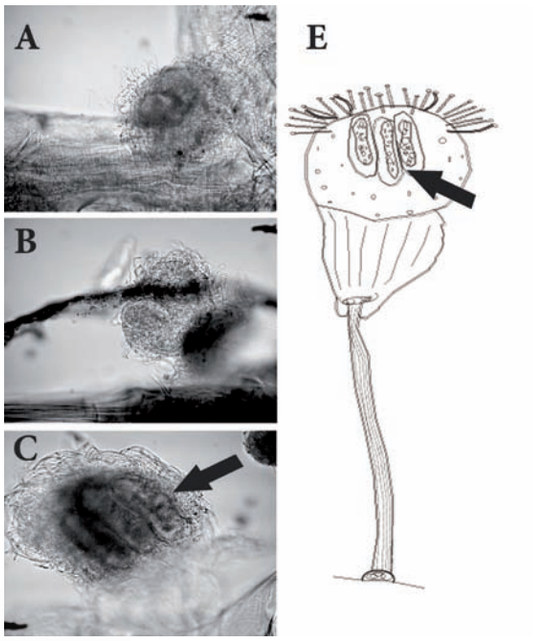


Fig. 3.I.3. Pelagacineta hebensis light micrograph of a specimen. (A) Buds were shown in the body. (B) Monogemmic budding (C) Polygemmic budding. (D) Schematic individual with asymetric developed buds that give rise to swarmers (arrow).

Table 3.I.2. Biometry of *Pelagacineta hebensis*. Measurements in µm. Ma = macronucleus; SD = standard deviation; SE = standard error. N=30.

	Mean	SD	SE	Min - Max
Body length	72.09	12.86	4.54	60.16 - 97.60
Body width	59.94	6.72	2.87	50.76 - 70.83
Number of tentacles	82.87	27.66	9.78	54.00 - 142.00
Tentacles length	32.31	6.33	2.24	21.80 - 43.70
Lorica length	91.70	6.91	2.44	84.60 - 108.00
Lorica max. width	99.13	9.58	3.38	88.36 - 118.70
Stalk length	173.82	50.56	17.87	85.60 - 233.00
Stalk width	14.36	1.43	0.50	12.50 - 16.92
Length of basal disc	20.75	0.20	0.07	20.50 - 221.05
Ma Length	34.94	2.59	0.91	31.20 - 40.36
Ma width	26.38	2.98	1.05	23.20 - 32.84
Bud length	19.02	1.14	0.40	17.40 - 20.80
Bud width	7.85	0.57	0.20	7.20 - 8.80
N. buds	2.25	0.88	0.31	1.30 - 3.00

3.I.4.1. Location on the basibiont

Overall, 39,030 copepods divided into 3,152 *C. helgolandicus*, 14,930 *C. carinatus*, 1,240 *C. chiercheae*, 10,785 *A. clausii*, 2,680 *P. hebes* and 6.242 *T. longicornis* were examined for protozoans. The suctorian ciliates were exclusively

found attached to the surface of *P. hebes*. A total of 114 males carried about 643 epibionts whereas 228 females bore about 1,461 (Table 3.I.3). Ciliates were encountered on the buccal appendages in a very low percentage. The preferred sites of attachment differed among sexes. The percentage of attachment of the epibionts in males, in decreasing order of importance was: leg 5 (L_5), leg 4 (L_4), urosome (U), metasome (M), leg 3 (L_3), caudal ramus (CR), cephalosome (C) and genital segment (G). In females was: G, U, CR, M, C, L_4, L_3, leg 2 (L_2) (Fig. 4).

Table 3.I.3. Number of *Paraeuchaeta hebes* examined for epibionts. NInf = number of non infected copepods. Inf = number of infected copepods. NºEpib = number of epibionts found on copepod surface. MInt±SE = Intensity average ± standard error. Max = maximum number of epibionts found on copepods surface. Min = minimum number of epibionts found on copepods surface.

P. hebes	NInf	Inf	NºEpib	MInt±SE	Max	Min
Males	733	114	643	5,64±0.52	30	1
Females	1605	228	1461	6,41±0.35	31	1
Total	**2338**	**342**	**2104**	**5,40±0.29**	**31**	**1**

3.I.4.2. Taxonomic position.

Phylum Ciliophora Doflein, 1901
subphylum Intramacronucleata Lynn, 1996
class Phyllopharyngea De Puytorac et al., 1974
subclass Suctoria Claparède & Lachmann, 1858
order Endogenida Collin, 1912
family Tokophryidae Jankowski in Small & Lynn, 1985
genus *Pelagacineta* Jankowski, 1978
Pelagacineta hebensis sp. n.

3.I.4.3. Diagnosis of Pelagacineta hebensis sp. n.

Pelagacineta hebensis has an ovoid body, often wider than long, with a length of 84.60-108.00 μm, and a width of 88.36-118.70 μm. A funnel-shaped lorica, thecostyle type, surrounds at least half of the lower body of the ciliate. The lorica is extended through the posterior part of the body in a narrow stalk, which is

finished on an oval basal disk. The surface of this stalk is covered with longitudinal striations. The tentacles are capitate and highly contractile. They all (54-142) start from different points of the body surface that is not in contact with the lorica. Macronucleus is oval and centrally located in the body however, sometimes, it is transversely elongated (31.20-40.36 μm long). Near to the macronucleus is placed a small, dense and spherical micronucleus. Endogenous budding in the apical area occurs in a unique bud (monogemmic) or more than one (polygemmic). The buds will develop into asymmetric and elongated swarmers with a mean length of 17.40-20.80 μm. The host is *Paraeuchaeta hebes* (Copepoda). *P. hebensis* may be mainly found on the female host on genital segment, urosome, caudal ramus and metasome. On the male host they may be mainly found on the leg 5, leg 4, urosome and metasome. Its geographical distribution is on the continental shelf at Ría de Vigo (N.E. Atlantic waters, Galician coast, Spain).

3.I.4.4. Phylogenetic analysis

Amplified sequences of 18S rRNA ranged from 521 to 788 bp. These sequences are available on GenBank under the accession numbers. BLAST search showed close homology (95%) with the 18S rRNA of *Paracineta limbata* (Maupas, 1881) Collin, 1912 and distant homology (86%) with *Acineta flava* Kellicott, 1885. The 18S rRNA genealogy showed that the class Phyllopharingea contains a monophyletic subclass, Suctoria (Albaina & Irigoien 2006, Gong et al. 2008, Gong et al. 2009, Pan et al. 2012). The families Discophrydae, Prodiscophrydae and Helioprhydae, were clustered in the order Evaginogenida with a strong bootstrap support (99%). Nevertheless, the proximity between *Discoprhya collini* Root, 1914 and *Prodiscophrya* sp. Kormos, 1935 (supported by a strong bootstrap, 99%) suggested that they are more probably, the same species (Fig. 3.I.4). Ephelotidae was grouped with a strong bootstrap support (96%) within Exogenida. Moreover, Endogenida included two families (Tokoprhydae and Acinetidae) with a moderate bootstrap support (66% ML). Contrary to expectations, the ML tree inferred from the 18S rRNA data set of Phyllopharyngea revealed that our specimens (*Pelagacineta hebensis*) belong to a highly supported clade (bootstrap values of 100), with *Paracineta limbata* (Fig. 3.I.4) within Tokophrydae (Endogenida). Moreover, the position of *Acineta flava* remained unresolved.

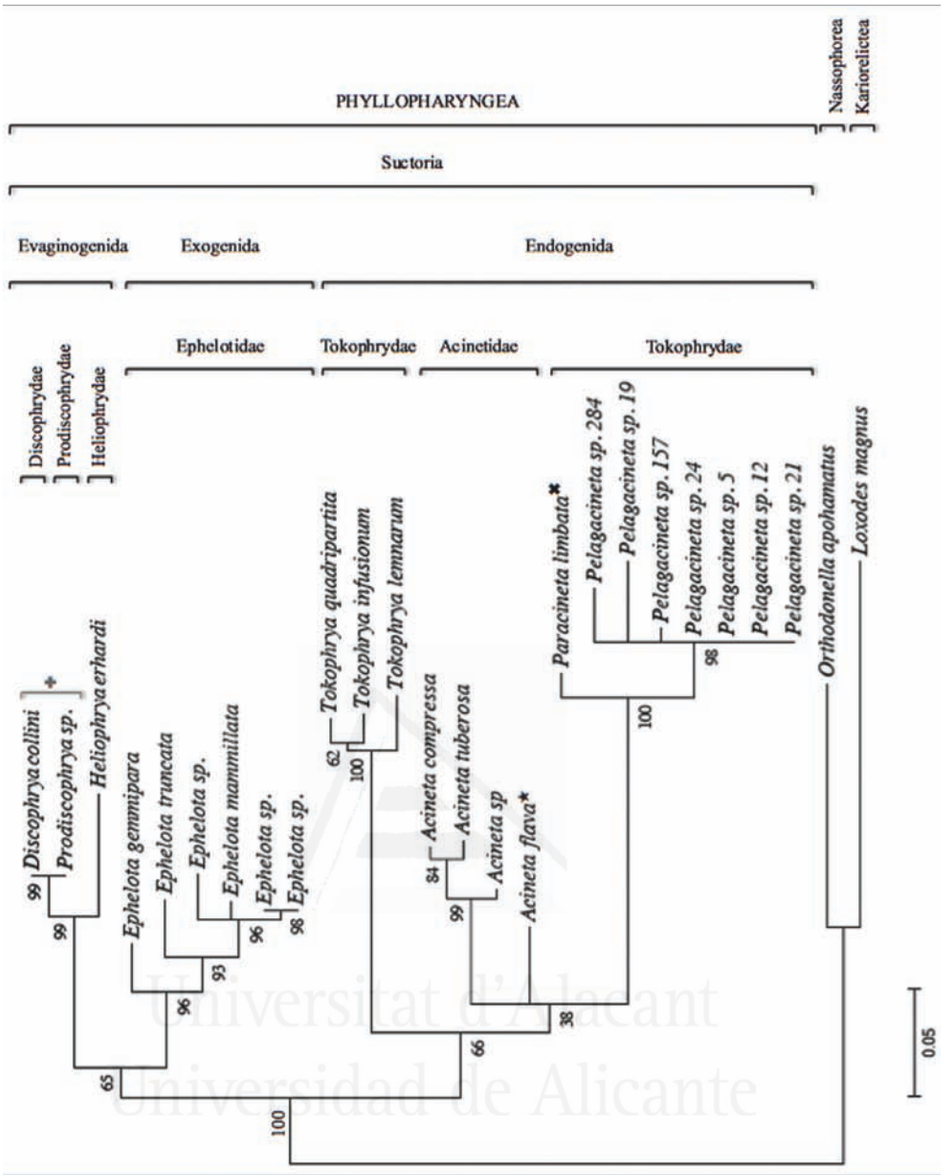


Fig. 3.I.4. Maximum likelihood tree based on 18S rRNA gen showing the phylogenetic relationship of Pelagacineta hebensis sp. n. among Suctoria. ML (the number showed in the tree) bootstrap support values over 30% are given. Sequences inferred by the minimum evolution algorithm within MEGA 5. Analysis of 18S rDNA sequences after 1000 bootstraps.

3.I.5. Discussion

The suctorian ciliate observed on the copepod *Paraeuchaeta hebes* belongs to the genus *Pelagacineta*. Like their congeners, they are marine loricate ciliates with a thecostyle lorica. This ovoid and transversely rounded ciliate did not present actinophores and possessed a unique group of capitate and contractile tentacles that in other species of this genus may appear forming two groups. The

stalk expands anteriorly to form the lorica. Macronucleus (Ma) elongated and often branched. Reproduction by multiple endogenous budding. Swarmers ovoid, partially ciliated with several longitudinal kineties. Attached to copepods or marine algae (Curds 1987).

The ciliates found could belong to the genus *Paracineta* or *Pelagacineta.* Notwithstanding, the specimens here studied differed from *Paracineta* in the arrangement of tentacles, which normally are placed in the apical end of the body's suctor of this species. However, when the lorica is too small tentacles are able to radiate from the other areas. Conversely, the tentacles of our specimens are placed anywhere on the surface of the body which is not covered by the lorica. The distinguishing feature of the *Paracineta* is the exogenous budding, while the most marked feature in our specimens is the endogenous budding, mono or polygemmic with asymmetric buds. The mode of asexual reproduction has been largely used to group these ciliates into the subclass Suctoria (Lynn 2008). Our specimens are different from *Paracineta gaetani* Sewell, 1951 in the length of the stalk, the Ma shape and the asexual reproduction. *P. gaetani* is characterized by a rigid stalk, which is shorter than the lorica, their Ma is spherical and their budding is exogenous. A stalk three times longer than the lorica, transversally ovoid and rounded macronucleus and endogenous budding marks the specimens here studied.

Among *Pelagacineta,* four species have been described: *Pelagacineta campanula* Schröder, 1907, *P. interrupta* Jankowski, 1978, *P. dibdalteria* Parona, 1881 and *P. euchaetae* Sewell, 1951. Differences among them are summarized in Table 3.I.4. From a fore said comparison Table 3.I.4 it is noted that our specimens slightly resemble *P. campanula,* which have a dorso-ventrally compressed and discoidal body in contrast with a not compressed and ovoid body in the samples studied. While the basal disk of the stalk is striated in *P. campanula*, in our specimens is longitudinally striated. An elongated and very branched Ma is typical in *P. campanula* whereas, our individuals present an oval Ma. An outermost circle of tentacles, that are patently shorter, surrounds the tentacles in *P. campanula*. This last feature is absent in our studied samples. Taking into account the morphological differences between the suctorians analysed here and other similar

species, we have concluded that the observed suctorian belong to a new species, which we have named *Pelagacineta hebensis* in reference to the copepod where they were found. Consequently, these data constitute both the registration of a new basibiont and geographical distribution for the genus *Pelagacineta*.

	Species of Pelagacineta				
	P. interrupta	***P. campanula***	***P. dibdalteria***	***P. euchaetae***	**This study**
Body length	100-140	100-150	50-60	50-90	60-97
Body shape		Dorso-ventrally compressed and discoidal			Not compressed and ovoid
N. groups of tentacles	2	1	2	2	1
N. tentacles	10-40 (each group)	-		-	54-142
Tentacle length	-	-	-	36	39-79
Stalk length	2-3 times lorica lenght	1-3 times lorica lenght	≤ lorica length	< lorica length	1-3 time lorica length (84-233 long)
Stalk width	20-30	-	-	-	12-16
Ma shape	Variable (horseshoe, C, X, ramified)	Elongated and highly	Sausage-shaped	Variable	Oval (elongate, elongated curved)
N. buds	1-4	Multiple	-	56 long	1-3
Lorica	Funnel-like	Cup-shaped (130-140 long)	Cup-shaped	257 long	Funnel- like (84-89 long)
Habitat	*Euchaeta Metridia* (antarctic)		Marine algae	*Euchaeta* (antarctic)	*Paraeuchaeta hebes* N.E. Atlantic

Table 3.I.4. Comparison between the different species of Pelagacineta. N = number.

3.I.5.1. Phylogenetic analysis

In previous studies (Albaina & Irigoien 2006, Gong et al. 2008, Gong et al. 2009, Pan et al. 2012), the Subclass Suctoria was strongly confirmed as a monophyletic clade containing three major Orders so far represented by 18S rRNA phylogeny. This is consistent with the traditional taxonomy based on the shared morphological characters (Lynn 2008). The 18S rDNA have been broadly used as a

taxonomic tool to clarify the taxonomy of Phyllopharyngea at the species level (Albaina & Irigoien 2006, Gong et al. 2008, Gong et al. 2009, Pan et al. 2012). Our genealogy showed that the Subclass Suctoria contained three monophyletic orders Evaginogenida, Exogenida and Endogenida which are strongly supported by some monographic works (Dovgal 1996, Dovgal 2002, Lynn 2008). Despite the fact that *Dyscophrya collini* and *Prodiscophrya* sp. have been included in the Order Evaginogenida, very close to *Heliophrya erhardi* Dragesco et al., 1955, they showed identical sequences (Pan et al. 2012). Hence we considered that these two species that belong to different families should be revised. According to the phylogenetic tree here obtained, *Acineta flava* clade has been unsolved. This species could be belonging to whatever three genus that appeared next to it (*Tokophrya*, *Acineta* or *Pelagacineta*). Nevertheless, in an attempt to resolve the position of *A. flava*, we removed *Tokophrya infusionum* (Stein, 1859) Buetschli, 1889 and *T. lemnarum* Stein, 1859 from the phylogenetic analysis. Surprisingly, *A. flava* was grouped with *T. quadripartita* Claparede and Lachmann, 1859 (bootstrap of 45%) and clearly appeared separated from the other *Acineta* analyzed (73%). This finding leads us to suspect that *A. flava* should also be reviewed. Regarding Exogenida, which is represented by *Ephelota* species, showed monophyly whitin the subclass Suctoria (Albaina & Irigoien 2006), whereas Tokophryidae (Exogenida) demonstrated paraphyly. According with Lynn (2008) seven genera have been included in the Tokophryidae family where we can found *Pelagacineta* and *Tokophrya*. Our phylogenetic tree showed that *Pelagacineta* genus could be included in Acinetidae as Fernandez-Leborans (2009) showed in their review. Thereby, Tokophryidae and Acinetidae will be monophyletic clades into Suctoria. Unexpectedly, our results also showed that *Paracineta limbata* belongs to the Endogenida Order as a sister taxon of *Pelagacineta hebensis*. This close proximity between this two species could be explained if *P. limbata* was erected to the *Pelagacineta* genus. Due to the asexual reproduction is an important feature, which group species of the Suctoria, *P. limbata* should be clustered with *Ephelota* species because this species showed exogenous budding. Moreover, we strongly suggested that *P. limbata* sequences should be revised.

The present study provides new phylogenetic information about Suctorians, taking into account that only 16 sequences of this species-rich group have been sequenced and represented on 18S rDNA phylogenetic trees. To determine details of their relationships in these highly specialized organisms, more data are definitely needed.

3.I.5.2. Specificity, distribution on the host and ecology

Despite the large number of copepods examined, *Pelagacineta hebensis* was only found on *Paraeuchaeta hebes*, a crustacean for which it seems to show a clear preference. In the report of Fernandez-Leborans (2009) *P. euchaetae* was detected on *Calanus helgolandicus,* which was one of the dominant species in our samples, however in our coastal region *C. helgolandicus* was free of this epibionts. The rest of the copepods here studied were the dominant species in the samples collected (Gregori et al. 2013) however, only *P. hebes* carried this epibiont demonstrating the specificity above mentioned.

Gender preferences have also been detected since an important number of epibionts, were most frequently attached to females. This phenomenon has widely been accepted as a feature among basibiont females (Xu & Burns 1991, Carman & Dobbs 1997, Walkusz & Rolbiecki 2007, Fernandez-Leborans 2010). Moreover, protozoan epibionts are able to show preferences on certain parts of the crustacean basibionts e.g. *Ophryodendron* sp. Claparède and Lachmann, 1859 on the caudal ramus of *Lichomolgus singularipes* Humes and Ho, 1968 (Humes & Ho 1968). Walkusz and Rolbiecki (2007) found some individuals of *Paracineta* sp. attached exclusively on the prosome of *Metridia longa* Lubbock, 1854 and *Paraeuchaeta norvegica* Boeck, 1872. Furthermore, Skovgaard et al. (2005) described that *Ephelota* spp. Wright, 1858 were distributed over the cephalothorax, genital segment, abdomen and caudal branches of the copepod. Among females of *Paraeuchaeta hebes*, suctorians were most frequently attached to the genital segment, urosome, caudal ramus and metasome. By contrast in males the attachment was Leg 5, Leg 4, urosome and metasome. This different distribution could be related with the reproduction behaviour where genital segment of females are related with Leg 5 of males. Likely when males deposit their sperm sac

on females genital pore, they became infected with the epibiont. In this sense different behaviour during mating could establish differences found between sexes. The location of *Pelagacineta hebensis* concentrated on the posterior part of the basibiont body coincided with that reported by Sherman and Schaner (1965), Evans et al. (1979), Walkusz and Rolbiecki (2007), Fernandez-Leborans (2009). The posterior locations on the copepod basibiont could protect the epibionts from the water friction (when copepod swimming). Moreover, swimming appendages could provide epibionts with food, faecal particles facilitating the capture of food by the suctorian, thereby increasing the density of ciliates in these regions (Fernandez-Leborans 2009, Fernandez-Leborans 2010).

Despite the fact that apparently, there was not a detrimental effect of the presence of *P. hebensis* on the basibionts since they do not penetrate into the copepod's tegument, some indirect negative effects can occur on them. Suctorians can affect negatively the basibiont survival (with heavy colonization), increasing the predation risk of the basibiont (by modifying chemical signals acting on mobility, decreasing sensory activity, increasing energy costs, substantial shift of interactions among species). Their effects on biological functions are also wide, because of nutrient competition, inhibition of moulting, increased of weight and friction with water, trans-epidermal impeded exchanges, etc.(Fernandez-Leborans 2010, Wahl et al. 2012). In this way Weissman et al. (1993) recorded slower sinking rates in *Acartia hudsonica* Pinhey, 1926, when infested with solitary peritrich, *Rhabdostyla* sp. Kent, 1881, suggesting that the epibiont may increase burden drag forces, thereby impeding locomotion and increasing energy expense by the host.

In summary, our investigation has provided evidence that the new epibiont species *Pelagacineta hebensis* sp. n is found in adult individuals of the copepod *Paraeuchaeta hebes* from NE Atlantic waters. It is the first time that this copepod is observed as a basibiont for suctorian species. The new epibiont is described both with morphological and molecular techniques, contributing to enlarge the DNA sequences available for the class Phyllopharyngea. Finally, our results show that the suctorian *P. hebensis* has a marked preference for sexual appendages and the posterior part of the body of *P. hebes* females. Next studies should test if the

oceanographic conditions affect the colonization of *P. hebensis* on *P. hebes*, as well as to determine if the sexual fitness of the copepod is affected by the presence of the suctorian epibiont.

3.II. New host records for ellobiopsidae in NE Atlantic waters and demographic values and filogeny

3.II.1. Abstract

Copepods are one of the most important crustaceans in the coastal upwelling system off Galician waters (Ría of Vigo, NE Atlantic). The parasite of the genus *Ellobiopsis* was found penetrating various parts of the copepods body. Demographic infection values among copepods species and among copepods sexes have been provided. Thus, the proportion of the infected copepods was significantly different within communities, while between them no differences were found. Moreover, no significant differences were found in the proportion of infected animals between sexes. The abundance and distribution of this parasite on the copepod surface was analysed, taking into account the sex of the crustacean, revealing some preference for females and also a different attachment point in both sexes. The morphological study allowed us to identify this parasite as *Ellobiospis chattoni*.

3.II.2. Introduction

Numerous parasites have been associated with Copepods, which without doubt are the most abundant organisms on earth. They also are a key component because are able to connect the microbial food web to the rest of marine pelagic food chain by feeding on microzooplankton. Thus, they play an important role in aquatic ecosystems (Sherr & Sherr 2002), (Calbet & Saiz 2005), (Mauchline 1998). In terms of biomass, species diversity, niche exploitation and distribution, copepods constitute one of the most prevalent assemblages of marine organisms (Amin 1985).

Parasitism is a common event in both, terrestrial and aquatic ecosystems, where parasites have been recognized as controlling factors for their population host (Bush et al. 2001{Skovgaard, 2006 #101, Albaina & Irigoien 2006)}. Hosts are usually damaged by its parasites, interiorly or on the surface, depending on parasite type. Whereas macroparasites such as Nematoda, Acanthocephalans, Monogenea and Isopoda are able to harm both the interior or the surface body of its hosts, microparasites are able to nourish themselves (as parasites) on their

copepod host (Køie 1993, Shields 1994, Køie et al. 1995, Skovgaard 2004, Arnason 2007, Skovgaard & Daugbjerg 2008, Gregori et al. Submmited). In this way, some reports have been demonstrated that *Ellobiopsis* are dangerous parasites of copepods. They can adversely affect fertility in females and cause feminisation in males (Shields 1994, Albaina & Irigoien 2006).

Among *Ellobiopsis* Coutière, 1911, *E. chattoni* Caullery, 1910, *E. elongata* Steuer, 1932 and *E. fagei* Hovasse, 1951 have been described. These species share characteristic morphology with a well-defined stalk, a trophomere and one, or two gonomeres for *E. chattoni* and *E. elongata* respectively. *E. fagei*, has been classed as intermediate between the other two species and even has been suggested as synonymous with *E. chattoni* (Shields 1994). The *Ellobiopsis* life cycle is composed of dispersion phase, a flagellated spore that settles on or adjacent seta of a buccal appendage of its hosts and then develops into two segments, the trophomere (proximal) and the gonomere (it is the distal one and also the reproductive body of the animal). The trophomere penetrates through the body cuticle and it seems that is used in the absorption of nutrients. The sporulation occurs as certain body size in which the gonomere becomes tightly constricted from the trophomere and groups of cells are released to the environment (Shields 1994).

This enigmatic parasite (*E. chattoni*) is widely distributed on a global basis and it has been reported from various species of Calanoids in the Mediterranean Sea (Caullery 1910), North Sea (Caullery 1910, Jepps 1937), Adriatic Sea (Hoenigman 1958), Indian Ocean (Krishnaswamy 1950, Wickstead 1963, Santthakumari & Saraswathy 1979), northeastern Pacific Ocean (Hoffman & Yancey 1966) and Bay of Biscay (Albaina & Irigoien 2006).

Few studies have been examined the host and environmental factors that contribute to the infection dynamics of the Ellobiopsidae. Moreover, their systematic position remains unknown, but it could be grouped within Alveolates (Gómez et al. 2009). The present work aimed to shed light on the possible importance of parasitism in copepods by focusing on the occurrence and specificity of *Ellobiopsis* sp. in NW Atlantic Ocean mesozooplankton.

3.II.3. Material and Methods

3.II.3.1. Collection and processing samples

Zooplankton samples were caught in the Ría de Vigo in Galician waters (NW Spain) on board of the RV "*Mytilus*" (Fig. 1). In 2008, ten surveys were undertaken,in summer (2nd, 4th, 9th and 11th of July) and autumn (26th of September; 1st, 3rd, 9th, 10th and 14th of October). Two samples were collected on each transect by double oblique towing, using a 750 mm diameter bongo net equipped with 375 µm mesh. At a ship's speed of 2 knots, the bongo net was first lowered and stabilized near the bottom for a period of 15 min, then hauled to the surface at 0.5 ms^{-1}. Bongo net was also equipped with a current meter to determine the filtered volume. Zooplankton samples were fixed on board with 70% ethanol and stored at -20 ºC to avoid DNA degradation (Passmore et al. 2006).

For the quantitative measures of parasites we studied the samples collected at summer (Fig. 3.I.1) on 2nd, 4th, 9th, and 11th of July. At least 1,000 copepods were counted using a Stempel pipette in different aliquots from a sub-sample obtained using a Folson splitter (Omori & Ikeda 1984). Then, infected and uninfected individuals were separated under a stereomicroscope (20x). When protozoa were found, they were isolated with their host, measured and photographed using a Nickon DS-Fi1 digital camera mounted on a Nikon ZMZ800 stereomicroscope. Scanning electron microscopy (SEM) preparations in a Philips XL 30 were used to clarify the morphological examination and insertion on the copepod body. Six species of copepods were studied for parasites *Calanus helgolandicus* Claus, 1863, *Calanoides carinatus* Krøyer, 1849, *Centropages chierchiae* Giesbrecht, 1889, *Acartia clausii* Giesbrecht, 1889, *Paraeuchaeta hebes* Giesbrecht, 1888 and *Temora longicornis* Müller O.F., 1785.

Infection parameters were estimated using quantitative parasitology program following the instructions provided by Bush et al. (1997) and Rósza et al. (2000). Following the recommendations of these authors proceeded to make the comparison of different parasitological parameters such as prevalence, mean intensity of infection and distribution. These comparisons were made in both, communities and species within each community. Moreover, we contrasted whether the infection parameters were different among sexes.

Furthermore, distribution of the parasites on the surface body of copepods among sex were analysed as well as their developmental stage that was divided into 1 = initial stage of development; 2 = prior to gonemere development and 3 = with gonomere. Finally, qualitative analyses from samples randomly selected were examined for the presence of infected organisms.

3.II.4. Results

A total of 7,609 males and 31,421 females copepods were examined for parasites. Sixty-eight copepods were infected with *Ellobiopsis* distributed in 48, 11, 3, 3, and 3 *Calanus helgolandicus*, *Calanoides carinatus, Centropages chierchiae, Acartia clausii* and *Temora longicornis*, respectively. The number of infected copepods females was higher than males Table 3.II.1. The number of infected animals was not homogenously distributed between species thus, females of *C. helgolandicus* were the most infected copepods followed, in decreasing order, by *C. carinatus*, *A. clausii* and *T. longicornis* and *C. chierchiae*. On the other hand, among males the most infected copepods also were *C. helgolandicus*, *C. carinatus*, and *C. chierchiae*.

Table 3.II.1.Distribution of infected copepods sorted by sex and species.

Copepod species	Infected Females	Infected Males	Total of infected copepods
Calanus helgolandicus	39	9	**48**
Calanoides carinatus	6	5	**11**
Acartia clausii	3	-	**3**
Temora longicornis	3	-	**3**
Centropages chierchiae	2	1	**3**
Total	**53**	**15**	**68**

The studied copepods belongs to different mesozooplankton communities that were analysed in (Roura et al. 2013). Accordingly six characteristic mesozooplankton communities in the Ría de Vigo during the upwelling season following the bathymetric gradient were defined. Three in early summer and three in autumn, named as coastal, frontal and oceanic. Complete information about their composition is available in the above-mentioned work.

Demographic infection values calculated for copepods population and copepods sex are given in (Fig. 3.II.2). We did not find significant differences in

the proportion of the infected hosts between summer communities (Fig. 3.II.1; Table 3.II.2). From the comparison of the typical levels of infection among communities no significant differences were found (Fig. 3.II.2 and Fig. 3.II.3).

Table 3.II.2. Infection parameters where Com = Community; S = copepod species studied; NT = number of studied hosts; NM = number of male hosts; NF = number of female hosts; IT = number of infected hosts studied; IM = number of infected males; IF = number of infected females; ITT = nº of total parasites per host; ITM = nº of parasites per male; ITF = nº of parasites per female; %P[CI] = total population prevalence [confidence interval]; %M[CI] = male prevalence [confidence interval]; %F[CI] = female prevalence [confidence interval]; ITp±SE = Mean intensity of infection population ± standard error; ITpM±SE = Mean intensity of infection in males ± standard error; ITpF±SE = Mean intensity of infection females ± standard error;

Com	S	NT	NM	NF	InT	InM	InF	ITT	ITM	ITF	%P[CI]	%M[CI]	%F[CI]	ITp ± SE	ITpM±SE	ITpF ± SE
SF	*C. helgolandicus*	1357	236	1121	24	3	21	35	5	30	1.7 [1.16-2.59]	1.3 [0.35-3.68]	1.8 [1.19-2.81]	1.46 ± 0.72	1.67 ± 1.15	1.43 ± 0.68
	C. carinatus	7625	1090	6535	7	4	3	8	5	3	0.1 [0.05-0.21]	0.4 [0.11-0.97]	0.04 [0.01-0.14]	1.14±0.41	1.25 ± 0.58	1
	C. chierchiae	700	363	337	1	-	1	1	-	1	0.1 [0.02-0.82]	-	0.3 [0.02-1.70]	1.00	-	1.00
	A. clausii	2904	425	2479	1	-	1	2	-	2	0.03[0-0.22]	-	0.04 [0-0.25]	2.00	-	2.00
	P. hebes	966	315	651		-			-			-			-	
	T. longicornis	2188	708	1480	3	-	3	4	-	4	0.1 [0.03-0.42]	-	0.2 [0.04-0.62]	1.33 ± 0.58	-	1.33 ± 0.58
SC	*C. helgolandicus*	566	94	472	10	2	8	23	2	21	1.7 [0.93-3.18]	2.1 [0.38.7.14]	1.7 [0.79-3.30]	2.3 ± 1.49	1.00	2.63 ± 3.53
	C. carinatus	627	118	509	-	-	-	-	-	-	-	-	-	-	-	-
	C. chierchiae	357	215	142	1	-	1	2	0	2	0.3 [0.02-1.60]	-	0.7 [0.04-3.73]	2.00	-	2.00
	A. clausii	6828	979	5849	2	-	2	3	0	3	0.02 [0-0.011]	-	0.03 [0-0.013]	1.50 ± 0.71	-	1.50 ± 0.71
	P. hebes	177	75	102	-	-	-	-	-	-	-	-	-	-	-	-
	T. longicornis	3915	889	3026	2	-	2	2	0	1	0.1 [0-0.31]	-	0.1 [0-0.38]	1.00	-	1.00
SO	*C. helgolandicus*	1229	202	1027	14	4	10	23	10	13	1.1 [0.65-1.90]	1.9 [0.67-4.99]	1.0 [0.50-1.79]	1.64 ± 1.15	2.50 ± 1.73	1.30 ± 0.67
	C. carinatus	6678	1170	5508	3	1	2	3	1	2	0.04 [0.01-0.14]	0.1 [0-0.53]	0.03 [0-0.14]	1.00	1.00	1.00
	C. chierchiae	183	87	96	1	1	-	1	1	-	0. 5 [0.03-3.99]	1.1 [0.06-6-06]	-	1.00	1.00	-
	A. clausii	1054	140	914	-	-	-	-	-	-	-	-	-	-	-	-
	P. hebes	1537	457	1080	-	-	-	-	-	-	-	-	-	-	-	-
	T. longicornis	139	46	93	-	-	-	-	-	-	-	-	-	-	-	-

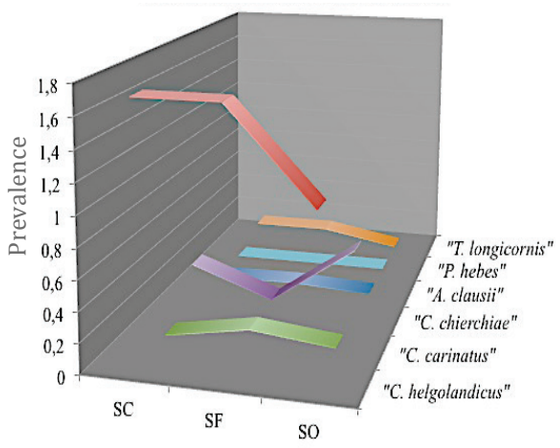


Fig. 3.II.1. Prevalence variation values among summer communities where 6 species of copepods were studied for parasites.

Table 3.II.3. P-values from the Fisher's exact test that compared prevalences among summer communities where summer coastal = SC; summer frontal = SF; summer ocean = SO. Ordinate axis (MedTotalIntensit) = median of the total intensity. No significant differences were found.

Fisher's exact test for comparing prevalences				
Species	**SC**	**SF**	**SO**	**P-Value**
C. helgolandicus	1.7	1.7	1.1	0.383
C. carinatus	0	0.1	0.04	0.58
C. chierchiae	0.3	0.1	0.5	0.546
A. calusii	0.02	0.03	0	1.00
P. hebes	0	0	0	1.00
T. longicornis	0.1	0.1	0	0.474

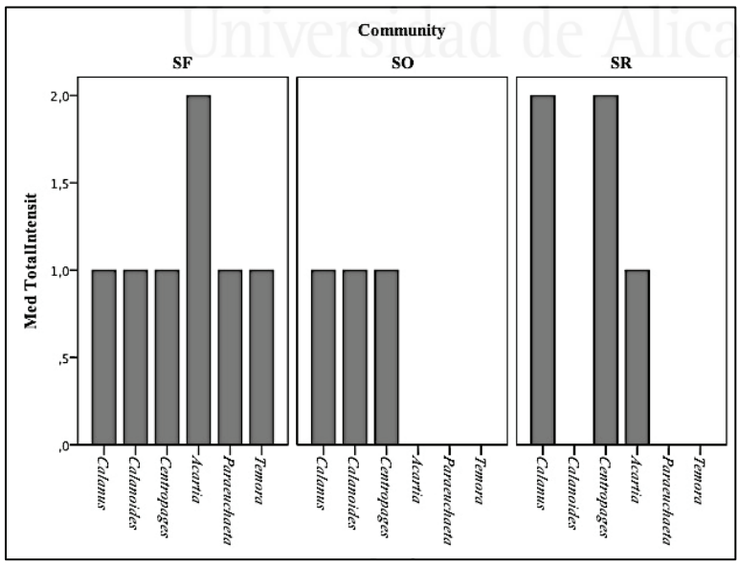


Fig. 3.II.2. Median intensity among communities. Summer coastal = SC; summer frontal = SF; summer ocean = SO. Ordenate axis = median of the intensity classed among communities and species.

Table 3.II.4.. P-values from the Moos's median tests for comparing median intensities among communities. Summer coastal = SC; summer frontal = SF; summer ocean = SO. Ordenate axis = median of the total intensity classed among communities and species.

Mood's median test for comparing median intensities				
Species	**SC**	**SF**	**SO**	**P-Value**
C. helgolandicus	2.00	1.00	1.00	0.137
C. carinatus	0.00	1.00	1.00	1.00
C. chierchiae	2.00	1.00	1.00	1.00
A. calusii	1.50	2.00	0.00	1.00
P. hebes	0.00	0.00	0.00	1.00
T. longicornis	1.00	1.00	0.00	1.00

Within summer communities we found significant differences in the proportion of the infected hosts, these differences were analysed separately by species pairs (Table 3.II.5) where in SC *C. carinatus*, *A.clausii* and *T. longicornis* were the species that contributed to the aforementioned differences. In SF community the proportion of infected hosts were significantly different among all species. Finally, in SO *C. carinatus*, *A. clausii* and *P. hebes* were the species that contributed to the differences found within this community. The total number of parasites among parasitized hosts was not significantly different among communities (Fig. 3.II.3). Finally, within communities the proportion of infected males and females of the same species of the copepod was not significantly different. These differences were not found in the number of parasites or distribution among parasitized population.

Concerning to parasite distribution between different parts of the body copepods we found a higher occurrence on the anterior appendices. In decreasing order of occurrence we found: first maxilla (Mx1), first antenna (A1), maxilleped (Mxpd), mandible (Md), second antenna (A2), second maxilla (Mx2), leg 1 (L1), and caudal ramus (CR) (Table 3.II.6).

Table 3.II.5. Fisher's exact test for comparing prevalences within communities among species of copepods where SC = summer coastal; SF = summer frontal; SO = summer ocean. * = Significant results ($\alpha = 0.05$).

Fisher's exact test for comparing prevalences	
SC	
Sample prevalences:	
C. helgolandicus: 0.017 (10 infected individuals out of 576)	*
C. carinatus: 0.000 (0 infected individuals out of 627)	
C. chiechiae: 0.003 (1 infected individuals out of 358)	
A. clausii: 0.000 (2 infected individuals out of 6830)	
P. hebes: 0.000 (0 infected individuals out of 177)	
T. longicornis: 0.001 (2 infected individuals out of 3197)	
Exact p-value (2-sided) = 0.000	
SF	
Sample prevalences;	
C. helgolandicus: 0.017 (24 infected individuals out of 1381)	*
C. carinatus: 0.001 (7 infected individuals out of 7632)	
C. chiechiae: 0.001 (1 infected individuals out of 701)	
A. clausii: 0.000 (1 infected individuals out of 2905)	
P. hebes: 0.000 (0 infected individuals out of 966)	
T. longicornis: 0.001 (3 infected individuals out of 2191)	
Exact p-value (2-sided) = 0.000	
SO	
Sample prevalences;	
C. helgolandicus: 0.011 (14 infected individuals out of 1243)	*
C. carinatus: 0.000 (3 infected individuals out of 6681)	
C. chiechiae: 0.005 (1 infected individuals out of 184)	
A. clausii: 0.000 (0 infected individuals out of 1054)	
P. hebes: 0.000 (0 infected individuals out of 1537)	
T. longicornis: 0.000 (0 infected individuals out of 139)	
Exact p-value (2-sided) = 0.000	

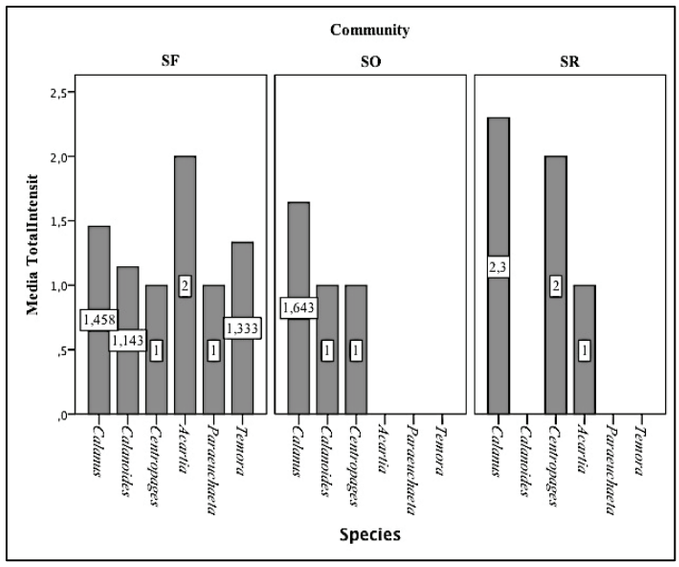


Fig. 3.II.3. Intensity mean among species of copepods analysed in their community where SC = summer coastal; SF = summer frontal; SO = summer ocean. The number of parasites among parasitized hosts was no significant.

Table 3.II.6. Distribution of the attachment points among different parts of the copepod body on 62 analysed animals. N = number of copepods analysed; P = number of parasites per segment of the copepod body; I±SE = Intensity of the gonomeres ± standard error; %G = Percentage of parasites. Mx1 = first maxilla; A1 = first antenna; Mxpd = maxilleped; Md = mandible; A2 = second antenna; Mx2 = second maxilla; L1 = leg 1; CR = caudal ramus.

	N	P	I±SE	%G
Mx1	15	20	1.33±0.82	28.98
A1	14	16	1.14±0.53	23.19
Mxpd	10	10	1	14.49
Md	9	9	1	13.04
A2	8	8	1	11.59
Mx2	4	4	1	5.79
L1	1	1	1	1.44
CR	1	1	1	1.44
Total	62	69	1.11±0.32	100

Different developmental stages were found on a single host (Fig. 3.II.4), but they not exceeded 8 parasites per individual. This number may be even higher,

because younger specimens of the parasite may go overlooked under stereomicroscope due to their extremely small size (Fig. 3.II.5). Their distribution on the various anatomical parts of the copepod body is shown in Fig. 3.II.6.

In general, the most common developmental stage observed was the initial stage (about of 40%) followed by the "prior to the gonomere development" (about 35%). The last developmental stage named "reproductive stage (with gonomere)" showed the lower percentage, about 24%.

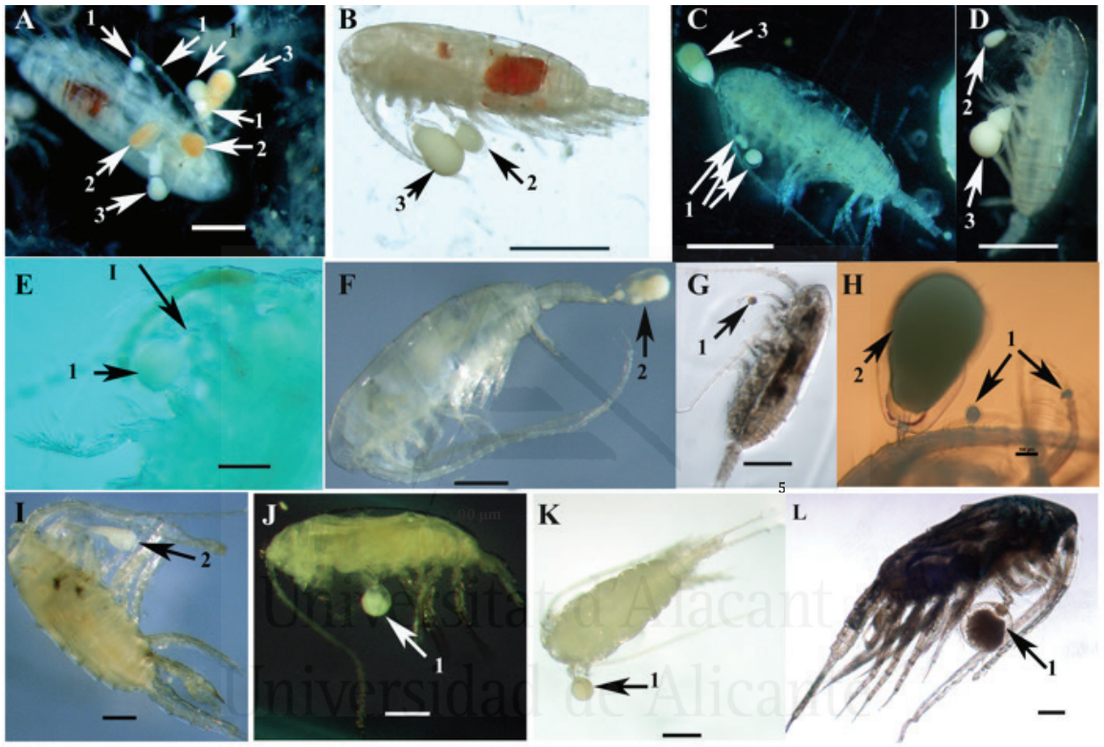


Fig. 3.II.4. Different copepod species infected with *Ellobiopsis chattoni* Parasites were found in different developmental stages as: prior developmental stage (1); prior to the development of the segmented form (2); reproductive stage (3). (A-E) *Calanus helgolandicus* a multi-infected with more than one developmental stage of the parasite normally found concentrated on their feeding appendages. (F) *C. helgolandicus* with an exceptional insertion point of the parasite. (G) *Calanoides carinatus* infected with only one parasite. (H) Two detailed developmental stages in different sizes. (I) *Centropages chierchiae* infected with one parasite. (J) *Acartia clausii* infected with prior developmental stage of the parasite. (K-L) *Temora longicornis* female and male respectively infected with one parasite on the stage 1. Scale bars for: (A, E, F, G) = 500 µm; (B, C, D) = 1 mm; (I, J, K) = 200 µm; (H, L) = 100 µm.

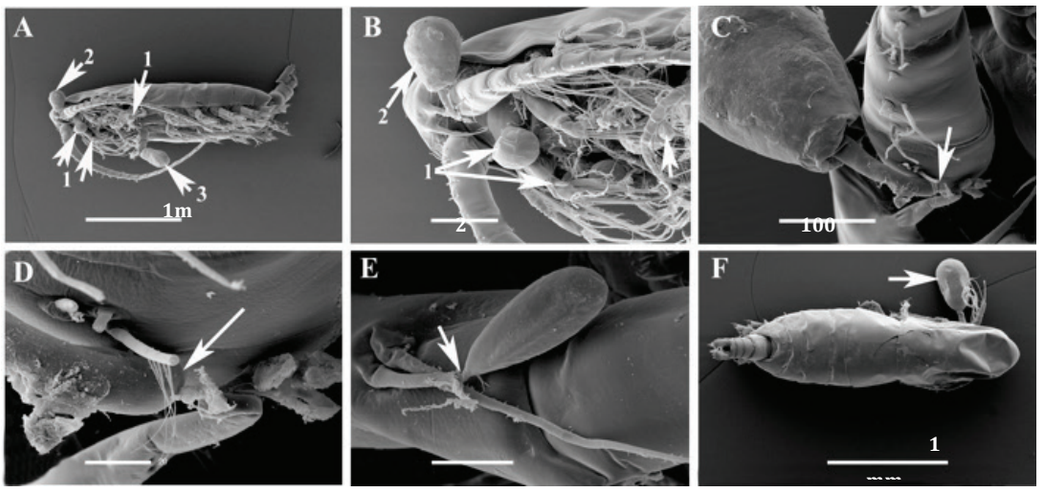


Fig. 3.II.5. Images analysed from the scanning electron microscopy where *Calanus helgolandicus* appeared with different developmental stages of *Ellobiopsis chattoni*: prior developmental stage (1). prior to the development of the segmented form (2); reproductive stage (3). (A, B, F) Are general views of the parasites in different parts of the copepod's body. (C-E) Detailed insertion points of the trophomere into the cuticle of the copepods where the surface is damaged ; (E) Is one of the examples of the younger specimens of the parasite that may go overlooked under stereomicroscope due to their extremely small size

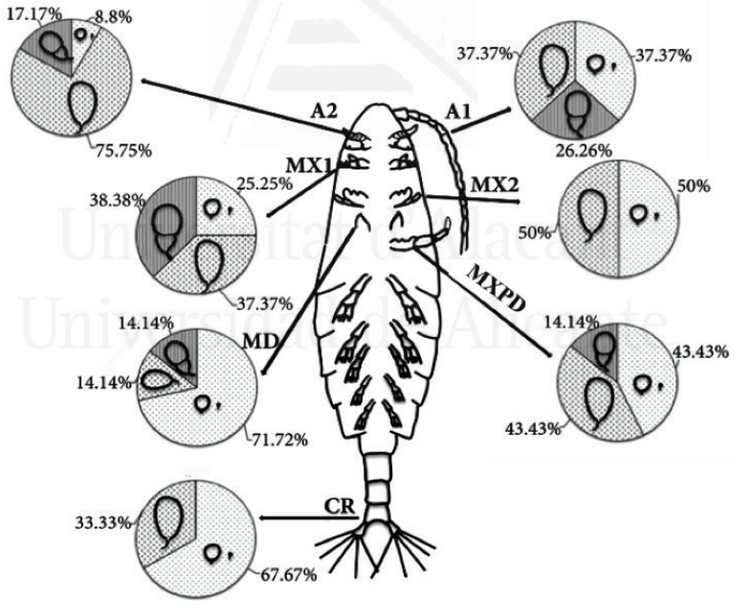


Fig. 3.II.6. Percentages of developmental stages found in different anatomical parts of the copepod bodies where A1 = first antenna; A2 = second antenna; Mx1 = first maxilla; Mx2 = second maxilla; Md = mandible; Mxpd = maxilleped; CR = caudal ramus. = Initial stage of development; = Prior developmental stage; = Prior to the development of the segmented form; = Reproductive stage.

The presence of a single gonomere suggests that the Ellobiopsid found in the present investigation agree with the morphological characters that defined it as *Ellobiopsis chattoni*, as Caullery, 1910 and Shields, 1994 described.

From the qualitative analyses we found that *Calanus helgolandicus* was the most infected copepod found with the higher intensity (Fig. 3.II.7 A-B). Detailed analyses of these samples revealed us a new infected copepod species that was not previously described as host of *Ellobiopsis*. Thus, one *Metridia lucens* Boeck, 1865 female was found infected in autumn ocean on 26/09/08 station 5 in the column water (Table 3.II.7 and Fig. 3.II.8). There we can appreciate where infected animals, through copepod species, were found. Moreover, some of these samples belong to the samples previously studied. The spatial distribution of infected copepods through the time is showed in (Fig. 3.II.8).

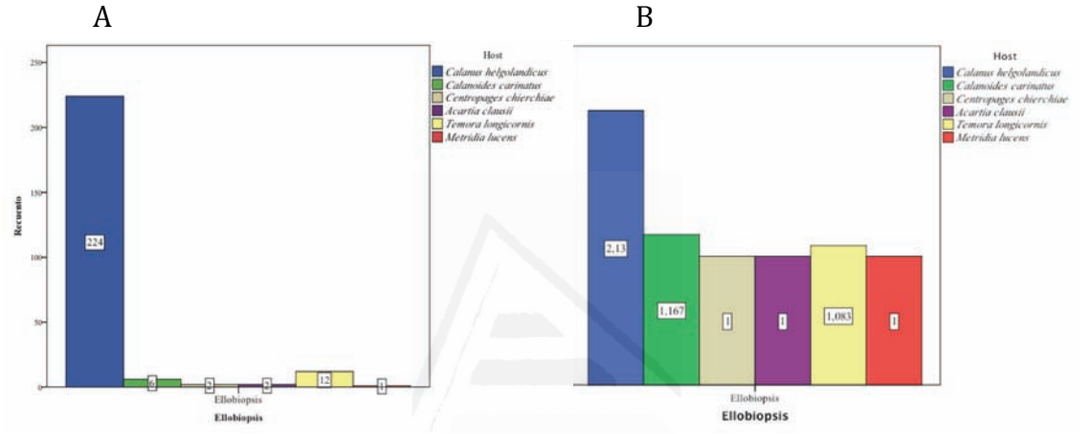


Fig. 3.II.7. A) Number of copepods infected found in samples ramdomly selected. B) Mean intensity of parasites in different copepod species from the randomly studied samples.

Com.	Day/Station/strata	Copepod species Females					Copepod species Males			
		1	2	4	6	7*	1	2	3	6
SC	2-7-08/2/2		1	1	1		1			2
	4-7-08/2/1									1
	4-7-08/2/2	1		1	1		2			
SF	2-7-08/5/2	11	3							
	2-7-08/4/2	30	1		6		3		1	
	4-7-08/3/1									
	4-7-08/3/2	21			1		2			
	9-7-08/5/2	7								
SO	4-7-08/5/1	21						1		
	4-7-08/5/2	16					2		1	
	9-7-08/5/1	82					18			
AO	26-9-08/5/1	5				1				
AC	14-10-08/4/1	2								
Total		**196**	**5**	**2**	**9**	**1**	**28**	**1**	**2**	**3**

Table 3.II.7. Summary of the qualitative results. Com. = community; SC = summer coastal; SF = summer frontal; SO = summer ocean; AO = autumn ocean; AC = autumn coastal. Day/station/strata = Day = date of the sampling; Station = location of the sampling: 5 = transect 5; 4 = transect 4; 3 = transect 3; 2 = transect 2; Strata: 1 = water column; 2 = surface. Copepod species: 1 = *Calanus helgolandicus*; 2 = *Calanoides carinatus*; 3 = *Centropages chierchiae*; 4 = *Acartia clausii*; 6= *Temora longicornis*; 7*= *Metridia lucens* (new record).

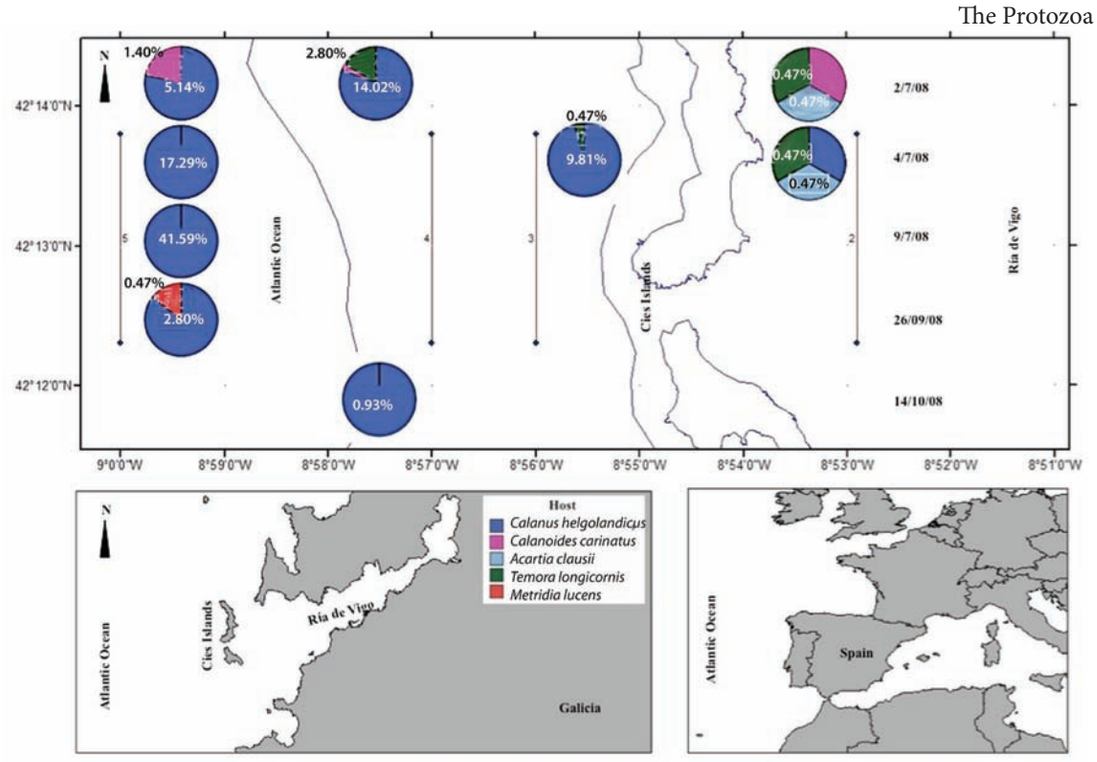


Fig. 3.II.8. Spatial distribution of the infected copepods from qualitative sampling Transects from 5, 4, 3, outside off Ría of Vigo and 2 inside.

3.II.5. Discussion

Ellobiopsis spp. have been noted in many species of Calnoid as *Calanus helgolandicus, Pseudocalanus elongates* Boeck, 1865, *Ctenocalanus vanus* Giesbrecht, 1888 and *Temora longicornis* in Bay of Biscay in North Atlantic Ocean (Albaina & Irigoien 2006). *Acartia* clausii was recorded as host of this parasite in Bay of Marseille, NW Metiterranean Sea (Gómez et al. 2009) as well as *Calanus* sp. (copepodites), *Temora stylifera* Dana, 1849 and *Calocalanus* sp. in NW Mediterranean sea (Skovgaard & Saiz 2006). *Calanus finmarchicus* Gunnerus, 1770 was reported carrying *Ellobiopsis* sp. in Loch Striven Scotland, Norvegian Sea and Kongsfjorden Greenland Sea (Marshall et al. 1934, Timofeev 2001, Walkusz & Rolbiecki 2007). *Undinula vulgaris* Dana, 1849 in Zanzibar Channel of Africa (Wickstead 1963), *Metridia longa* Lubbock, 1845 in Kachemak Bay, Alaska (Hoffman & Yancey 1966), *Calanus glacialis* Jaschnov, 1955 in Kongsfjorden Greenland Sea (Walkusz & Rolbiecki 2007) and *Undinula vulgaris* in Bay of Bengal, India (Santhakumari & Saraswathy 1979). More infected copepods species are summarized in Shields (1994) and Walkusz and Rolbiecki (2007). Thus, as far as

we know this is the first time that *Ellobiopsis chattoni* has been found on *Calanoides carinatus*, *Centropages chierchiae* and *Metridia lucens* in NE Atlantic waters extending the host range for *E. chattoni*.

It is widely accepted that mortality is considered one of the main factors able to modulate life-history traits and structure of planktonic communities (Verity & Smetacek 1996). This population feature is also one of the most difficult parameters to quantify in nature where the marine environment is extremely patchy, diluted and three-dimensional affected by different oceanographic events. In this sense it is know that some protistan parasites of copepods as *Syndinium turbo* Chatton, 1910 obligatorily kill their hosts and produce infective spores in massive numbers. In contrast, *Ellobiopsis* keeps their hosts alive, but it reduces fecundity in females or cause feminization in males because parasites are able to decrease reserves in host that are available for reproduction (Shields 1994, Albaina & Irigoien 2006, Skovgaard & Saiz 2006). The castration effect in females should not be neglected since this effect not only keeps infected females without eggs production, but also because mating with a sterile female reduces successful mating of healthy males. Thus, sterility caused by parasites plays an important role on the host population regulation but their impact remains understudied.

The pathology caused by this parasite in the copepod host is little known. Nevertheless the stalk penetration into the appendages of the host causes localized damage to the carapace and the surrounding musculature (Shields 1994). Moreover, with high levels of infection this damage could be increased significantly even dramatically for host survival. On the one hand, the accumulation of *Ellobiopsis* in mouth appendages presumably would impede proper feeding of the animal causing starvation. On the other hand, this great intensity of parasites could increase burden drag forces, thereby impeding locomotion and increasing energy expense by the host (Fernandez-Leborans 2010). This last effect could also affect to the buoyancy and the ability of the copepod to stay in the most favourable level in the water column. Additionally, the animal could be more easily preyed regulating their population by predator-prey interaction induced by parasites.

Concerning prevalence infection in copepods our results are comparable with that found in the published literature on the topic Table 3.II.8.

According to Rózsa et al. (2000) differences found in the different comparisons, among prevalence, median of the intensity and average intensity of infection, in a variety of sampled hosts do not allow us to answer which of the samples is more parasitized. In this sense, the proportion of infected hosts was similar in the 3 communities since that at least 3 species of parasitized copepods were found, however Calanus helgolandicus remained higher values of infection. The infection levels showed to be homogeneous among the communities studied. Consequently we cannot conclude which was the most parasitized community. This fact could be due to the presence of these 6 copepod species through the summer communities where in turn they are the most abundant copepods in the studied area (Gregori et al. 2013). Different abundance of copepods was found through summer communities according to their life strategies. Thus in SC from higher to lower abundance we found *A. clausii*, *T. longicornis*, *C. helgolandicus*, *C. chiercheae*, *P. hebes* and *C. carinatus*.

The abundance was varying following the coastal-oceanic gradient. Accordingly in SF we found that *C. carinatus*, *C. helgolandicus*, *C. chiechiae*, *P. hebes* and *T. longicornis* increased its abundance while *A. clausii* diminished it. Finally, in SO community only *P. hebes* increased its abundance whereas the rest of the species decreased it.

Within SC community, differences found in the proportion of different species of infected copepods could be due to high number and abundance of *A. clausii* and *T. longicornis*. This community was mainly located in coastal waters zone where these neritic species prefer to be. Likely this high population concentration allowed *Ellobiopsis chattoni* to infect them. Contrary, we found that neritic-oceanic species as *C. carinatus* were not infected because their number and abundance were comparatively low respect to *C. helgolandicus* specie which were co-occurring.

Within SF community we observed that the proportion of infected animals among species were different. In fact, SF is a very variable community that was located between the coastal and oceanic domains. In this intermediate domain the continuous intrusions of animals from both communities, SC and SO, forced by the

Table 3.II.8. Reported prevalence of Ellobiopsis sp. In copepod expressed as prevalence (%). * References found in (Walkusz & Rolbiecki 2007). ** References from (Albaina & Irigoien 2006). ♀ = Only females were studied. • Prevalence average.

Host	%	Location	Reference
Calanus finmarchicus	0.3	Loch Striven, Scotland	Marshall et al. (1934)*
Undinula vulgaris	26	Zanzibar Channel of Africa	Wickstead (1963)*
Metridia longa	5-22.4	Kachemark Bay, Alaska	Hoffman and Yancey (1966)
Undinula vulgaris	8.3	Bay of Bengal, India	Santhakumari and Saraswathy (1979)**
C. finmarchicus	15-45	Norvegian Sea	(Timofeev 1997, 2002)
C. helgolandicus	6.8♀	Bay of Biscay, Spain	Albaina and Irigoien (2006)
C. finmarchicus	0.06	Kongsfjorden, Greeland Sea	Walkusz and Rolbiecki (2007)
C. glacialis	0.09		
Acartia clausii *A. danae* *Acrocalanus gibber* *Paracalanus aculeatus*	0.56-1	Persian Gulf, Kuwait	(Fahmi & Hussain 2003)
Calanus helgolandicus	1.5•	NE Atlantic Ocen (Galician waters) Spain	Present study
Calanoides carinatus	0.07•		
Centropages chierchiae	0.3•		
Acartia clausii	0.025•		
Temora longicornis	0.1•		
Metridia lucens	No data		

upwelling/downwelling currents might favour conditions of *E. chattoni* transmission through different copepod species.

The most neritic-coastal copepods decreased in number and abundance in SO, while the most oceanic ones increased. *P. hebes* increased their abundance due to habitat preference (the oceanic domain), but we did not find any animal infected with the parasite studied. On the other hand, *A. clausii* neither show any parasite, probably due to their decreasing abundance. Finally, although *C. carinatus* decreased their abundance, it remained high comparing with *C. helgolandicus*.

Herein we studied 6 dominant copepod species from different zooplankton samples where we found infected animals with specimens of *Ellobiopsis*. We

assumed that a single *Ellobiopsis* species is responsible for the infection of multiple host species, however it has been reported that different copepod species appear to have different susceptibilities to the ellobiopsid infection. The different prevalences that were showed by the co-occurring species could explain different susceptibilities of the copepods to be infected. In spite of having different host susceptibilities to the infection, of the 6 co-occurring populations of copepods selected for the study, only *C. helgolandicus* showed the highest prevalence in both, within communities and through them. This supports the host specificity of *Ellobiopsis chattoni* that it has been suggested in previous studies (Wickstead, 1963 in (Albaina & Irigoien 2006) (Hoffman & Yancey 1966); (Albaina & Irigoien 2006). Albaina and Irigoien (2006) studied 1706 females of *C. carinatus* whereas here we studied 12552 of them, so it is possible that they did not find any infected animal because their sample was not higher enough.

The infected hosts carry a similar number of parasites, this fact could be due to the earlier stage of development showed by the parasite that probably was being recruited. It could also be possible that the habitat (not *C. helgolandicus*) was not the best for the parasite growth and development. Because the development of the parasites is very fast we found the rest of the stages in the same host. This is not an isolated case, it is believed that different developmental stages can be found in the same host at the same time, but only one or two trophomeres are able to mature at one time (Jepps 1937 in (Shields 1994). Although the parasite-carrying capacity is limited per host more than one maturational stage in the same animal were found, likely due to the ability of the host to provide enough resources for both, parasites and for himself.

According to (Shields 1994, Albaina & Irigoien 2006) *Ellobiopsis* were found infecting more females than males due to their sex preference. The fact that we did not find these differences could be due to the low power of the analyses and the low number of copepods studied. It is clear that more than one variable (biotic and abiotic) are affecting the prevalence observed here. Which is the variable that better explain the results observed will be studied in future works.

The preferred attachment points of *E. chattoni* were the mouth appendages. As was previously suggested by (Shields 1994, Albaina & Irigoien 2006, Walkusz & Rolbiecki 2007) the feeding behaviour of the animal could explain it. They create

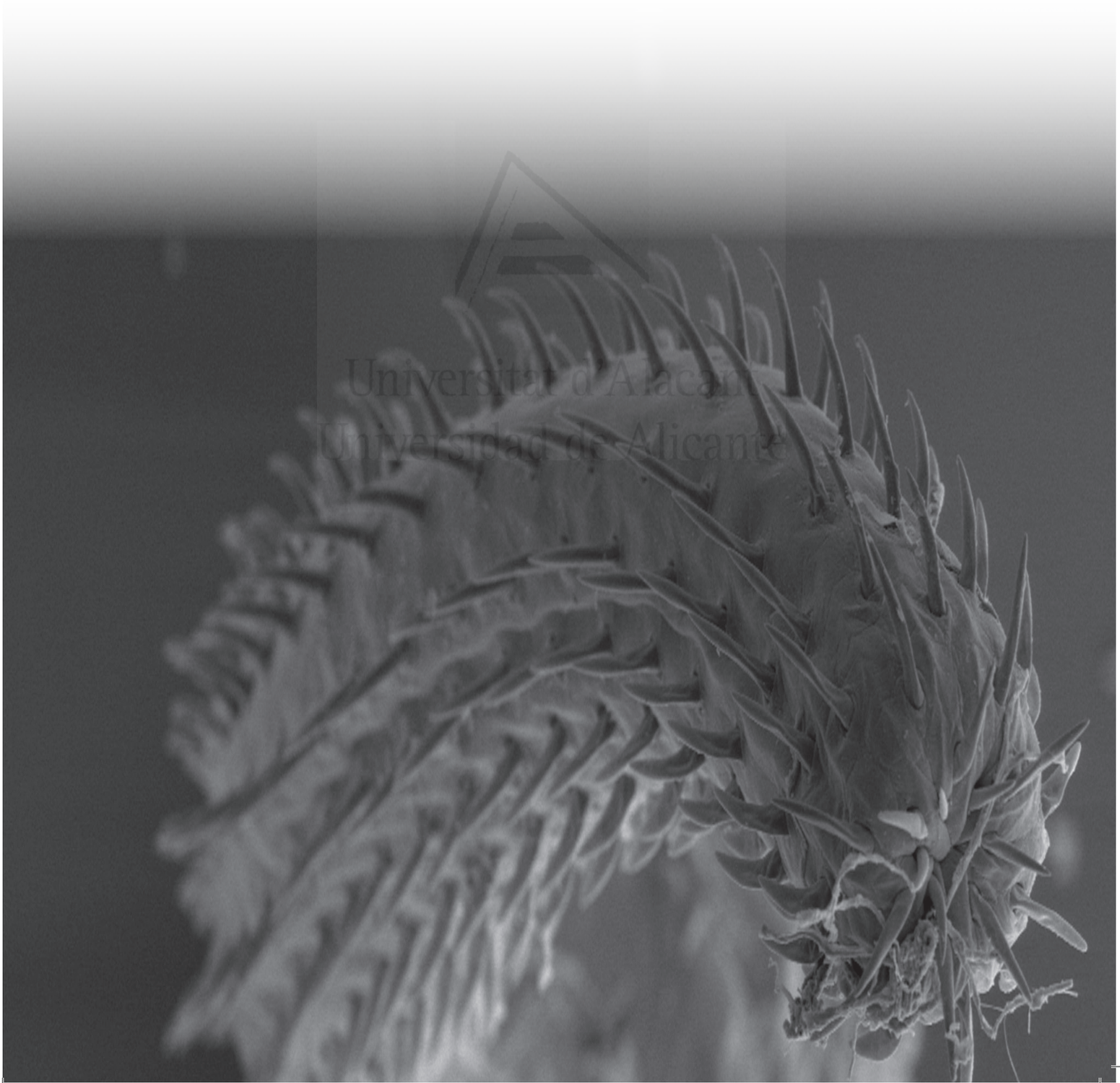
currents with their cephalic appendages directioning alimentary particles from the top of the animal to the mouth area. The spores of the parasite are catched within this current where the first exposed part of the animal corresponds with the described pattern. Thus, the first maxilla and first antenna becomes the first exposed. The swimming legs carry few if any parasites as well as the furca. This pattern differs slightly from the exposed by Albaina and Irigoien (2006) where the Antennule was the first exposed part of the copepods body.

In this study, parasite prevalence was quantified by examining the morphology of fixed zooplankton. Many parasites, as the initial developmental stages of *Ellobiopsis* need to reach a minimum size before they are detectable during traditional microscopic studies nevertheless, as ectoparasite it can be more accurately detected through microscopic examination. Furthermore, parasites may be lost especially when both, they are fixed with methanol and the zooplankton concentration is extremely high. Hence, observed parasite prevalence is definitely an underestimate of total prevalence. This fact is easily detectable from the observed qualitative database presented here. In there we can appreciate that the number of infected animals was higher than detected with a quantitative analyses. Moreover, this examination allowed us to detect new hosts (*Metridia lucens*). Due to that we are able to conclude that observed prevalences are an underestimate of total ones. Thus, more sensitive methods should be developed an applied to better assess total parasite prevalence. Detection of specific parasites may be facilitated by molecular probes, or, perhaps using the next generation DNA sequencing (Le Roux et al. 1999, Audemard et al. 2002, Shendure & Ji 2008).

Experimental infection studies are needed to elucidate lot of questions that remains unknown as for example, whether a single strain of *Ellobiopsis* is able to infect different copepod species. In addition, further molecular and ultrastuctural studies, including the survey of different seasons, hosts and geographical locations will address the question of whether *E. chattoni* constitutes an independent species, widespread on a global basis, or a species complex. Moreover, experimental studies should be addressed to explore different susceptibilities to the hosts for this parasite and contrast the specificity of the parasite towards *Calanus helgolandicus*, specifically in females. Furthermore, concerning transmission, how the spores are able to detect and attach to the host. In addition

to this, the study of the physiological, behavioural and immunological responses of copepods to avoid this attachment it could improve and increase our knowledge. Finally, more efforts and studies should aim to elucidate the influence of oceanographic processes.

The Acanthocephala



4. THE ACANTHOCEPHALA

4.I. *NYCTIPHANES COUCHII* AS INTERMEDIATE HOST FOR THE ACANTHOCEPHALAN *BOLBOSOMA BALAENAE* IN TEMPERATE WATERS OF THE NE ATLANTIC

4.I.1. Abstract

Cystacanths of the acanthocephalan *Bolbosoma balaenae* were found encapsulated in the cephalothorax of the euphausiid *Nyctiphanes couchii* from temperate waters in the NE Atlantic Ocean. Euphausiids were caught in locations outside the Ría of Vigo in Galicia, NW Spain, and prevalence of infections was up to 0.1%. The parasite was identified by morphological characters. Cystacanths were 8.09 ± 2.25 mm total length (mean ± SD) and had proboscises that consisted of 22 to 24 longitudinal rows of hooks, each of which had 8 or 9 hooks per row including 2 or 3 rootless ones in the proboscis base and 1 field of small hooks in the prebulbar part. Phylogenetic analyses of 18S rDNA and cytocrome c oxidase subunit I revealed a close relationship with other taxa of the family Polymorphidae. The results extend northwards the known distribution of *B. balaenae*. Taxonomic affiliation of parasites and trophic ecology in the sampling area suggest that *N. couchii* is the intermediate host for *B. balenae*, and we suggest that the whales *Balaenoptera physalus* and *B. Acutorostrata* are its definitive hosts. This life cycle is probably completed with or without paratenic hosts.

4.I.2. Introduction

The most frequent parasites in marine zooplankton appear to be protists hosted by copepods (Skovgaard & Saiz 2006). However, there are other groups of parasites, mostly platyhelminths, nematodes and acanthocephalans, which are also common and play a major part in both host life cycles and ecosystem food webs (Marcogliese 1995, 2002, 2004). Among zooplankton communities, euphausiids play an important role as intermediate hosts in the pelagic realm (Marcogliese 1995). They are able to attain massive biomasses and can form vast and dense swarms occupying one of the lowest trophic levels. Additionally they can be used

by different types of parasites to reach their definitive host (Mauchline 1980, 1984, Marcogliese 2002).

Nyctiphanes couchii Bell, 1853 is the most abundant euphausiid in European Atlantic waters and occurs very close to the European continental shelf. Locations where they are most abundant are near the Spanish coast, the Celtic Sea, the coasts of Ireland, Scotland and north eastern England and the entrance to the Skagerrak (Lindley 1977). In the NE Atlantic Ocean it is one of the main prey items for different cephalopods and fish species, which in turn become part of the diet of other vertebrates that serve as definitive hosts for a number of parasites. In addition, euphausiids are also a sizeable ingredient of the diet of some marine mammals (Raga et al. 2009). Despite the fact that parasites have great ecological importance, their recruitment (especially for acanthocephalans) in the lower levels of the food web and the role that they play is poorly understood.

Adult polymorphid acanthocephalans are intestinal parasites of marine mammals, fish-eating birds and waterfowl. *Bolbosoma* Porta, 1908 and *Corynosoma* Lühe, 1904 are two of the main genera of intestinal parasites that infect marine mammals (Aznar et al. 2006). The life cycle of *Bolbosoma* species is thought to involve pelagic marine zooplankton, especially pelagic euphausiids and copepods, as an intermediate host (Hoberg et al. 1993) and different species of fish as paratenic (transport) hosts (Raga et al. 2009). The juvenile forms of acanthocephalans are cystacanths, which are morphologically similar to the mature worms but differ from them in the size of the trunk and the degree of development of the sexual organs (Zdzitowiecki 1991, Hoberg et al. 1993). Moreover, these juvenile forms are widely considered to be the infective stage for definitive hosts. These cystacanths appear contracted with an introverted proboscis and neck inside cysts of the intermediate and paratenic hosts. The genus *Bolbosoma*, established for Acanthocephala from whales, contains 15 species (Amin 1985) and has a worldwide distribution (Measures 1992). Despite this, no previous data on the presence of *B. balaenae* cystacanths in euphausiids from the NE Atlantic Ocean are available. With the exception of Shimazu (1975) and Tsimbalyuk (1980), *Bolbosoma* has not been reported in euphausiids. Shimazu (1975) described the larvae of *B. caenoforme* Heitz, 1920 from *Thyssanoessa longipes* Brandt, 1851 and *T. raschi* Sars, 1864 in the North Pacific Ocean. On the

other hand, Tsimbalyuk (1980) found *Bolbosoma* sp. infecting *Thyssanoessa* sp. Recently Gómez-Gutiérrez et al. (2010) reported 3 Polymorphidae larval stages of *Nyctiphanes simplex* Hansen, 1911 (probably *Bolbosoma* or *Corynosoma)* off the northwestern coast of Mexico. Considering the background of the identification of parasites in the mesozooplankton communities from the NE Atlantic Ocean, the aims of this paper are to (1) undertake the taxonomic and molecular diagnosis of cystacanths of *Bolbosoma balaenae* (Gmelin, 1790) found in communities of *Nyctiphanes couchii*, thereby ascertaining the role of krill in the life cycle of acanthocephalan parasites from northwest of the Iberian Peninsula, (2) provide data on the occurrence of cystacanths of *B. Balaenae* in the *N. couchii* population and (3) explore the potential life cycle strategies for these cystacanths to reach their definitive host.

4.I.3. Material and Methods

4.I.3.1. Collection and processing samples

The zooplankton sample was collected off the coast of Ría de Vigo in Galician waters, NW Iberian Peninsula, in 1 tow of length 1 nautical mile (from 42.21° N, 09° W to 42.23° N, 09° W) (Fig. 4.I.1 T5 transect) with the RV *Mytilus* in July 2008.

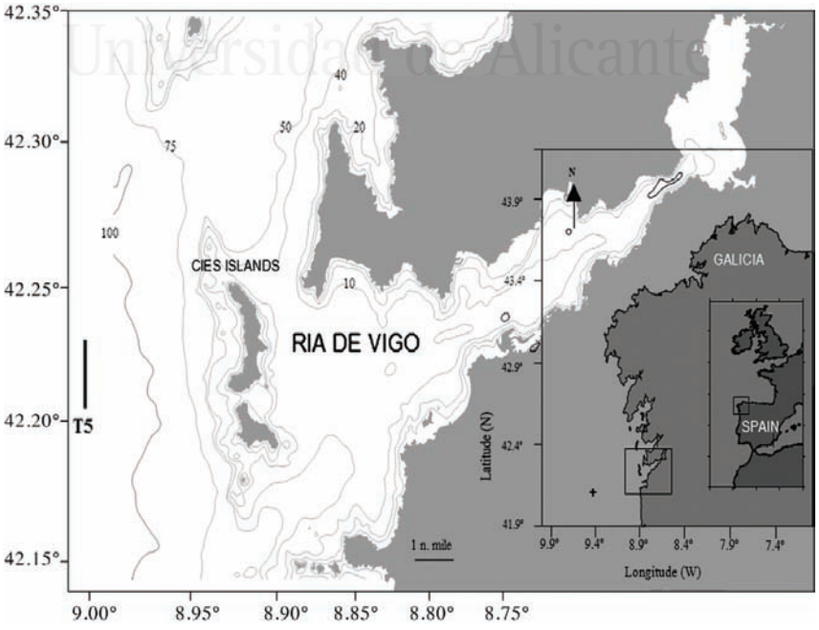


Fig. 4.I.1. Sampling transect (T5) off the Ría de Vigo in Galician waters, NE Atlantic

The sample was collected by double oblique towing, using a 750 mm diameter bongo net with 375 µm mesh size. At a ship speed of 2 knots, the bongo net was first lowered and stabilized near the bottom for a period of 15 min, then hauled to the surface at 0.5 m^{-1}. The bongo net was equipped with a current meter, which allowed the volume of water filtered during the haul to be calculated, thus permitting zooplankton abundance (as nº organisms m^{-3}) to be estimated. The sample was filtered with a 500 µm mesh sieve and fixed onboard with 100% ethanol. In the laboratory samples were transferred to 70% ethanol and stored at −20°C. The abundance of the different zooplankton taxa was calculated after counting a subsample obtained from a Folsom splitter (Omori & Ikeda 1984). Organisms were identified to the lowest possible taxonomic level. Species diversity was calculated by using the Shannon-Wiener and Species Evenness Indices (Omori & Ikeda 1984, Guisande et al. 2006). The number of euphausiids was estimated by the method of calculating precise replica (Andrew & Mapstone 1987).

All the zooplankton components of the sample were examined for acanthocephalans with a stereomicroscope (20x). When cystacanths were found, they were extracted from the host by dissection. Cystacanths were analyzed by using morphological methods. The morphological characters used for identification followed (Delyamure 1955, Petrochenko 1958, Yamaguti 1963, Zdzitowiecki 1991, Peterson 1998). The most important diagnostic characters for generic and specific assignation of species within Polymorphidae are the proboscis armature and the pattern of trunk spination, particularly of the anterior trunk (Zdzitowiecki 1991, Aznar et al. 2006) and references therein). Since the proboscis, the neck and part of the anterior trunk were introverted in all specimens, we cut the trunk posterior to the proboscis receptacle and dissected it to evert the proboscis and the anterior trunk. This body part was dissected and cleared with lactophenol to improve the visibility of structures. The rest of the body was cleared before DNA extraction. We cleared them by using different concentrations of glycerine and 70% ethanol in order to observe the degree of sexual development. The sample was initially washed in a clearing solution that consisted of 1 part pure glycerine and 3 parts 70% ethanol for 5 min. For the second wash we used equal volumes of glycerine and 70% ethanol for 5 min. For the third wash we used 3 parts glycerine and 1 part 70% ethanol for 10 min, and the final wash contained

100% glycerine. This method allowed us to extract DNA from the body because the organisms were not destroyed. The following measurements were recorded: body length and width, foretrunk width (the typical bulb of *Bolbosoma*, see (Amin & Margolis 1998) and length of receptacle and proboscis. When possible, the number of rows of hooks and number of hooks per row were counted, and hooks were measured. Prevalence and intensity were estimated as described by Bush et al. (1997). The CI for prevalence was calculated by the method described by Agresti and Coull (1998) using an R macro developed by S. DoraiRaj (http:// rss. acs. unt. edu/ Rdoc/ library/ binom/ html/binom. confint. html, accessed 13 October 2011). Comparisons of prevalences were performed by using Fisher's exact tests (Rózsa et al. 2000).

4.I.3.2. Genomic DNA extraction and PCR amplification

Genomic DNA was isolated by using Qiagen DNeasy™ Tissue Kit according to manufacturer's instructions. DNA quality and quantity was checked in a spectrophotometer (ND-1000, Nanodrop Technologies) and in 1% agarose gel. The primers LCO1490 (5-GGT CAA CAA ATC ATA AAG ATA TTG G-3) and HCO2198 (5-TAA ACT TCA GGG TGA CCA AAA AAT CA-3) (Folmer et al. 1994) were used to amplify approximately 700 bp of the cytochrome oxidase *c* subunit I (COI), and the primers 18SU467F (5-ATC CAA GGA AGG CAG CAG GC-3) and 18SL1310R (5-CTC CAC CAA CTA AGA ACG GC-3) (Suzuki et al. 2008) were employed to amplify approximately 900 bp of the small 109 subunit (18S) ribosomal RNA gene.

PCR reactions were performed in a total volume of 25 µl containing 1 µl of genomic DNA (150 to 200 ng), PCR buffer at 1x concentration, 1.5 mM $MgCl_2$, 0.2 mM nucleotides (Roche Applied Science), 0.3 µM primers and 0.625 U *Taq* DNA polymerase (Roche Applied Science). The cycling protocol for the COI gene was 2 min at 94°C, 35 cycles with 30 s at 94°C, 30 s at 50°C and 1 min at 72°C, followed by 7 min at 72°C. The cycling protocol for the 18S rRNA gene was 2 min at 94°C, 35 cycles with 30 s at 94°C, 1 min at 55°C and 2 min at 72°C, followed by 7 min at 72°C. All PCRs were carried out in a TGradient thermocycler (Biometra) and a negative control (no DNA) was included for each set of PCR reactions. PCR products were separated on a 2% agarose (in 1x Trisacetic EDTA buffer) gel, stained with

ethidium bromide and scanned in a GelDoc XR documentation system (Bio-Rad Laboratories).

4.I.3.3. DNA sequencing and phylogenetic analysis

The positive PCR products were cleaned for sequencing by using ExoSAP-IT© (USB) as supplied by the manufacturer. Sequencing was performed by Secugen (Madrid, Spain). The chromatograms were analyzed with ChromasPro v. 1.41 (Technelysium). To complete the partial sequence of the 18S rDNA gene, the specific primer Bolbo-F1 (5-CTA TCG CCA ACG CTT TAT CT-3) was designed by using the program Primer-3 (Rozen & Skaletsky 2000) Sequences were subject to basic local alignment search tool (BLAST) analyses with BLASTn against available sequences from GenBank, through web servers of the National Center for Biotechnology Information (USA). For phylogenetic analyses the sequences obtained were aligned with other acanthocephalan sequences available on GenBank. Taxa used for 18S rDNA and COI analyses are listed in Table 4.I.1. Species and GenBank accession number of taxa used for (a) 18S rDNA and (b) COI analyses.; the rotifer *Rotaria rotatoria* was used as the outgroup.

Taxon and authority	Accession number
(a) 18S rDNA analysis	
Acanthocephaloides propinquus (Dujardin, 1845)	AY830149
Andracantha gravida (Alegret, 1941)	EU267802
Corynosoma magdaleni (Montreuil, 1958)	EU267803
Corynosoma strumosum (Rudolphi, 1802)	EU267804
Echinorhynchus gadi (Zoega, 1776)	AY218123
Gorgorhynchoides bullocki (Cable & Mafarachisi,	AY830154
Hexaglandula corynosoma (Travassos, 1915)	EU267808
Ibirhynchus dimorpha (Schmidt, 1973)	GQ981436
Pararhadinorhynchus sp.	HM545903
Polymorphus minutus (Goeze, 1782)	EU267806
Pseudocorynosoma constrictum (Van Cleave, 1918)	EU267800
Taxon and Authority	GenBank nº
Rhadinorhynchus sp.	AY062433
Southwellina hispida (Van Cleave, 1925)	EU267807
Transvena annulospinosa (Pichelin & Cribb, 2001)	AY830153
Outgroup: *Rotaria rotatoria* (Pallas, 1766)	AY218121
(b) COI analysis	
Andracantha gravida	EU267822
Arhythmorhynchus frassoni (Molin, 1858)	EU189484
Corynosoma strumosum	EF467871
Echinorhynchus gadi	AY218095
Hexaglandula corynosoma	EU189488
Ibirhynchus dimorpha (Schmidt, 1973)	GQ981438
Polymorphus brevis (Van Cleave, 1916)	EF467861
Profilicollis botulus	EF467862
Pseudocorynosoma anatarium (Van Cleave, 1945)	EU267821
Rhadinorhynchus sp.	DQ089712
Southwellina hispida	FJ824189
Transvena annulospinosa	DQ089711
Outgroup: *Rotaria rotatoria* (Pallas, 1766)	EU499879

Table 4.I.1. Species and GenBank accession number of taxa used for (a) 18S rDNA and (b) COI analyses.

Alignment was accomplished by using the Clustal W algorithm in MEGA v. 3.1 software (Kumar et al. 2004) with settings at defaults: gap opening/gap extension penalties = 15/6.66 for both pairwise and multiple alignments, and with transitions weighted at 0.5. Maximum parsimony analysis was conducted by using the close neighbour interchange (CNI) heuristic option with initial trees by random addition of 1000 replicates, a search level of 1 and bootstrap values calculated over 1000 replicates.

Minimum evolution analysis was performed by using the Nucleotide Maximum Composite Likelihood model, the CNI heuristic option with a search level of 2, and bootstrap values were calculated over 1000 replicates. Nucleotide sequence data reported in this paper are available in the GenBank under the Accession No. JQ040303–6.

4.I.4. Results

The composition, total abundance and density of taxa collected in the zooplankton samples, as well as the Shannon-Wiener Index and Species Evenness Index values for the entire assemblage, are shown in Table 4.I.2. The most abundant taxon was *Nyctiphanes couchii* (Euphausiacea) which contributed ~49.09% to the total zooplankton community.

Taxon and authority	n	A	%
Amphipoda			
Gammaridea (Latreille, 1802)	85	0.21	0.03
Brachyura zoeae	2462	6.00	0.78
Copepoda			
Acartia clausii (Giesbrecht, 1889)	7421	18.08	2.34
Calanoides carinatus (Kroyer, 1849)	72 258	176.08	22.78
Calanus helgolandicus (Claus, 1863)	35 155	85.67	11.08
Centropages chierchiae (Giesbrecht, 1889)	3966	9.67	1.25
Paraeuchaeta hebes (Giesbrecht, 1888)	8637	21.05	2.72
Euphausia			
Nyctiphanes couchii adults (Bell, 1853)	69 954	170.47	22.05
N. couchii calyptopis	34 432	88.78	11.49
N. couchii furcilia	49 387	120.35	15.57
Fish larvae	511	1.25	0.16
Mysidacea	1918	4.68	0.61
Cephalopoda			
Sepiola atlantica (Orbigny, 1839)	7	0.02	0.0022
Ophiuroidea larvae	511	1.25	0.16
Paguridae megalopa	479	1.17	0.15
Polychaeta larvae	5	0.01	0.0013
Siphonophora	1535	3.74	0.48
Stomatopoda			
Meiosquilla desmaresti (Risso, 1816)	479	1.17	0.15
Thaliacea	21 175	51.60	6.68
Total	317 174	772.94	100
Shannon-Wiener index (*H*)	1.555		
Species Evenness index	0.149		

Table 4.I.2. Zooplankton groups represented in the lankton sample obtained off the coast of Ría de Vigo, Galicia, Spain. n = number of individuals; A = Abundance (n m^{-3}); % = percent composition of selected zooplankton categories. Total volume filtered in the tow was 410.37 m^3.

Adults of this species made up ~22.05% of the total sample, whereas furcilia and calyptopis larvae contributed ~15.57 and ~11.49%, respectively. Copepods contributed ~41.68% of the sample, in which *Calanoides carinatus* were the most abundant species of this group (~22.78%), followed by *Calanus helgolandicus* at 11.08%. Thaliacea was also relatively abundant and contributed ~6.68%. The remaining zooplanktonic taxa contributed just ~2.52% (Table 4.I.2).

A total of 70 uncoloured cystacanths putatively identified as belonging to the family Polymorphidae were removed from the thorax of adult individuals of *Nyctiphanes couchii* (Fig. 4.I.2 A). All cystacanths had the neck and proboscis invaginated within the foretrunk and the proboscis receptacle, respectively. After dissection, diagnostic details for a specific assignation were revealed, and all specimens were identified as *Bolbosoma balaenae*. No evidence of genital development was observed in any specimen. A brief morphological description follows.

The cystacanth body was cylindrical (length: 8.09 ± 2.58 mm [mean ± SD], n = 60; width at middle length: 0.41 ± 0.08 mm, n = 47), and had a funnel-shaped bulb (width: 0.68 ± 0.13 mm, n = 59) at the foretrunk (Fig. 4.I.2 B), with a wide apex and narrow base (Fig. 4.I.2 C). A somatic armature was present, and the single field of trunk spines was restricted to the prebulbar part or the foretrunk and composed of 4 to 6 irregular circles of small spines adjacent to the neck (no. of specimens observed = 38). The bulb was unarmed. The proboscis was cylindrical (length: 0.69 ± 0.48 mm, n = 45; width: 0.15 ± 0.08 mm, n = 41) and had 22 to 24 rows of hooks with 8 to 9 hooks per row, 6 with roots and 2 to 3 rootless (30 specimens observed) (Fig. 4.I.2 D). Hook (H) lengths (in µm) were as follows: H1 (39.9 ± 7.8, n = 29); H2 (44.3 ± 8.1, n = 29); H3 (55.2 ± 11.4, n = 27); H4 (59.2 ± 7.3, n = 26); H5 (61.1 ± 6.1, n = 25); H6 (63.8 ± 6.0, n = 25); H7 (67.7 ± 9.2, n = 26); H8 (68.5 ± 5.9, n = 25); H9 (66.2 ± 0.2, n = 2). Voucher specimens were deposited at the Museo do Mar de Galicia, Vigo, Spain, with the accession number MDMG8012011.

The mean prevalence (95% CI) of *Bolbosoma balaenae* in *Nyctiphanes couchii* was 0.045% (0.035 to 0.057%). Considering only adults of *N. couchii*, the prevalence was 0.100% (0.079 to 0.127%). All host individuals harboured single specimens of *B. balaenae*. A comparison of prevalence between *N. couchii* and

arthropod taxa in the zooplankton sample with at least 1,000 individuals is shown in Table 4.I.3. Only 2 copepod species had a significantly lower pre valence tan that of the total sample of *N. couchii*, but all copepod species had significantly lower prevalence than that of *N. couchii* when only adults of the latter species were considered (Table 4.I.3).

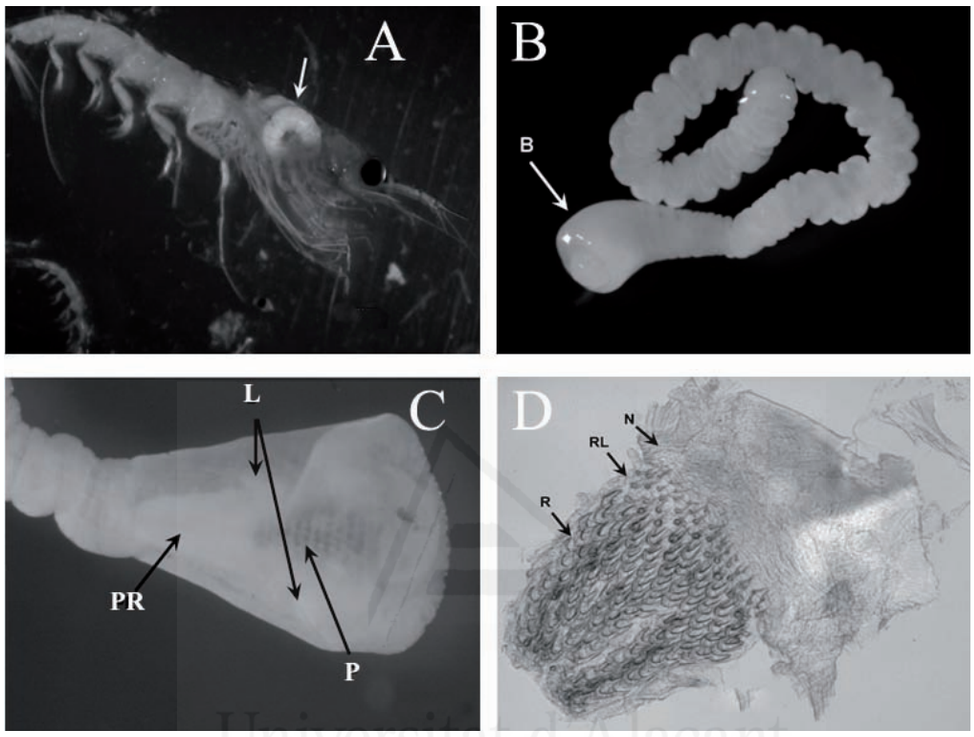


Fig. 4.I.2. *Bolbosoma balaenae*. (A) Cystacanth (arrow) inside the cephalothorax of *Nyctiphanes couchii*. Scale bar = 1 mm. (B) General view of the cystacanth. Arrow: the bulb characteristic of Bolbosoma. Scale bar = 500 µm. (C) Detail of the anterior part of the body showing the funnel-like-shaped bulb that contains the proboscis (P), its receptacle (PR) and lemniscis (L). Scale bar = 200 µm. (D) General view of the proboscis after dissection of the cystacanth showing the neck (N), rootless hooks (RL) and hooks with roots (R). Scale bar = 100 µm.

Table 4.I.3. Results of Fisher's exact tests that compare the prevalence of the acanthocephalan *Bolbosoma balaenae* in the total sample and the sample of adults only of the euphausiid *Nyctiphanes couchii* with that in other arthropod taxa collected in samples of zooplankton caught off Ría de Vigo, Galicia, Spain. Only taxa with ≥1000 individuals are included. Brachyura zoeae were excluded because their small size precluded infections with B. balaenae (see also Table 2). n = number of individuals.

Taxon	n	Prevalence (%) (95% CI)	p-value (*N. couchii*) Total	Adult
Euphausiacea				
N. couchii	155 773	0.04 (0.03–0.06)		
Copepoda				
Acartia clausii	7421	0 (0–0.04)	0.078	0.002
Calanoides carinatus	72 258	0 (0–0.00)		
Calanus helgolandicus	35 155	0 (0–0.00)	<0.001	<0.001
Centropages chierchiae	3996	0 (0–0.08)	<0.001	<0.001
Paraeuchaeta hebes	8637	0 (0–0.03)	0.053	<0.001
Paraeuchaeta sp.	4797	0 (0–0.06)	0.277	0.023
Mysidacea sp.	1918	0 (0–0.16)	1.000	0.268

4.I.4.1. Molecular characterization

The amplified and sequenced 18S rDNA and COI regions of the acanthocephalans were 885 and 512 bp in length, respectively. The G+C contents of the sequenced genes were 48.5% for 18S rDNA and 36.4% for COI. Submission to the BLAST server showed that the most similar se quences in GenBank were the 18S rDNA of *Corynosoma strumosum*, *C. magdaleni*, *C. Enhydri* (Morozov, 1940) and *Andracantha gravida* with identity values of 99%, and the COI of *C. strumosum* with an identity value of 81%. Alignment of sequences showed that 490 sites (59%) were conserved, 333 (40.1%) were variable, 193 (23.2%) were parsimony informative and (16.7%) were singleton for 18S rDNA. Of the COI sites, 150 (30.8%) were conserved, (67.9%) were variable, 219 (45.0%) were parsimony informative and 30 (17.4%) were singleton. Phylogenetic analyses using the minimum evolution (ME) and minimum parsimony (MP) methods yielded a similar tree topology, placing the species within the Polymorphidae. Phylogenetic analyses of the 18S rDNA revealed a close relationship to the sequences of *C. strumosum* and *A. gravida*, with bootstrap values of 100% for ME analysis (not shown) and 96% for MP analysis (Fig. 4.I.3). For COI phylogenetic analyses, a similar dendrogram was obtained but it was supported with low bootstrap values. The trees also showed that the Polymorphidae form a monophyletic group, which is supported by high bootstrap values (>95%).

4.I.5. Discussion

Within the Polymorphidae, the main diagnostic characters to identify taxa are the number and arrangement of proboscis hooks and the patterns of trunk armature (Schmidt 1973, Amin & Margolis 1998, Aznar et al. 2006). For identification purposes, one advantage of these characters is that they are already visible at the cystacanth stage and remain unmodified during the adult development (Van Cleave 1953). This is the reason why some *Bolbosoma* species currently considered as valid have been described from cystacanths alone, e.g. *B. caenoforme* and *B. heteracanthis* (see (Petrochenko 1958)). Amin (1985) and Golvan (1994) listed 14 valid species within the genus *Bolbosoma*. Twelve species are common to both classification schemes, i.e. *B. balaenae*, *B. bobrovoi* Krotov &

Delyamure, 1952, *B. brevicolle* Malm, 1867, *B. caenoforme* Heitz, 1920, *B. capitatum* Linstow, 1880, *B. hamiltoni* Baylis, 1929, *B. Heteracanthis* Heitz, 1917, *B. nipponicum* Yamaguti, 1939, *B. physeteris* Gubanov, 1952, *B. scomberomori* Wang, 1980, *B. tuberculata* Skrjabin, 1970) and *B. Vasculosum* Rudolphi, 1819.

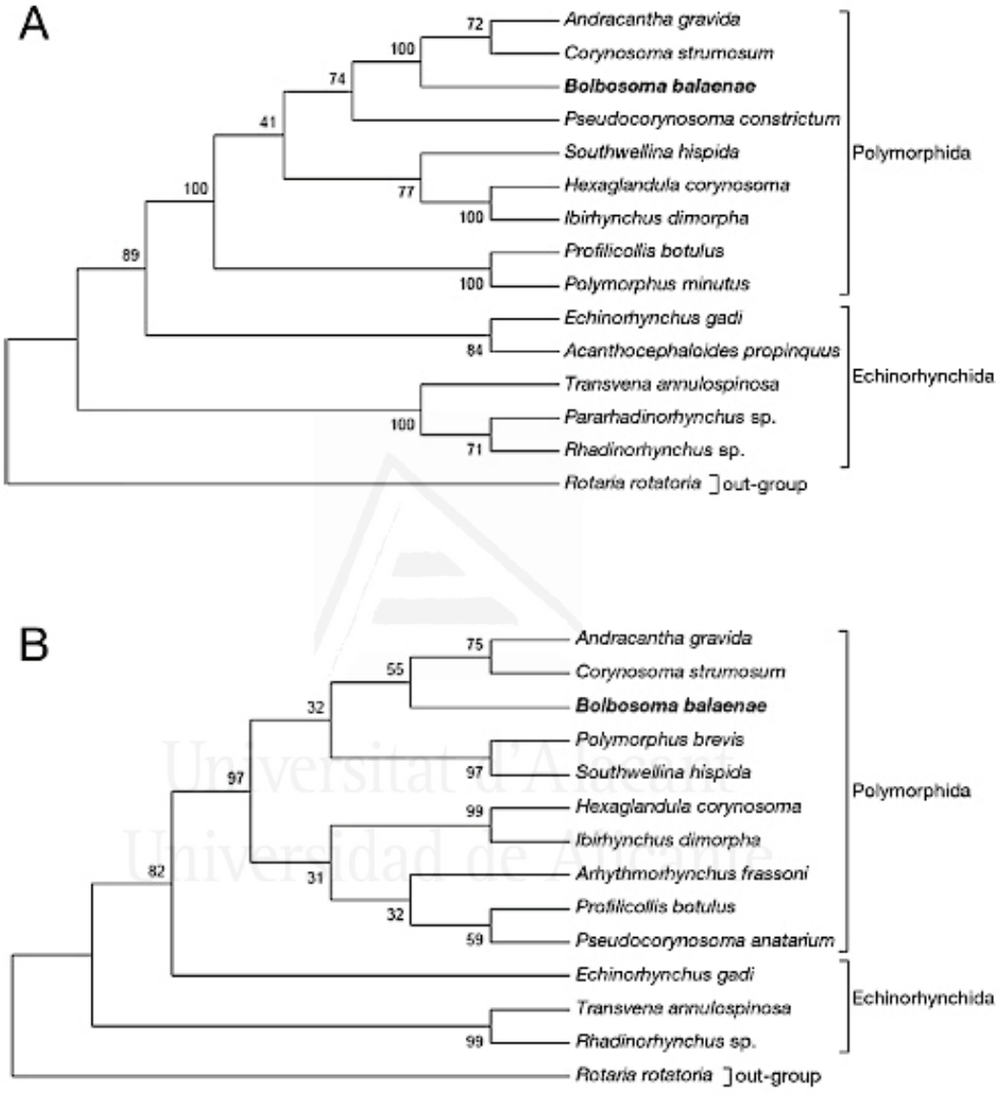


Fig. 4.I.3. *Bolbosoma balaenae*. Phylogenetic relationship among acanthocephalan sequences inferred by the minimum evolution algorithm within MEGA 4.1. (A) Analysis of 18S rDNA sequences. (B) Analysis of COI sequences. Numbers indicate the bootstrap confidence values of 1000 replicates

However, (Amin 1985) considered *B. thunni* Harada, 1935 as a valid species whereas Golvan (1994) considered that it could be synonymous with *B. vasculosum*, as did more recent authors (Costa et al. 2000). Amin (1985) also considered 2 subspecies within *B. turbinella* Diesing, 1851, i.e. *B. turbinella*

turbinella Diesing, 1851 and *B. turbinella australis* Skrjabin, 1972. Golvan (1994), however, regarded these subspecies as separate species. Later, Amin and Margolis (1998) synonymized *B. capitatum* and *B. physeteris*.

Regardless of these slight differences between classification schemes, *B. balaenae* is the only species of *Bolbosoma* that has a single field of trunk spines restricted to the prebulbar foretrunk; in the remaining species, spines cover the bulb to a variable extent (Meyer & Bronn 1933, Van Cleave 1953, Petrochenko 1958, Zdzitowiecki 1991, Measures 1992, Amin & Margolis 1998, Costa et al. 2000) and references therein. The number of circles of prebulbar spines seems to vary among specimens of *B. balaenae*; Meyer and Bronn (1933) mentioned 6 circles, whereas Zdzitowiecki (1991) mentioned up to 10 circles, and Van Cleave (1953) reported specimens even without spines, but he made it clear that this was a natural condition. Accordingly, the diagnostic character of somatic armature would allow a ready assignment of the cystacanths collected in this study as *B. balaenae*; the number of circles of spines observed in our sample may be considered within the range of natural variability of this species. In addition, our identification is confirmed based on the patterning of proboscis hooks. The proboscis of cystacanths collected in this study had 22 to 24 rows of hooks with 8 to 9 hooks per row, 6 with roots and 2 to 3 rootless. As far as we know, this combination of characters is shared only with previous descriptions of *B. balaenae.* Only 3 species, *B. australis*, *B. brevicolle* and *B. nipponicum*, have a number of rows of hooks that overlaps with that of *B. balaenae*; however, the number of hooks per row in these species is lower (range, 5 to 7) (see (Meyer & Bronn 1933, Van Cleave 1953, Petrochenko 1958, Zdzitowiecki 1991, Measures 1992, Amin & Margolis 1998, Costa et al. 2000) and references therein).

Phylogenetic analyses shows that this species is well embedded in the family Polymorphidae and is included as a sister taxa to *Corynosoma strumosum* and *Andracantha gravida*. The phylogenetic trees herein obtained are in accordance with previous molecular and morphological phylogenetic hypotheses for acanthocephalans, which support the monophyly of this group (García-Varela et al. 2000, Garcıa-Varela et al. 2002, García-Varela et al. 2011). The absence of DNA sequences deposited in the GenBank for *Bolbosoma balaenae* only allows placing the species within the phylogeny of the group. However, the sequences

deposited are useful as reference material for future comparisons with sequences of adults of *B. balaenae*. Interestingly, *Corynosoma*, *Andracantha* and *Bolbosoma* are the only genera within the Polymorphidae in which the majority or all species occur in marine hosts: *Corynosoma* mainly in pinnipeds, *Andracantha* in cormorants and *Bolbosoma* in cetaceans (see (Hoberg et al. 1993, Aznar et al. 2006). The position of a member of *Bolbosoma* in the phylogenetic tree of the Polymorphidae opens new avenues to interpret the history of associations between these acanthocephalans and marine mammals. In particular, it is possible that the ancestor that was the origin of these forms came from a single colonization event in the marine realm. The phylogenetic trees obtained here are in accordance with the results described previously by (García-Varela et al. 2000, Garcıa-Varela et al. 2002, García-Varela et al. 2011).

Apart from 3 sporadic records in oceanic odontocetes, i.e. the northern bottlenose whale *Hyperoodon ampullatus* Lacépède, 1804 see (Delyamure 1955), spinner dolphins *Stenella longirostris* Gray, 1828 and spotted dolphins *S. attenuata* Gray, 1846 see (Dailey & Perrin 1973), *Bolbosoma balaenae* has frequently been reported in at least 7 mysticetes species worldwide, including the bowhead whale *Balaena mysticetus* Linnaeus, 1758, sei whale *Balaenoptera borealis* Lesson, 1828, common minke whale *B. acutorostrata* Lacépéde, 1804, fin whale *B. Physalus* Linneus, 1758, blue whale *B. musculus* Linneaus, 1758, humpback whale *Megaptera novaeangliae* Borowski, 1781 and grey whale *Eschrichtius robustus* Lilljeborg, 1861 (Zdzitowiecki 1991, Dailey et al. 2000), and references therein. In the Atlantic Ocean *B. balaenae* has been found in bowhead, common minke, sei, blue and fin whales. Fin whales occupy principally a wide area between the 40° and 55°N latitudes and migrate southward during winter months (Raga et al. 1986). It is a common species in coastal waters where upwelling events occur(Jefferson et al. 1993, Aguilar 2009). It is considered a generalist and euryphagous. In the northern hemisphere its diet is mainly composed of krill *Meganyctiphanes norvegica* Sars, 1856 and *Thysanoessa inermis* Kryer, 1846, other zooplanktonic crustaceans, fishes and small squids (Aguilar 2009), especially schooling fish such as capelin, herring, cod, sardine and mackerel (Nemoto 1959, Klumov 1963 and Mitchell 1975, all as cited in (Measures 1992). Consequently this whale probably acquires *B. Balaenae* from infected fish that probably serve as

paratenic hosts. Reports of juvenile *Bolbosoma* sp. in fish implicate members of Scombridae, Scorpa e n idae, Carangidae, Trichiuridae, Gempylidae, Salmonidae, Berycidae, Lophotidae, Gadidae and Belonidae (Measures 1992), www. nhm. ac.uk/ research- curation/ research/projects/ host-parasites/ index. html.

The known geographic distribution of the euphausiid *Nyctiphanes couchii* includes Galician waters in NW Spain, where it is relatively abundant (Lindley 1977). As a component of the mesozooplankton, the krill is part of the diet of decapods, cephalopods (Pascual et al. 1996), fish and marine mammals (Mauchline 1980). This places in one of the lowest trophic levels, allowing the transmission of the cystacanths towards a definitive host throughout predator-prey interactions (Marcogliese 1995).

This is the first time that *Bolbosoma balaenae* has been found in the euphausiid *Nyctiphanes couchii*, which probably acts as an intermediate host. Hoberg et al. (1993) and Gómez-Gutiérrez et al. (2010) suggested that euphausiids and copepods could be the intermediate hosts of the genus *Bolbosoma* and Acanthocephala in general. Nevertheless, only 2 studies have reported the genus *Bolbosoma* in euphausiids ((Shimazu 1975, Tsimbalyuk 1980). In our samples we found *Bolbosoma* only in adults of *N. couchii*, despite the fact that we studied a great number of copepod species and other zooplankton components. This reinforces the idea proposed by Nickol et al. (2002) in which different genera of Polymorphidae are specialized in a specific fraction of zooplankton. Consequently, it is possible that *B. balaenae* is specific for this species of krill (*N. couchii*) in the NE Atlantic Ocean.

We did not find *Bolbosoma balaenae* larvae in the Euphausiacea larvae (calyptopis and furcilias, which were 0.8 to 2.20 mm and 2.2 to 5.5 mm in total length, respectively), nor in the copepod species or other taxa from our sample; this fact could be due to the large size of these cystacanths (8.09 mm). We speculate that only adults of *Nyctiphanes couchii* could harbour them in their body cavity (12 to 17 mm length). The larger copepods in our sample were *Calanus helgolandicus*, *Calanoides carinatus* and *Paraeuchaeta hebes*, and none exceeded a total length of 3 mm. The smaller ones were *Acartia clausii*, *Centropages chierchiae* and *Temora longicornis* (Müller O.F., 1785), whose size is always smaller than 2 mm. Despite finding Mysidacea with a similar size to euphausiids in our sample,

we did not find cystacanths inside. Probably this is due to the low numbers in our sample or because they occupy a different niche and have a different behaviour. Consequently, it seems that the smaller crustaceans cannot harbour cystacanths nor act as intermediate host for these acanthocephalans.

Additionally, none of the examined *Nyctiphanes couchii* showed more than one cystacanth per individual. Therefore, it seems that krill are able to harbour only one cystacanth. Nevertheless it is desirable to carry out experimental infections to validate the null hypothesis of host size as a limiting factor for infection intensity or determine whether the dilution of the pelagic realm could be that factor. It is true that multiple infections can reduce survival rates and perhaps we did not find more than one cystacanth in a host because these hosts died. Also, we only found cystacanths in the cephalothorax of krill, as did Shimazu (1975), Sars (1885), Lindley (1977) and Gómez-Gutiérrez et al. (2010), who also found Polymorphidae acanthocephalans in different species of krill. On the other hand, different Polymorphidae cystacanths have been reported in crabs with frequencies of more than one individual per crab. Nickol et al. (2002) detected infections of *Arhythmorhynchus* Lühe, 1911 and *Hexaglandula* Petrochenko, 1950 in fiddler crabs. In that case they found a crab that harboured a maximum of 3 cystacanths while the rest of the crabs examined harboured only 1 per individual. Balboa et al. (2009) detected the same larval stage harboured by several Brachyura Linnaeus, 1758 and Anomura Macleay, 1838, which showed differences in the intensity of infection. Experimental infections are required to determine whether size is the limiting factor for infection intensity.

The low prevalence (0.1%) observed in adults of *Nyctiphanes couchii* is usually considered a feature of an invertebrate intermediate host, especially in zooplankton communities (Marcogliese 1995). This is mainly because of the effect of dilution in the pelagic realm where finding a suitable intermediary host may be difficult. In spite of this low prevalence, most predators ingest large quantities of crustaceans and thus acquire prevalent and heavy infections (Marcogliese 1995). The information on larval acanthocephalans in euphausiid communities is very scanty and most of this information comes from studies on lake and river ecosystems where euphausiids, being marine organisms, are not found. Moreover the low prevalence is common for intermediate crustacean hosts (Uznanski &

Nickol 1980, Ashley & Nickol 1989). Despite different ecosystem and transmission pathways, the prevalence (0.1%) agree with those found for different acanthocephalan genera and platyhelminthes compiled in zooplanktonic communities (Marcogliese 1995). Although there is information on other acanthocephalan species infecting zooplanktonic groups, no data on their prevalence is available Hubschman 1983 and Wilson & Hubschman 1985 as cited in (Marcogliese 1995, Dezfuli 1996, Bush et al. 2001, Kakizaki et al. 2003).

Owing to the high abundance of *Balaenoptera physalus* off the west coast of the Iberian Peninsula, we suggest that this whale could be a definitive host of *Bolbosoma balaenae* in this geographical region, which supplies and supports an infective population in the euphausiids. We also consider that the minke whale and other mammals could be additional suitable definitive hosts of this parasite in the NE Atlantic Ocean. However, we cannot rule out the possibility that the life cycle of *B. balaenae* could also involve pelagic fishes and cephalopods as paratenic hosts, which in turn are an important commercial resource in the area studied (http://conselleriamar.xunta.es/ web/ pesca/datos-producion).

Owing to the high importance of the euphausiids within the marine pelagic trophic webs, and taking into account that they are a key component, directly and/or indirectly, in the dietary habits of many cephalopods, fishes and marine mammals, we suggest that it would be interesting to undertake further studies focused on the possible interactions between the recruitment of *Bolbosoma balaenae* to *Nyctiphanes couchii* driven by the upwelling events that occur in this area of the NE Atlantic Ocean.

4.II. *NYCTIPHANES COUCHII* AS INTERMEDIATE HOST FOR *RHADINORHYNCHUS* SP. (ACANTHOCEPHALA, ECHINORHYNCHIDAE) FROM NW IBERIAN PENINSULA WATERS

4.II.1. Abstract

In the mesozooplanktonic community of the coastal upwelling system of the Ría de Vigo (NW Spain), the euphausiid Nyctiphanes couchii has been identified for the first time in temperate waters of the NE Atlantic as the intermediate host for cystacanths of *Rhadinorhynchus* sp. Parasites were identified using morphological characters described in 20 cystacanths. The hooks of the proboscis were arranged in 14 rows of 26 hooks each, while the hooks of the basal circle were only slightly erected and were longer than remaining spines. A maximum-likelihood estimation (ML) tree inferred from the 18S rRNA data set of *Palaeacantocephala* revealed that our specimens belong to a highly supported clade with *Rhadinorhynchus* sp., *Pararhadinorhynchus* sp. and *Transvena annulospinosa*. Nonetheless, our morphological and phylogenetic analyses suggested that the status of *Rhadinorhynchus pristis* should be re-examined. The prevalences of parasites were 0.0019% and 0.0001% for frontal and coastal summer communities, and 0.0068% and 0.0008% for coastal and oceanic autumn communities, respectively. The presence of these cystacanths in different mesozooplankton communities throughout the study suggests that the recruitment of parasites may be affected by the oceanography.

4.II.2. Introduction

Acanthocephalans, or thorny-headed worms, are in adult stages obligate endoparasites of the intestine of vertebrates (Nickol 1985, Marcogliese 1995, Garey et al. 1996, Nickol et al. 2002, Taraschewski 2005). The cystacanths of this genus are the infective stage. Morphologically, they are similar to the mature worms, but differ in the size of the trunk and the degree of development of the sexual organs (Zdzitowiecki 1991, Hoberg et al. 1993).

Among zooplankton communities, euphausiids play an important role as intermediate hosts in the pelagic realm (Marcogliese 1995). They are able to attain

massive biomasses that form vast and dense swarms occupying one of the lowest trophic levels. Moreover, they can be used by different parasites to reach their definitive host (Mauchline 1980, 1984, Marcogliese 2002). *Nyctiphanes couchii* is the main euphausiid in the European continental shelf and one of its areas of higher concentrations is situated near the Spanish coast (Roura et al. 2013). This species is also one of the main prey items of different fish species, which in turn are involved in the diet of potential definitive hosts (Pascual et al. 1996, Marcogliese 2002). Some reports e.g. (Sars 1885, Shimazu 1975, Lindley 1977, Tsimbalyuk 1980, Gómez-Gutiérrez et al. 2010, Gregori et al. 2012) recognised some species of cystacanths infecting the thoracic organs of adults of several species of krill. Moreover, it is well-known that within the Palaeacanthocephala, a few members of *Rhadinorhynchus* Lühe, 1911 have experienced considerable speciation in aquatic environments (Taraschewski 2005), where they are able to infect important comercial fishes such as Scombridae (Hogans et al. 1983, Rêgo et al. 1985, Rego 1987), Xiphidae (Hogans et al. 1983), Belonidae, Carangidae, and Bramidae in the Atlantic Ocean (www. nhm. ac. uk/ research-curation/ research/ projects/host-parasites/ database/ index. jsp).

As far as we know, no previous data on the presence of *Rhadinorhynchus* sp. Rudolphi, 1802 in euphausiids from the NE Atlantic are available. With the exception of (Rodrigues et al. 1975) and (Rêgo et al. 1985), *Rhadinorhynchus* has not been reported in this area. In addition, these authors found adults of this genus infecting scombrid fishes.

Despite parasites having a great ecological and economic significance in NE Atlantic waters, their recruitment to the zooplankton level is poorly understood. Therefore, the aims of this study were to (1) report the role of euphausiids in the life cycle of *Rhadinorhynchus* sp. in NW Iberian Peninsula waters; (2) provide data about parasite morphology, genetic and demographic infection values; and (3) discuss the controversy with the genetic identification of *R. prisits*.

4.II.3. Material and Methods

4.II.3.1. Collection and processing samples

The zooplankton samples were caught in the Ria de Vigo in Galician waters, NW Iberian Peninsula, on board the RV '*Mytilus*' (Fig. 4.II.1). Ten surveys were

undertaken in the summer (2, 4, 9 and 11 July) and autumn (26 September, and 1, 3, 9, 10 and 14 October) of 2008. Samples were collected by double oblique towing, using a 750 mm diameter bongo net equipped with 375 μm mesh. At a ship's speed of 2 knots, the bongo net was first lowered and stabilized near the bottom for a period of 15 min, then hauled to the surface at 0.5 m s^{-1}. The Bongo was equipped with a current meter, which enabled calculation of the volume of water filtered during the haul, thus permitting an estimation of zooplankton abundance ($nºm^{-3}$). The sample was filtered using a 500 μm sieve and fixed on board with 100% ethanol. Samples were later transferred to 70% ethanol in the laboratory and stored at −20°C.

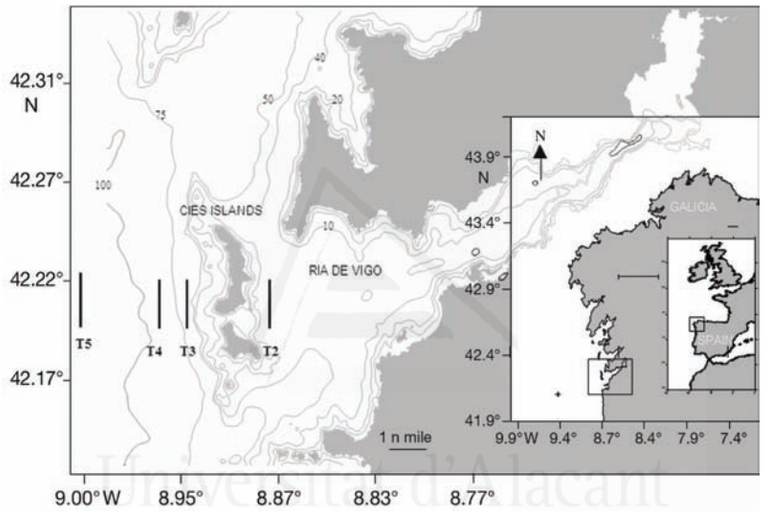


Fig. 4.II.1. Sampling area off the Ría de Vigo in Galician waters, NE Atlantic. T2–5: transects 2–5

4.II.3.2. Zooplankton estimation

The abundance of the different zooplankton taxa was estimated after counting a sub-sample using a Folsom splitter. Organisms were identified to the lowest possible taxonomic level. Species diversity was calculated using the Shannon-Weaver and Evenness indices (Omori & Ikeda 1984, Guisande et al. 2006). The number of euphausiids was estimated using the method of calculating precise replica (Andrew & Mapstone 1987).

4.II.3.3. Collection and processing of cystacanths

All zooplankton components of the samples were examined for acanthocephalans using a stereomicroscope (20x). Parasites were removed from the host using dissection material under the stereomicroscope. Cystacanths were identified by examining the body and proboscis according to Petrochenko (1956, 1958), Cable and Linderoth (1963), (Zdzitowiecki 1989) and Arai (1989). The number and distribution patterns of the proboscis armature and variations in the spination of the anterior part of body are considered the most important determining features (Miller & Dunagan 1985). Morphological study was carried out to determine the cystcanth species. As the cystacanths normally presented the proboscis, neck, and part of the anterior trunk introverted, we dissected them to evert these structures. The body was cleared using the protocol described by (Gregori et al. 2012) because this method does not damage DNA. The caudal part of the body was used for DNA extraction.

Scanning electron microscopy preparations in a Philips XL 30 were used to clarify the morphological examination. Infection parameters were estimated following Bush et al. (1997) and Rózsa et al. (2000). Sterne's exact 95% confidence interval (CI) was calculated for prevalence Reiczigel (2003).

4.II.3.4. Genomic DNA extraction and PCR amplification

Genomic DNA was isolated using the Qiagen DNeasyTM Tissue Kit according to the manufacturer's instructions. DNA quality and quantity was checked in a NanoDrop® ND-1000 spectrophotometer and in 1% agarose gel. The primers 18SU467F (5'-ATC CAA GGA AGG CAG CAG GC-3') and 18SL1310R (5'-CTC CAC CAA CTA AGA ACG GC-3') (Suzuki et al. 2008) were employed to amplify approximately 900 bp of the small subunit (18S) ribosomal RNA gene. PCRs were performed in a total volume of 25 µl containing 1 µl of genomic DNA (150–200 ng), PCR buffer at 1°— concentration, 1.5 mM $MgCl_2$, 0.2 mM nucleotides (Roche Applied Science), 0.3 µM primers and 0.625 U Taq DNA polymerase (Roche Applied Science). The cycling protocol for 18S rRNA gene was 2 min at 94°C, 35 cycles with 30 s at 94°C, 1 min at 55°C and 2 min at 72°C, followed by 7 min at 72°C.

All PCRs were carried out in a TGradient thermocycler (Biometra) and a negative control (no DNA) was included for each set of PCRs.

4.II.3.5. DNA sequening and phylogenetic analysis

Positive PCR products were cleaned for sequencing using ExoSAP-IT© (USB Corporation). Sequences were subjected to BLASTn analyses against available sequences from GenBank, through web servers of the National Center for Biotechnology Information (USA). All 18S rRNA sequences present in GenBank of the Class Palaeacanthocephala were downloaded for phylogenetic analyses (n = 49). Additionally, 2 rotiferan sequences were downloaded as an outgroup, due to their close relationship with the acanthocephalans (García-Varela et al. 2000). Table 4.II.1 shows the species used for phylogenetic analyses and their accession numbers. These 18S rRNA sequences were aligned using MAFFT v5.7 (Katoh et al. 2002) with default settings. GBlocks (Castresana 2000) were then used to identify and remove highly divergent regions and poorly aligned positions. Afterwards, a substitution model was selected under Akaike's information criterion (Akaike 1974) as implemented in jModeltest (Posada 2008). The GTR+I+G (Tavaré 1986) model was chosen to infer the evolutionary history using the máximum likelihood (ML) method. The analysis involved 51 nucleotide sequences with a total of 582 conserved sites in the final data set. Bootstrap probabilities with 1000 replications were calculated to assess reliability on each node of the ML tree. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011).

4.II.4. Results

A total of 20 cystacanths infecting *Nyctiphanes couchii* were found in different samples. Their prevalences (95% CI) are presented in Table 4.II.2. Estimations of accompanying zooplankton taxa and abundance, Shannon-Weaver index and species evenness where larvae were found are recorded in Table 4.II.3. Complete information about mesozooplankton composition in each community is available in (Roura et al. 2013). Cystacanths were removed from the thoracic organs (Fig. 4.II.2 A) of *N. couchii* adults. The body of the cystacanths was cylindrical and in most samples their neck and proboscis were invaginated. A

detailed description is given for male and female specimens pooled because sex could not always be determined. Morphometric measurements are given as means ± SD [range], with sample size in parentheses.

Table 4.II.1. Species and GenBank accession number of taxa used for 18S rDNA analyses.

Taxon	GenBank nº
Southwellina hispida	JX014228, EU267809
Arhythmorhynchus brevis	AF064812
Pseudocorynosoma constrictum	EU267800
Ibirhynchus dimorpha	CQ981436
Hexaglandula corynosoma	EU267808
Pseudocorynosoma anatarium	EU267801
Polymorphus minutus	EU267806
Profilicollis botulus	EU267805
Polymorphus sp.	AF064815
Polymorphus altmani	AF001838
Andracantha gravida	EU267802
Corynosoma enhydri	AF001837
Corynosoma magdaleni	EU267803
Corynosoma strumosum	EU267804
Bolbosoma vasculosum	JX014225
Bolbosoma balaenae	JQ040306
Plagiorhynchus cylindraceus	AF001839
Centrorhynchus conspectus	U41399
Centrorhynchus sp.	AY830155
Centrorhynchus microcephalus	AF064813
Serrasentis sagittifer	JX014227
Gorgorhynchoides bullocki	AY830154
Transvena annulospinosa	AY830153
Pararhadinorhynchus sp	HM545903
Rhadinorhynchus pristis	JQ061133
Rhadinorhynchus sp.	AY062433
Acanthocephalus dirus	AY830151
Acanthocephalus lucii	AY830152
Filisoma bucerium	AF064814
Filisomaa rizalinum	JX014229
Echinorhynchida sp	EU732662
Acanthocephaloides propinquus	AY830149
Echinorhynchus gadi	AY218123, U88335, JX014222
Rhadinorhynchus pristis	JX014226
Rhadinorhynchus lintoni	JX14224
Pomphorhynchus laevis	JX014223, AY218124, AY423346
Pomphorhynchus tereticollis	AY423347
Dentitruncus truttae	JX460865
Illiosentis sp	AY830158
Pseudoleptorhynchoides lamothei	EU090950
Koronacantha pectinaria	AF092433, AY830157
Leptorhynchoides thecatus	AF001840
Pomphorhynchus bulbocoli	AF001841
Outgroup: *Rotaria rotatoria*	AY218121
Plationus patulus	DQ297712

Table 4.II.2. *Nyctiphanes couchii* population divided in three different larval stages in each community during 2008, where cystacanths were found from Ría de Vigo, Galicia, NW Atlantic, Spain in different sampling belonging to SF = Summer Frontal, SC = Summer Coastal, AO = Autumn Ocean, AC = Autumn Coastal communities. T5B = Transect number 5 at bottom. T2B = Transect 2 at bottom. T3B = Transect 3 at bottom N = number of individuals estimated; AB = Abundance ($n*m^{-3}$); Population % [CI] = prevalence in the population followed by the confidence interval (95%), Adults % [CI] = prevalence in adults followed by the confidence interval (95%).

Community	SF		SC		AO						AC	
	T5B, 2 Jul		T2B, 2 Jul		T5B, 26 Sep		T5B, 1 Oct		T5B, 9 Oct		T3B, 10 Oct	
	N	Ab	N	Ab	N	Ab	N	Ab	N	Ab	N	Ab
Calyptopis	36433	88.78	1423426	2097.96	2496	11.30	4060	25.52	475	2.13	165120	436.46
Furcilia	49388	120.35	37415	55.15	4512	20.44	480	3.02	175	0.78	92160	243.61
Adults	69955	170.47	328	0.48	3600	16.31	2620	16.47	2175	9.76	1920	5.07
Population	155775	380.60	1461169	2154.59	10608	48.05	7160	45.01	2825	13.67	259200	685.14
No. infected	3		1		12		1		1		2	
Population % [CI]	0.0019 [0.0004–0.002]		0.0001 [0.0000–0.00013]				0.068 [0.0372–0.0445]				0.0008 [0.0001–0.0008]	
Adults % [CI]	0.0043 [0.0125–0.0009]		0.3047 [0.0000–0.0169]				0.0014 [0.0912–0.2797]				0.1042 [0.0126–0.3758]	

Table 4.II.3. Mesozooplankton taxa collected in each community during 2008, where cystacanths were found from Ría de Vigo, Galicia, NWAtlantic, Spain, in different samplings: summer frontal (SF), summer coastal (SC), autumn ocean (AO) and autumn coastal (AC). T5B: transect number 5 at bottom; T2B: transect 2 at bottom; T3B: transect 3 at bottom. N: number of individuals estimated; Ab: abundance (no. m_{-3}). Volumes filtered per transect were 410.37, 678.48, 220.78, 159.07, 222.92, and 3783.2 m_3, respectively. –: taxon not found.

Community	SF		SC		AO						AC	
	T5B, 2 Jul		T2B, 2 Jul		T5B, 26 Sep		T5B, 1 Oct		T5B, 9 Oct		T3B, 10 Oct	
	N	Ab	N	Ab	N	Ab	N	Ab	N	Ab	N	Ab
MEROPLANKTON												
Cephalopoda												
Loliginidae	–	–	8	0.01	2	0.01	–		1	0.004	4	0.01
Octopus vulgaris	–	–	1	0.001	1	0.005	3	0.01	2	0.01	13	0.03
Sepiolidae	7	0.08	1	0.001	–	–	–	–	–	–	3	0.01
Cirripedia												
Cirripedia larvae	–	–	4267	6.29	–	–	–	–	–	–	170880	451.69
Echinodermata												
Echinoidea larvae	–	–	1422	2.10	–	–	–	–	–	–	3840	10.15
Ophiuroidea larvae	512	1.28	28444	41.92	–	–	–	–	–	–	353280	933.82
Fish												
Fish eggs	–	–	–	–	–	–	–	–	–	–	1920	5.07
Fish larvae	512	1.25	1046	1.54	29	0.13	2	0.01	6	0.03	1969	5.20
Gastropoda												
Gastropoda larvae	–	–	12800	18.87	–	–	–	–	25	0.11	24960	65.98
Isopoda												
Aegidae	–	–	50	0.07	–	–	–	–	–	–	24	0.06
Decapoda												
Alpheidae zoeae	–	–	1422	2.10	–	–	–	–	25	0.11	–	–
Brachyura juvenile	–	–	7	0.01	2	0.01	1	0.01	–	–	–	–
Brachyura megalopa	–	–	–	–	–	–	–	–	–	–	1920	5.07
Brachyura zoeae	2463	6.00	45511	67.08	–	–	40	0.25	25	0.11	24960	65.98
Crangonidae zoeae	–	–	1422	2.10	–	–	–		–	–	–	
Paguridae megalopa	480	1.17			–	–	–	–	–	–	–	
Paguridae zoeae	–	–	4267	6.29	–	–	20	0.13	–	–	9600	25.38
Palaemonidae zoeae	–	–	1422	2.10	–	–	–	–	–	–	–	
Pisidia longicornis megalopa	–	–	–		–	–	–	–	–	–	1920	5.07
Pisidia longicornis zoeae	–	–	15644	23.06	–	–	–	–	–	–	17280	45.68
Porcellana platycheles zoeae	–	–	5689	8.38	–	–	–	–	–	–	15360	40.60
Processidae zoeae	–	–	533	0.92	–	–	–	–	–	–	600	11.41
Jaxea nocturna	–	–	1422	2.10	–	–	–	–	–	–	–	–
Amphipoda												
Caprellidea	–	–	6	0.009	–	–	4	0.02	1	0.004	–	–
Gammaridea	85	0.201	65	0.10	6	0.03	2	0.01	24	0.11	176	0.46
Stomatopoda												
Meiosquilla desmaresti	480	1.17	–	–	–	–	–	–	–	–	–	–
Polychaeta												
Polychaeta larvae	5	0.01	49	0.07	35	0.16	6	0.04	8	0.04	21	0.06
HOLOPLANKTON												
Appendicularia	–	–	7111	10.48	–	–	–	–	–	–	71040	187.78
Amphipoda												
Hyperiidea	–	–	1	0.001	5	0.02	9	0.06	17	0.08	13	0.03
Chaetognatha	–	–	11942	17.60	1861	8.43	1220	7.67	775	3.48	96000	253.76
Cnidaria	–	–	–	–	–	–	–	–	–	–	5760	15.22
Cladocera												
Evadne nordmanni	–	–	14222	20.96	–	–	–	–	–	–	36480	96.43
Podon intermedius	–	–	36978	54.50	–	–	–	–	–	–	55680	147.18
Hydrozoa												
Siphonophora	1536	3.74	5317	7.84	74	0.33	–	–	300	1.35	1920	5.07

Table 5.II.3 (continued)

Community	SF		SC		AO						AC	
	T5B, 2 Jul		T2B, 2 Jul		T5B, 26 Sep		T5B, 1 Oct		T5B, 9 Oct		T3B, 10 Oct	
	N	Ab	N	Ab	N	Ab	N	Ab	N	Ab	N	Ab
Euphausiacea												
Nyctiphanes couchii calyptopis	36433	88.78	1423426	2097.96	2496	11.30	4060	25.52	475	2.13	165120	436.46
N. couchii furcilia	49388	120.35	37415	55.15	4512	20.44	480	3.02	175	0.78	92160	243.61
N. couchii adult	69955	170.47	328	0.48	3600	16.31	2620	16.47	2175	9.76	1920	5.08
Copepoda												
Calanoidea												
Acartia clausi	7421	18.08	93867	138.35	1263	5.721	260	1.63	3350	15.03	99840	263.91
Candacia armata	–	–	1422	2.10	–	–	–	–	–	–	–	–
Calanoides carinatus	72259	176.08	2844	4.19	1600	7.25	20	0.13	575	2.58	7680	20.30
Calanus helgolandicus	35156	85.67	1422	2.10	1853	8.39	100	0.63	475	2.13	–	–
Centropages chierchiae	3966	9.67	14222	20.96	–	–	20	0.13	75	0.34	1920	5.07
Centropages typicus	–	–	–	–	–	–	–	–	–	–	1920	5.07
Clausocalanus spp.	–	–	–	–	–	–	100	0.63	100	0.45	3840	10.15
Diaixis pygmaea	–	–	1422	2.10	–	–	–	–	25	0.11	–	–
Isias clavipes	–	–	1422	2.10	–	–	–	–	–	–	1920	5.07
Mesocalanus tenuicornis	–	–	–	–	253	1.14	–	–	–	–	–	–
Metridia lucens	–	–	1422	2.10	1432	6.48	–	–	100	0.45	–	–
Paracalanus parvus	–	–	–	–	253	1.14	–	–	575	2.58	1920	5.07
Paraeuchaeta hebes	8637	21.05	–	–	7326	33.18	220	1.38	850	3.81	1920	5.07
Paraeuchaeta sp.	4797	11.69	–	–	20463	92.68	1700	10.69	2225	9.98	15360	40.60
Pseudocalanus elongatus	–	–	–	–	–	–	20	0.13	25	0.11	–	–
Subeucalanus crassus	–	–	–	–	–	–	20	0.13	–	–	–	–
Temora longicornis	–	–	28444	41.92	–	–	–	–	–	–	9600	25.38
Cyclopoidea												
Oithona plumifera	–	–	–	–	–	–	–	–	250	1.12	5760	15.22
Mysidacea	1919	4.68	–	–	528	2.39	360	2.26	475	2.13	1920	5.07
Thaliacea												
Salpida	21175	51.60	4267	6.29	3874	17.54	1780	11.19	1900	8.52	13440	35.53
Polychaeta												
Tomopteris spp.	–	–	–	–	–	–	–	–	25	0.11	–	–
Total	317185	773.93	1814417	2674.37	51466	233.11	13067	82.15	15084	67.66	1321863	3503.90
Shannon's Index (*H'*)	1.55	0.507	2.12	1.49	2.31	2.37						
Evenness index	0.54	0.139	0.71	0.48	0.69	0.64						

4.II.4.1. Description

Trunk long, uniformly cylindrical, 10.5 ± 3.9 mm [5.3–19.5 mm] long (n = 18) x 0.55 ± 0.37 mm [0.33–1.87 mm] wide (n = 15), spinose anteriorly. Trunk spines in 2 fields separated by unarmed zone (Fig. 4.II.2 B-C). Anterior trunk spines adjacent to neck, arranged in 2 or 3 circles, the 3rd ventrally not complete, posterior field restricted to ventral area (Fig. 4.II.2 B), with ca. 10–14 rows of spines, reaching 23 ± 3.5% [15–28%] of trunk length (n = 15). Trunk spines 82 ± 20 µm [53–118 µm] long (n = 163 from 12 specimens), slightly longer in the posterior region, embedded in cuticular sheath. Neck cylindrical, 0.22 ± 0.10 mm [0.13–0.39 mm] long x 0.18 ± 0.06 mm [0.12–0.23 mm] wide (n = 5). Proboscis slender, cylindrical, 2.26 ± 0.43 mm [1.55–3.32 mm] long x 0.20 ± 0.06 mm [0.11–0.33 mm] wide (n = 16) (Fig. 4.II.2 D). Hooks arranged in 14 (rarely 13) rows of 26 hooks (rarely 23–25) each (n = 16). Hooks of basal circle only slightly erected and longer than remaining spines (Fig. 2D). Range of hook (H) length (in

μm) at base as follows (n = 10 specimens except in H24–26, for which n = 6): H1: 93–131; H2: 62–99; H3: 79–98; H4: 67–110; H5: 74–110; H6: 69–111; H7: 78–120; H8: 87–114; H9: 88–124; H10: 94–128; H11: 90–123; H12: 91–122; H13: 94–118; H14: 86–110; H15: 77–111; H16: 85–123; H17: 96–122; H18: 87–113; H19: 92–116; H20: 85–114; H21: 77–122; H22: 85–111; H23: 68–108; H24: 85–99; H25: 68–98; H26: 66–88. Proboscis receptacle considerably longer than proboscis, 3.8 ± 0.5 mm [2.9–4.7 mm] long (n = 16) x 0.18 ± 0.06 [0.22–0.28] mm wide (n = 4). Lemnisci not extending past receptacle, generally hidden behind it but apparently reaching half of proboscis receptacle (n = 2). Terminal gonopore. A voucher specimen was deposited at the Natural History Museum of London, UK, with the accession number NHMUK 2013.4.2.1.

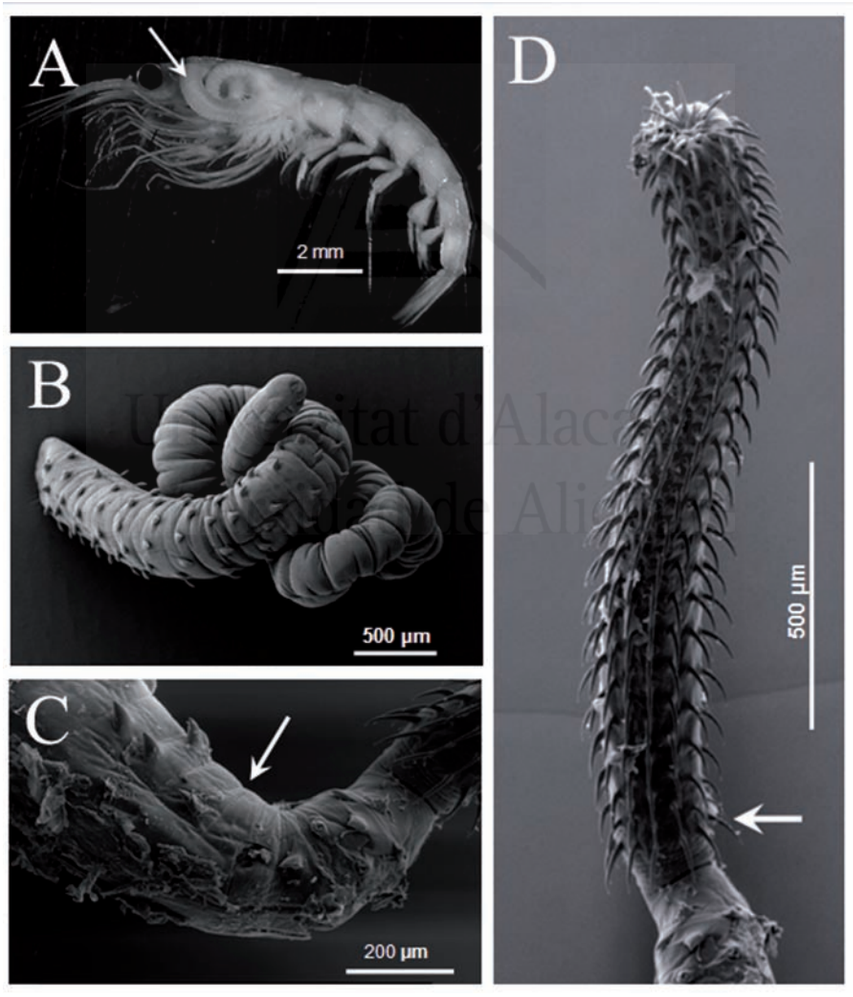


Fig. 4.II.2. Cystacanth of *Rhadinorhynchus* sp. from *Nyctiphanes couchii*. (A) Single infection with one cystacanth of Rhadinorhynchus sp. in the cephalothorax cavity (arrow). (B) The spines of the anterior part of the body, general view. (C) Two fields of spines separated by a space without spines (arrow). (D) Cystacanth's proboscis. Basal circle perpendicular hooks (arrow)

Amplified sequences of 18S rRNA ranged from 814 to 819 bp. These sequences are available on GenBank under the accession numbers JQ061133–JQ061136. BLAST search showed close homology (98%) with the 18S rRNA of *Rhadinorhynchus* sp. and *Pararhadinorhynchus* sp. (Johnston & Edmonds, 1947). The ML tree inferred from the 18S rRNA data set of Palaeacantocephala revealed that our specimens belong to a highly supported clade (bootstrap values of 1000), with *Rhadinorhynchus* sp., *Pararhadinorhynchus* sp. And *Transvena annulospinosa* (Pichelin & Cribb, 2001) (Fig. 4.II.3). Unexpectedly, sequences of *Rhadinorhynchus pristis* and *R. lintoni* (Cable & Linderoth, 1963), recently described by (Verweyen et al. 2011), are nested in a highly supported group with *Pomporhynchus* (Monticelli, 1905) species, displaying homologies of 99% with those species. Comparing our data against the sequences of Verweyen et al. (2011) revealed homology of only 84%. The ML tree showed the monophyly of Polymorphida and the paraphyly of Echinorhynchida, the 2 orders found within Palaeacantocephala. In fact, Rhadinorhynchidae was the most polyphyletic family among the Echinorhynchida.

4.II.5. Discussion

Cystacanths found in *Nyctiphanes couchii* can be undoubtedly assigned to the genus *Rhadinorhynchus* based on the patterns of trunk armature and proboscis morphology see (Petrochenko 1956, Golvan 1969, Amin et al. 2011). *Pararhadinorhynchus* and *Transvena*, which belong to the same clade as *Rhadinorhynchus* (Fig. 4.II.3), can be readily distinguished using their taxonomy because a key character for species of the genus *Pararhadinorhynchus* is the absence of trunk spines. The morphotype of species of *Transvena* is distinguished from other acanthocephalans because their trunk possesses a single ring of posteriorly pointing spines, at or near the junction between the neck and trunk (Pichelin & Cribb 2001). In contrast, all our examined specimens possess extended fields of spines on the trunk.

According to the most recent key to species of *Rhadinorhynchus* (Amin et al. 2011), our specimens belong to a group of 20 species that combine 2 character states that are apparently stable: (1) trunk spines in 2 fields clearly separated by an unarmed zone; and (2) dorsal spines absent in the posterior field. Among these,

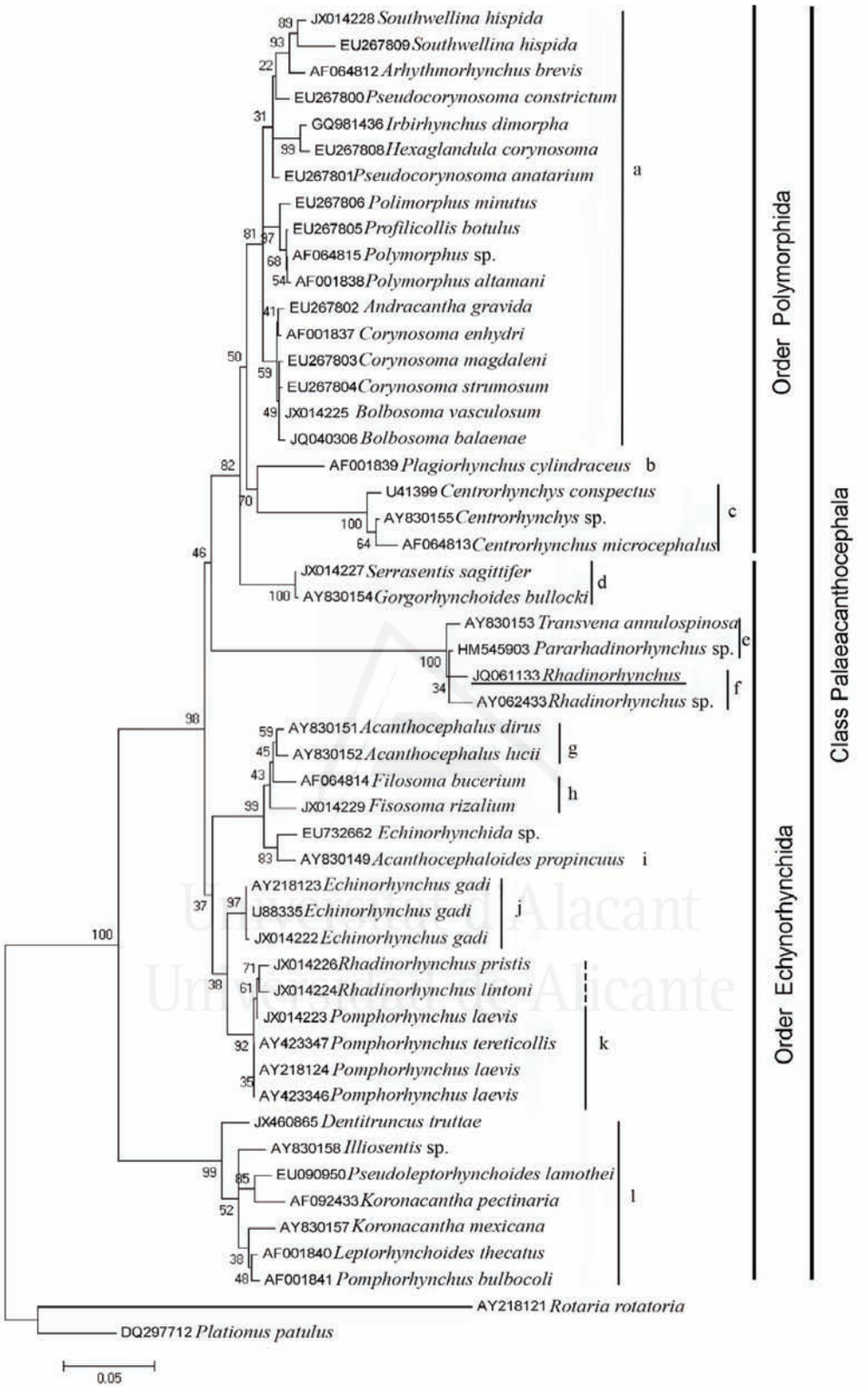


Fig. 4.II.3. Maximum likelihood consensus tree after 1000 bootstraps showing the phylogenetic relationships of the Palaeacanthocephala using 18S rRNA sequences and Rotifera as the outgroup. Abbreviations: a, Polymorphidae; b, Plagiorhynchidae; c, Centrorhynchidae; d, Rhadinorhynchidae; e, Transvenidae; f, Rhadinorhynchidae; g, Echinorhynchidae; h, Cavisomidae; i, Arhythmacan - thidae; j, Echinorhynchidae; k, Pomphorhynchidae; l, Illiosentidae. Underlined species correspond with cystacanths found infecting *Nyctiphanes couchii*

only a single species, namely *Rhadinorhynchus saltatrix* (Troncy & Vassiliades, 1973), exhibits the combination of a proboscis armature with 14 rows of hooks and a field of posterior trunk spines with >10 spine rows (Amin et al. 2011). The description of *R. saltatrix* is well detailed but based only on a few specimens, 5 males and 8 females (Troncy & Vassiliades 1973). Our specimens agree with the description made by Troncy & Vassiliades (1973) except that the number of hooks per row that they report is 24 in *R. saltatrix* while 26 hooks per row are more frequently found in our specimens. However, it is important to note that our specimens closely resemble *R. pristis* and *R. selkirki* (Van Cleave, 1921), except that the number of spine rows in the posterior field of the trunk is smaller (≤10) in these species (see Amin et al. 2011). From the above results a key question is the range of variability of this spiny field, and the factors that may influence this trait. For instance, there is evidence of clear sexual differences of this trait in many species (see e.g. Petrochenko 1958, Cable & Linderoth 1963, Troncy & Vassiliades 1973). In contrast, the status of *Rhadinorhynchus pristis* is currently rather confused. Amin et al. (2011) list 7 species that, in their opinion, were erroneously identified as *R. pristis*, but they consider *R. selkirki* as a valid species. In contrast, Chandler (1934) and Petrochenko (1956) consider *R. selkirki* as synonym of *R. pristis*. To compound the problem, other available descriptions of *R. pristis* (e.g. (Arai 1989, Bunkley-Williams & Williams 1996, Amin & Margolis 1998) were not included in the revision by Amin et al. (2011) and do not fulfil the diagnostic traits of this species sensu these authors.

According to the above morphological discussion, we should tentatively identify our specimens as *Rhadinorhynchus saltatrix*, pending a critical reexamination of *R. pristis* and related species.

Once the morphological iden tification was confirmed, genetic homology using 18S rRNA se quences allowed us to assign the cystacanths to the genus *Rhadinorhynchus* (Fig. 4.II.3). The 18S (SSU sequences) have been broadly used in different research as a taxonomic tool to clarify the taxonomy of acanthocephalans at the species level (Near et al. 1998, García-Varela et al. 2000, Herlyn et al. 2001, Garcıa-Varela et al. 2002, Near 2002, García-Varela & Nadler 2005, 2006, García-Varela & González-Oliver 2008, Gregori et al. 2012). At the genetic level, our identification disagrees with the results obtained by Verweyen et al. (2011), whose

sequences correspond with *R. Pristis* and *R. lintoni*. Phylogenetic analyses showed almost the same topology, but the position of *Rhadinorhynchus* species is markedly different. Our sequences appeared in a well-supported clade with members of the family Transvenidae, *Transvena annulospinosa* and *Rhadinorhynchus* sp. as reported by García-Varela & Nadler (2005) and García-Varela & González-Oliver (2008). However, *R. Pristis* and *R. lintoni* identified by Verweyen et al. (2011) appeared within the *Pomporhynchus* group. This contradiction is due to the absence of *Pararhadinorhynchus* sp. And *Rhadinorhynchus* sp. sequences in the Bayesian analysis carried out by Verweyen et al. (2011). The omission of these 2 sequences placed their *R. pristis* and *R. lintoni* far from *Transvenna annulospinosa*, which was a clade strongly supported with a bootstrap con fidence of 100% (García-Varela & Nadler 2005, García-Varela & González-Oliver 2008). Our results suggest that genetic identification of *Rhadinorhynchus* by Verweyen et al. (2011) should be revised. Another explanation would be that *Rhadinorhynchus* is a polyphyletic genus, since Rhadinorhynchidae is a polyphyletic family as shown by morphological, molecular and cladistic studies (Herlyn et al. 2001, Monks 2001, Garcıa-Varela et al. 2002, García-Varela & Nadler 2005, García-Varela & González-Oliver 2008). It is clear that the paraphyly of Rhadinorhynchidae requires reexamination and re classification and even the creation of new families. As suggested by Pichelin & Cribb (2001) and García-Varela & Nadler (2005), the group formed by *Serrasentis sagittifer* (Linton, 1889) and *Gorgorhynchoides bullocki* (Cable & Mafarachisi, 1970), plus *Golvanorhynchus* (Noronha, Fabio & Pinto, 1978), which form a sister group of Polymorphida, should be removed from the Rhadynorhynchidae. Our results support the transfer of *Leptorhynchoides* (Kostylev, 1924) and *Pseudoleptorhynchoides* (Salgado-Maldonado, 1976) (both Rhadinorhynchidae) to the family Illiosentidae, as suggested by García-Varela & González-Oliver (2008). This way the Illiosentidae would be a monophyletic clade. In our work the order Polymorphida is monophyletic, in contrast with the works of García-Varela & Nadler (2005), García-Varela & González-Oliver (2008) and Verweyen et al. (2011). This difference may result from the larger data set analysed in this work, 49 Palaeacantocephalan sequences, versus the 19, 20 and 36 used by García-Varela & Nadler (2005), García-Varela & Gon zález-Oliver (2008) and Verweyen et al. (2011), respectively. Apart from *R. pristis* and *R. lintoni*, our

phylogenetic analysis highlights the possible misidentification of *Pomporhynchus bulbocoli* (Linkins in Van Cleave, 1919) as a member of the monophyletic clade Illiosentidae and *Echinorhynchida* sp. (Cobbold, 1879) (Fig. 4.II.3).

This is the first time that *Rhadinorhynchus* sp. Has been found in the euphausiid *Nyctiphanes couchii*. Euphausiids are an essential and abundant nexus between the mesozooplankton and nekton, ingested by fishes, cephalopods (both acting as paratenic host) and birds (Deagle et al. 2007, Braley et al. 2010, Roura et al. 2012).

Reports of larval acanthocephalans acting as intermediate hosts in zooplankton are scarce. Among the Palaeacanthocephala some *Echinorhynchus corrugatus* have been found in *Euphausia krohnii* (Marcogliese 1995). *Bolbosoma caenoforme* has been found infecting *Thysanoessa longipes* and *T. raschii* (Shimazu 1975), whereas *Bolbosoma* sp. Porta, 1908 has been detected in *Thysanoessa* sp. Brandt, 1851 (Tsimbalyuk 1980). Lindley (1977) reported 3 larvae of Paleoacanthocephala infecting the euphausiid *T. longicaudata.* Recently, 3 larval stages of Polymorphida *(Bolbosoma* or *Corynosoma)* were reported within *Nyctiphanes simplex* on the northwestern coast of Mexico (Gómez-Gutiérrez et al. 2010). Finally, cystacanths of *Bolbosoma balaenae* were found in *Nyctiphanes couchii* in the NE Atlantic (Gregori et al. 2012). Therefore, only acanthocephalans the orders Echinorhynchida and Polymorphida have been found in euphausiids. This fact may be related to their final hosts, with Echinorhynchida infecting mainly teleost fishes and occasionally amphibians and reptiles, whereas Polymorphida infect mainly birds and marine mammals (Bush et al. 2001).

Rhadinorhynchus is a generalist at the definitive host level. However, at the zooplankton level, it demonstrated some specificity for krill. This specificity is reinforced because we did not find cystacanths of *Rhadinorhynchus* sp. in the larvae of *Nyctiphanes couchii* (calyptopis and furcilias with 0.8–2.20 and 2.2–5.5 mm in total length, respectively). We also did not find them in copepod species or other taxa, probably due to the large size of these cystacanths (~11.41 mm). We suspect that only adults of *N. couchii* could harbour them in their cephalothorax (12–17 mm length). Consequently, it seems that the smaller zooplankton organisms cannot harbour large cystacanths or act as intermediate hosts for these acanthocephalans. Moreover, the large size of the cystacanths we found probably

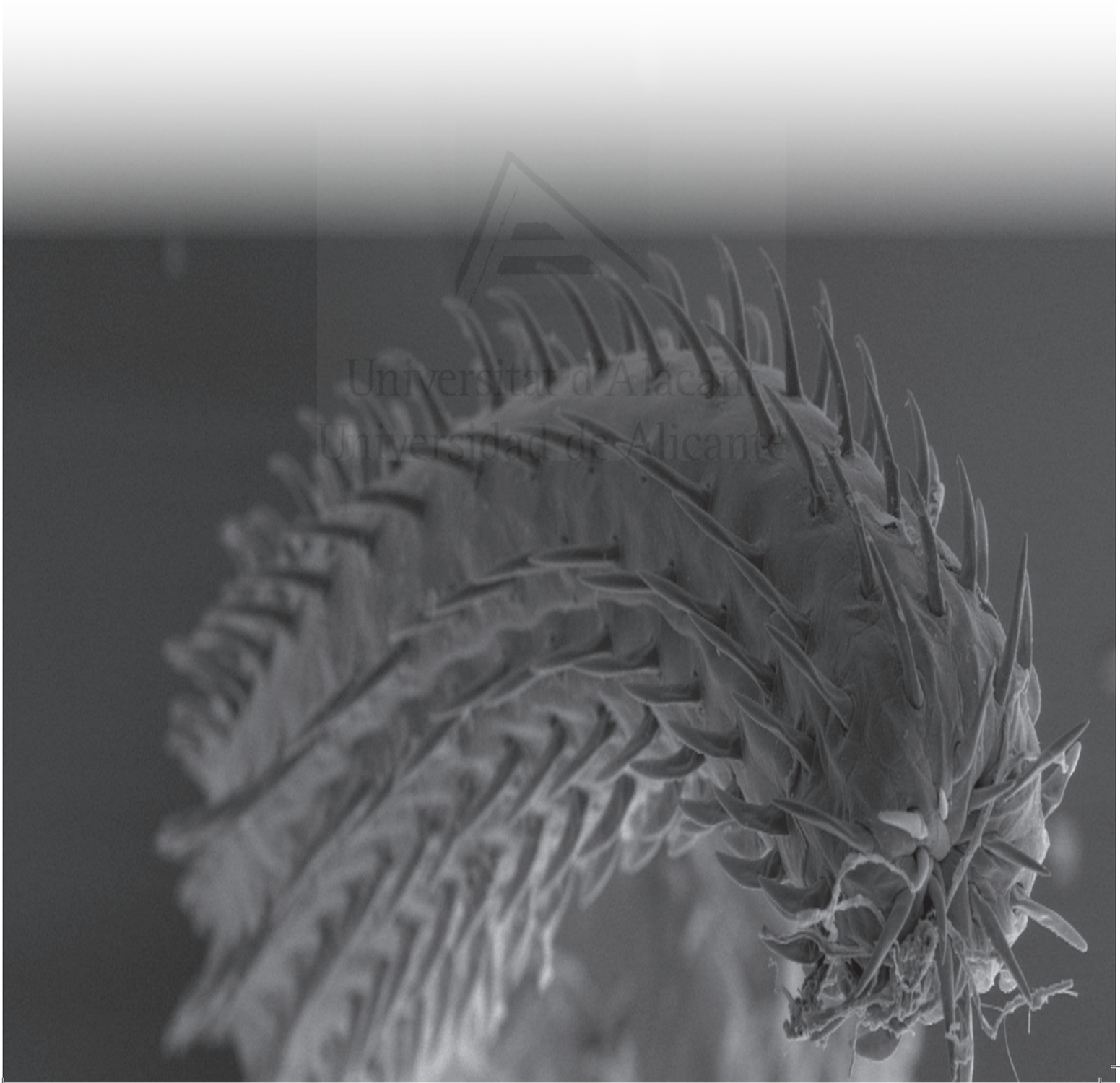
acts to limit the intensity to 1, because none of the examined *N. couchii* showed more than one cystacanth per individual, as described in Gómez-Gutiérrez et al. (2010) and Gregori et al. (2012).

The prevalence of *Rhadinorhynchus* sp. within the euphausiid population was very low (Table 4.II.2). This is usually considered a feature of the zooplankton trophic level because of the dilution effect of the pelagic realm where it becomes difficult to find a suitable intermediary host (Marcogliese 2002). Nevertheless, since most predators ingest large quantities of krill, euphausiids become significant intermediate or paratenic hosts that originate high infection rates and intensities in the final parasite's hosts(Marcogliese 1995, 2002). Despite the fact that low prevalence is a feature at the zooplankton level, information about it is very scarce. Nevertheless, our prevalences are similar to those reported by Shimazu (1975) with infection rates about 0.219% in *Thysa noessa longipes* and 13.33% in *T. raschii*. Gómez-Gutiérrez et al. (2010) reported an average of prevalence about 3.1% in *Nyctiphanes simplex* and Gregori et al. (2012) reported a prevalence of 0.10% in *N. couchii*.

The ecological impact of cystacanths can be better understood if we consider the whole mesozooplanktonic community where the sample was taken. Roura et al. (2013) defined 6 characteristic mesozooplankton communities in the Ría de Vigo during the upwelling season following the bathymetric gradient, 3 in early summer and 3 in autumn, named as coastal, frontal and oceanic. These 6 communities changed from summer to autumn due to a shift from relaxation-downwelled to upwelled conditions coupled with life-cycle changes in the zooplankton. We found cystacanths in the coastal (SC) and frontal (SF) summer communities as well as coastal (AC) and oceanic (AO) autumn communities (Table 4.II.3). A total of 2079 and 185107 adults of *Nyctiphanes couchii* were counted in SC and SF, respectively, and therefore the number of potential infected adults would be 6 and 8 in these 2 communities. In autumn communities under upwelling conditions the number of adults of *N. couchii* was 3363 and 16741 individuals in AC and AO. Accordingly, 4 and 24 would be the inferred number of infected adults in each community, respectively. These results suggest that the recruitment of parasites may be affected by the oceanography (Pascual et al. 2007).

In conclusion, we would like to emphasize that this is the first record of *Rhadinorhynchus* sp. in *Nyctiphanes couchii* in coastal waters of the NW Iberian Peninsula, with *N. couchii* probably acting through predator-prey interactions as an intermediate host. The results of our morphological and phylogenetic study, along with the available epidemiological information on *R. pristis* infection in Scombridae and Xiphidae fishes from the nearby Portugal coast, the Madeira Islands and the North Atlantic, suggest that the cystacanths herein described probably belong to this species (Rodrigues et al. 1975, Hogans et al. 1983, Vassiliades 1985, Rego 1987, Costa et al. 2004). However, we strongly recommend that a thorough review of the species, as well as the family Rhadinorhynchidae, should be carried out

The Platyhelminthes



5. THE PLATYHELMINTHES

5.I. *ANISAKIS SIMPLEX* COMPLEX (NEMATODA: ANISAKIDAE) IN ZOOPLANKTON COMMUNITIES FROM TEMPERATE NE ATLANTIC WATERS

5.I.1.Abstract

The euphausiid *Nyctiphanes couchii* and an unidentified mysid have been diagnosed, for the first time, as intermediate hosts for third-stage larvae (L_3) of the *Anisakis simplex* complex in the mesozooplanktonic community of the coastal upwelling system in Galicia (NW Spain). Parasite larvae were molecularly identified using the internal transcribed spacer (ITS) region. The prevalence of these parasites in the mesozooplankton was 0.0019%. The existence of parasites in a variety of mesozooplankton organisms suggests that the transmission routes of *A. simplex* and *A. pegreffii* are wider than expected. Moreover, the results demonstrated that these two *Anisakis* species are not specific for their intermediate hosts. Finally, our results shed light on the influence of oceanography over the recruitment of *A. simplex* complex, being different under upwelling or downwelling conditions.

5.I.2.Introduction

Cosmopolitan nematodes of the genus *Anisakis* Dujardin, 1845 are by far one of the most prevalent and host-ranged macroparasites in marine ecosystems. To date, three species are included in a complex of morphologically sibling species: *A. simplex sensu stricto (s.s.), A. pegreffii, and A. simplex* C. These species are hitherto morphologically indistinguishable at each developmental stage (from larval to adult). Accordingly, only molecular methods can be used to identify them at all developmental stages (Mattiucci & Nascetti 2006). The importance of Anisakis is due to the seafood security and safety concerns (EFSA 2010a). The ingestion of live anisakid larvae with raw or undercooked seafood can cause anisakiasis (Nagasawa 1990, Ishikura et al. 1993, Smith 1999, Du Plessis et al. 2004, Nieuwenhuizen et al. 2006). Furthermore, IgE-mediated reactions, with several clinical manifestations after eating well cooked but infected fish, have also been reported (Audicana et al. 1997, Caballero & Moneo 2004). Clinical symptoms include anaphylaxis, acute urticaria or angiodema in the course of gastro-allergic anisakiasis to allergic airbone asthma and dermatitis in the domestic and occupational settling after

work place exposure in the fish industry (EFSA 2010b, Kim et al. 2012, Pontone et al. 2012, Shiryaeva 2012).

From an ecological perspective, the evolutionary success of a multi-host complex life cycle within Anisakidae species has a marked phylogenetic basis (Mattiucci & Nascetti 2008). Moreover, it also has important constraints related to different factors that have favoured these indirect life cycles linked to the trade-offs between transmission strategies (Poulin 1998, Choisy et al. 2003). Actually, one of the most relevant findings is the fact that anisakids infect species that act as trophic bridges in marine food webs showing differential fitness among the host species of similar ecological value (Marcogliese 1995, Abollo et al. 1998, Abollo & Pascual 2001). All these strategies ensure a high parasite flow of larval stages from the mesozooplankton to the top predators. In this ecological framework euphausiids and mysids, naturally infected with larval (L_3) of Anisakidae, have been reported worldwide (Table 5.I.1).

Table 5.I.1. Records of *Anisakis* larvae in euphausiids and mysids. N = Number of hosts; n = number of infected host species; % = prevalence; *Anisakis* species can be seen next to its host *Nyctiphanes couchii*.

Location	Hosts	N	n	%	Reference
	Euphausiids				
Barents Sea	*Thysanoessa raschii*	?	1	?	Uspenskaya (1963)
Bering Sea and N Pacific	*T. raschii*	121	3	0.020	Oshima et al. (1969)
	T. longipes	405	2	0.005	
Northern N Pacific	*Euphausia pacifica*	54,000	1	0.002	Shimazu et al. (1970)
Northern North Sea. North of Scotland and Faroe	*T. inermis*	2,730	18	0.5-4	Smith (1971)
	T. longicaudata	950	3	0.7-1	
	Meganyctiphanes norvegica	3,178	1	0.031	Van Banning (in Smith 1971)
North West Pacific	*E. pacifica*			0.00002	Shimazu and Oshima (1972)
E China Sea	*E. pacifica*	28,219	2	0.007	Kagei (1974)
North Sea	*T. inermis*	?	2	?	Lindley (1977)
Central northern	*E. krohnii*				
Antartic Ocean	*E. vallentini*	11,233	2	0.018	Kagei (1979)
E China Sea	*E. nana*	?	1	?	Shimazu (1982)
NE Atlantic and Norh Sea	*T. inermis*	11,956	presence	0-4	Smith (1983)
	T. longicaudata	2,218	presence	0-1	
	T. raschii	6,587	presence	0-1.3	
	Nyctiphanes couchii	3,067	presence	?	
S Pacific	*N. australis*	11,850	3	0.0003	Hurst (1984)
St Lawrence Stuary	*T. raschii*	551,569	100	0.018	Hays et al. (1998)
	M. norvegica	9,681	1	0.010	
Prince William Sound. Alaska	*E. pacifica*	7,443	1	0.013	Smith and Snyder (2005)
	T. raschii	10,437	2	0.019	
NW coast of Mexico	*N. simplex*			0.0001	Gómez-Gutiérrez et al. (2010)
NE Atlantic Galician Waters	*N. couchii* (*A. simplex s.s.*)	69,954	1	0.0043	Present study
NE Atlantic Galician Waters	*N. couchii* (*A. pegreffii*)	69,954	1	0.0043	Present study
NE Atlantic Galician Waters	*N. couchii* (*A. simplex s.l.*)	69,954	1	0.0043	Present study
	Mysids				
Millport W. Scotland NE Atlantic	*Mesopodopsis slabberi*	131	1	0.763	Makings (1981)

Moreover, infected mysids have also been described by Køie (1993) who reported *Hysterothylacium aduncum* Rudolphi, 1802 from Isefjord. Marcogliese (1992, 1993) found ascaridoid nematodes in the opossum shrimp *Neomysis americana* Smith, 1873 from Sable Island pond (Eastern of Canada). In addition, Marcogliese (1993) found the spirurid *Ascarophis* sp. van Beneden, 1871 and *Paracuaria adunca* Creplin, 1846 in *Mysis stenolepis* Latreille, 1802 in Passamaquoddy Bay, New Brunswick. Finally, Jackson et al. (1997) reported infected mysids by *Pseudoterranova decipiens* Krabbe, 1878, *H. aduncum* and *P. adunca* in nine areas in the Northwest Atlantic. As far as we known, there is only a single report of *Anisakis simplex* in *Mesopodopsis slabberi* van Beneden, 1861 (Table 5.I.1) from West Scotland (Makings 1981).

The aim of this work was to undertake the diagnosis of the third-larval stage of anisakid nematodes found in mesozooplankton communities collected off NW Iberian Peninsula waters. Demographic and ecological findings were also discussed in relation to the coastal upwelling and the structure of mesozooplankton communities in the studied area.

5.I.3. Material and Methods

5.I.3.1. Collection and processing parasite larvae

Zooplankton samples were caught in the Ría de Vigo in Galician waters (NW Spain) on board of the RV "*Mytilus*" (Fig. 4.II.1). In 2008, ten surveys were undertaken, in summer (2nd, 4th, 9th and 11th of July) and autumn (26th of September; 1st, 3rd, 9th, 10th and 14th of October). Two samples were collected on each transect by double oblique towing (one at the surface layer and the other at the bottom) at a ship's speed of 2 knots, using a 750 mm diameter bongo net equipped with 375 μm mesh. Samples collected near the bottom were considered as integrated water-colum samples, because of the bongo nets methodology. Bongo net was also equipped with a current meter to determine the filtered volume. Zooplankton samples were fixed on board with 70% ethanol and stored at -20 ºC to avoid DNA degradation (Passmore et al. 2006).

Herein we analysed the surface and water column samples collected at transect 5 (T5, Fig. 4.II.1) during 2nd, 4th and 9th of July and 26th of September. Overall, eight samples with high abundance of euphausiids were thoroughly

screened under a stereomicroscope (20x) looking for nematodes. Mesozooplankton organisms were counted and identified to the lowest taxonomic level, as described in Gregori et al. (2013). Species diversity was calculated using the Shannon-Wiener Index (Omori & Ikeda 1984). When nematodes were found, they were extracted from the host by dissection. Seven larvae were used for morphological identification using features described by Hartwich (1974), Yoshinaga et al. (1987) and Berland (1989). These larvae were clarified with lactophenol and therefore it was not possible to use them for molecular procedures. Infection parameters were estimated following Bush et al. (1997) and Rósza et al. (2000). Sterne's exact 95% confidence interval (CI) was calculated for prevalence (Reiczigel 2003). Abundance of parasites was estimated, when prevalence could not be calculated (Bush et al. 1997).

5.I.3.2. Molecular methods

Genomic DNA was isolated from 11 L_3 using Qiagen DNeasy™ Tissue Kit according to manufacturer's instructions. DNA quality and quantity was checked in a spectrophotometer Nanodrop® ND-1000 (Nanodrop technologies, Inc) and in 1% agarose gel. The entire internal transcribed spacer (ITS) (ITS1, 5.8S rDNA gene and ITS2) was amplified using the primers NC5 (forward: 5'-GTAGGTGAACCTGCGGAAGGATCATT-3') and NC2 (reverse: 5'-TTAGTTTCTTCCTCC GCT-3') (Zhu et al. 2000).

PCR reactions were performed in a total volume of 25 μl containing 1 μl of genomic DNA (100 ng), PCR buffer at 1x concentration, 1.5 mM $MgCl_2$, 0.2 mM nucleotides (Roche Applied Science), 0.3 μM primers and 0.625 U Taq DNA polymerase (Roche Applied Science). The cycling protocol was 2 min at 94 ºC, 35 cycles with 30 s at 94 ºC, 30 s at 55 ºC and 1 min 15 s at 72 ºC, followed by 7 min at 72 ºC. PCR products were separated on a 2% agarose gel (in 1x Tris-acetic EDTA buffer), stained with ethidium bromide and scanned in a GelDoc XR documentation system (Bio-Rad Laboratories). PCR products were cleaned for sequencing using ExoSAP-IT reagent (USB Corporation) according to the manufacturer's instructions. Sequencing was performed by the company Secugen (Madrid). Chromatograms were analysed using ChromasPro version 1.5 Technelysium Pty Ltd. All generated sequences were searched for similarity using BLAST (Basic Local Alignment Search

Tool) through web servers of the National Center for Biotechnology Information (USA).

5.I.4. Results

According to the morphological features (Hartwich 1974, Yoshinaga et al. 1987, Berland 1989) 7 L_3 were identified as *Anisakis simplex sensu lato (s.l.)*. The average total length (TL) was 19.03 ± 3.20 mm [mean ± SD]. The smallest individual measured was 13.86 mm, while the largest one was 24.00 mm (TL). The amplification of the ITS region yielded a 1,000 bp amplicon, as recorded in D'Amelio et al. (2000). BLAST search showed sequence identity values of 100% with *A. simplex s.s*. (6 larvae) and with *A. pegreffii* (5 larvae). Nucleotide sequence data reported in this paper are available on GenBank under the Accession numbers from KF953967 to KF953977.

Taxonomic composition and abundance of the mesozooplankton samples herein analysed are detailed in Table 5.I.2. These samples belonged to summer frontal (SF), summer oceanic (SO) and autumn oceanic (AO) communities, which have recently been described in the Ría de Vigo (Gregori et al. 2013).

A total of 18 nematode L_3 larvae of the *Anisakis simplex* complex were found, 14 out of these 18 *Anisakis* belonged to the SF community. Five *Anisakis simplex s.l.* were found, one of them infecting *Nyctiphanes couchii* Bell, 1853 (Fig. 5.I.1 A) and two into *Salpa fusiformis* Cuvier, 1804 (Fig. 5.I.1 B-C), the remaining two were found free in the water column. Additionally, four *Anisakis simplex s.s.* were encountered. One of them was found laid apparently free in the haemocoel of an adult of *N. couchii* (Fig. 5.I.1 D), while the others were encountered free in the water column. Finally, five *Anisakis pegreffii* were found. One of them was located into the cephalothorax of an adult of *N. couchii* (Fig. 5.I.1 E-F) and the rest were found free in the water column.

In SO community, three L_3 were found. Two *Anisakis simplex s.l.* were encountered, one of them floating in the water column and the other infecting a mysid (Fig. 5.I.1 G). The last larvae belonged to *Anisakis simplex s.s*. and was also found in the water column. Finally, only one *Anisakis simplex s.s*. was found free in the AO community.

Demographic and parasitological values are summarized in Table 5.I.3. The abundances of *A. simplex s.l.*, *A. simplex s.s.* and *A. pegreffii* L_3 were 0.005, 0.007 and

Table 5.I.2. Composition, number and total abundance of mesozooplankton collected at transect 5 (T5, Figure 1). Shannon-Wiener index for the entire assemblage is shown. SF: Summer Frontal; SO: Summer Ocean; AO: Autumn Ocean communities were L_3 were found. N = number of animals; Ab = Abundance N/m^3. Filtered volume: 2nd of July column and surface = 410 and 685 m^3 respectively; 4th of July column and surface = 108 and 279 m^3 severally.

Communities								
	SF				SO			
Meroplankton	**2nd of July column**		**2nd of July surface**		**4th of July column**		**4th of July surface**	
	N	**Ab**	**N**	**Ab**	**N**	**Ab**	**N**	**Ab**
Cephalopoda								
Loliginids			1	0.15^{-3}				
Octopus vulgaris			1	0.15^{-3}				
Sepiolids	7	0.02						
Echinodermata								
Ophiuroidea larvae	512	1.25						
Fish								
Fish larvae	512	1.25	66	0.10	9	0.08	50	0.18
Gasteropoda								
Gasteropoda larvae					123	1.13		
Isopoda								
Aegidae	1	0.24^{-3}	1	0.15^{-3}				
Malacostraca								
Decapoda								
Alpheidae zoeae			560	0.82				
Brachyura juvenile			82	0.12	10	0.09	5	0.01
Brachyura megalopa			1,762	2.57	10	0.09	238	0.86
Brachyura zoeae	2,463	6.00	8,400	12.25				
Galatheidae zoeae								
Paguridae megalopa	480	1.17	560	0.82				
Paguridae zoeae			560	0.82				
Amphipoda Gammaridea	12	0.03	12	0.02	3	0.03	4	0.01
Amphipoda Hyperiidea	73	0.18			11	0.10	8	0.03
Stomatopoda								
Meiosquilla desmaresti	480	1.17			123	1.13	9	0.03
Platysquilla eusebia			560	0.82				
Polychaeta								
Polychaeta larvae	5	0.01			1	0.01	7	0.03

	SF				SO			
Holoplankton	**2nd of July column**		**2nd of July surface**		**4th of July column**		**4th of July surface**	
	N	**Ab**	**N**	**Ab**	**N**	**Ab**	**N**	
Chaetognatha			165	0.24	369	3.40	1,991	7.14
Siphonophora	1,536	3.74						
Euphausiacea								
Nyctiphanes couchii calyptopis	36,433	88.78	10,080	14.71	1,723	15.88	234	0.84
N. couchii furcilia	49,388	120.35	52,080	75.98	3,938	36.30	351	1.26
N. couchii adult	69,955	170.47	7,280	10.62	1,846	17.01	117	0.42
Maxillipoda								
Copepoda								
Calanoidea								
Acartia clausii	7,421	18.08	50,960	74.35	492	4.54	2,693	9.66
Candacia armata								
Calanoides carinatus	72,259	176.08	106,960	156.05	23,262	214.37	19,785	70.99
Calanus helgolandicus	35,156	85.67	21,280	31.05	16,246	149.72	10,185	36.54
Centropages chierchiae	3,966	9.67	10,080	14.71	738	6.81	702	2.52
Clausocalanus spp.			1,120	1.63			117	0.42
Mesocalanus tenuicornis								
Metridia lucens								
Paracalanus parvus								
Paraeuchaeta hebes	8,637	21.05	19,040	27.78	6,031	55.58	2,810	10.08
Paraeuchaeta sp.	4,797	11.69			6,646	61.25	3,512	12.60
Pseudocalanus elongatus								
Scolecithricella spp.			1,680	2.45				
Temora longicornis								
Calanoid copepodit								
Mysidacea	1,919	4.68	560	0.82	492	4.54	702	2.52
Salpida	21,175	51.60	10,773	15.72	8,661	79.82	35,680	128.01
Total N	317,186		304,622		70,736		79,203	
Shannon index (H')	**1.55**		**1.99**		**1.91**		**1.60**	
Eveness index	**0.15**		**0.61**		**0.64**		**0.52**	

Table 5.I.2. Continuation. 9th of July column and surface = 830 and 193 m^3 respectively; 26th of September column and surface = 221and 34 m^3 severally.

Communities								
	SO		SF		AO			
Meroplankton	9th of July column		9th of July surface		26th of September column		26th of September surface	
	N	Ab	N	Ab	N	Ab	N	Ab
Cephalopoda								
Loliginids					2	0.01		
Octopus vulgaris	3	0.36^{-3}			1	0.45^{-3}		
Sepiolids	1	0.12^{-3}	1	0.52^{-3}				
Fish								
Fish larvae	73	0.09	307	1.59	29	0.13	2	0.06
Isopoda								
Aegidae	6	0.01						
Decapoda								
Alpheidae zoeae	267	0.32	267	1.38				
Brachyura juvenile	4	0.48^{-3}			2	0.01		
Brachyura megalopa	4	0.48^{-3}					8	0.22
Brachyura zoeae	800	0.96	800	4.14				
Galatheidae zoeae							8	0.22
Scyllarus arctus zoeae	267	0.32						
Amphipoda Caprellidea							1	0.03
Amphipoda Gammaridea	30	0.04	8	0.04	6	0.03		
Amphipoda Hyperiidea	12	0.01	4	0.02	5	0.02		
Stomatopoda								
Meiosquilla desmaresti	8	0.01						
Polychaeta								
Polychaeta larvae	24	0.03			35	0.16		

	SO		SF		AO			
Holoplankton	9th of July column		9th of July surface		26th of September column		26th of September surface	
	N	Ab	N	Ab	N	Ab	N	Ab
Chaetognatha	5,408	6.52	2,933	15.18	1,861	8.43	1,558	45.69
Cnidaria							30	0.89
Siphonophora	267	0.32	267	1.38	74	0.33	15	0.44
Euphausiacea								
Nyctiphanes couchii calyptopis	2,933	3.53	1,600	8.28	2,496	11.30	83	2.43
N. couchii furcilia	1,333	1.61	4,533	23.46	4,512	20.44	8	0.22
N. couchii adult	267	0.32			3,600	16.31	98	2.88
Copepoda								
Calanoidea								
Acartia clausii	19,733	23.77	17,867	92.45	1,263	5.72	1,072	31.42
Candacia armata	267	0.32						
Calanoides carinatus	50,933	61.36	43,733	226.29	1,600	7.25		
Calanus helgolandicus	22,133	26.67	7,467	38.63	1,853	8.39	53	1.55
Centropages chierchiae	1,067	1.29	267	1.38				
Clausocalanus spp.	267	0.32	533	2.76			8	0.22
Mesocalanus tenuicornis					253	1.14	15	0.44
Metridia lucens	1,333	1.61	267	1.38	1,432	6.48	23	0.66
Paracalanus parvus	267	0.32			253	1.14	30	0.89
Paraeuchaeta hebes	7,467	9.00	7,200	37.25	7,326	33.18	513	15.05
Paraeuchaeta sp.	30,667	36.95	29,600	153.16	20,463	92.69	732	21.46
Pseudocalanus elongatus			800	4.14				
Temora longicornis							8	0.22
Calanoid copepodit			267	1.38				
Mysidacea	800	0.96	800	4.14	528	2.39	211	6.20
Salpida	24,356	29.34	36,823	190.53	3,874	17.54	2,743	80.43
Total N	170,996		156,344		51,466		7,218	
Shannon index (H')	**2.01**		**1.94**		**2.31**		**1.78**	
Eveness index	**0.59**		**0.62**		**0.69**		**0.58**	

0.01 L_3/m^3 respectively in SF community (2nd of July). In samples of the SO community (4th July) the abundance of *A. simplex s.s*. was 0.009 L_3/m^3 while the abundances of *A. simplex s.l*. were 0.003 L_3/m^3 for those found free in the water column and 0.001 L_3/m^3 for the one found among mysids. Finally, in AO community (26th September) the abundance of *Anisakis simplex* s.s. was 0.004 L_3/m^3.

Table 5.I.3. L_3 of *Anisakis simplex* complex found on each mesozooplankton community. Summer frontal (SF) and summer oceanic (SO) communities. SP: Species of parasite. IA: Infected animals. NKP: Number of non-infected krill population (*N. couchii*) population. NKA: Number of non infected krill's adult of *N. couchii.* NSP: Number of non-infected *Salpa fusiformis*. NMP: Number of non-infected mysid hosts %[CI]*: Krill Adults prevalence and confidence interval (which are the same for each species of the complex). %[CI]: Krill population prevalence and confidence interval (which also are the same for each species of the complex) Superscripts: host where parasites were found. NC: *N. couchii*. S: *Salpa fusiformis*.

SF					
SP	***Anisakis simplex* s.l.**	***A. simplex* s.s.**	***A. pegreffi***	**%[CI]***	**%[CI]**
IA	1^{NC}; 2^{S};	1^{NC}	1^{NC}		
NKP		155,775		0.0006 [0,00002 – 0.00357]	0.0019 [0.00040 – 0.00562]
NKA		69,955		0.0014 [0.00004 – 0.00796]	0.0043 [0.00088 – 0.0125]
NSP		31,948			0.00626 [0.00758 - 0.02261]
SO					
	***Anisakis simplex* s.l.**				
	1				
NMP	800				

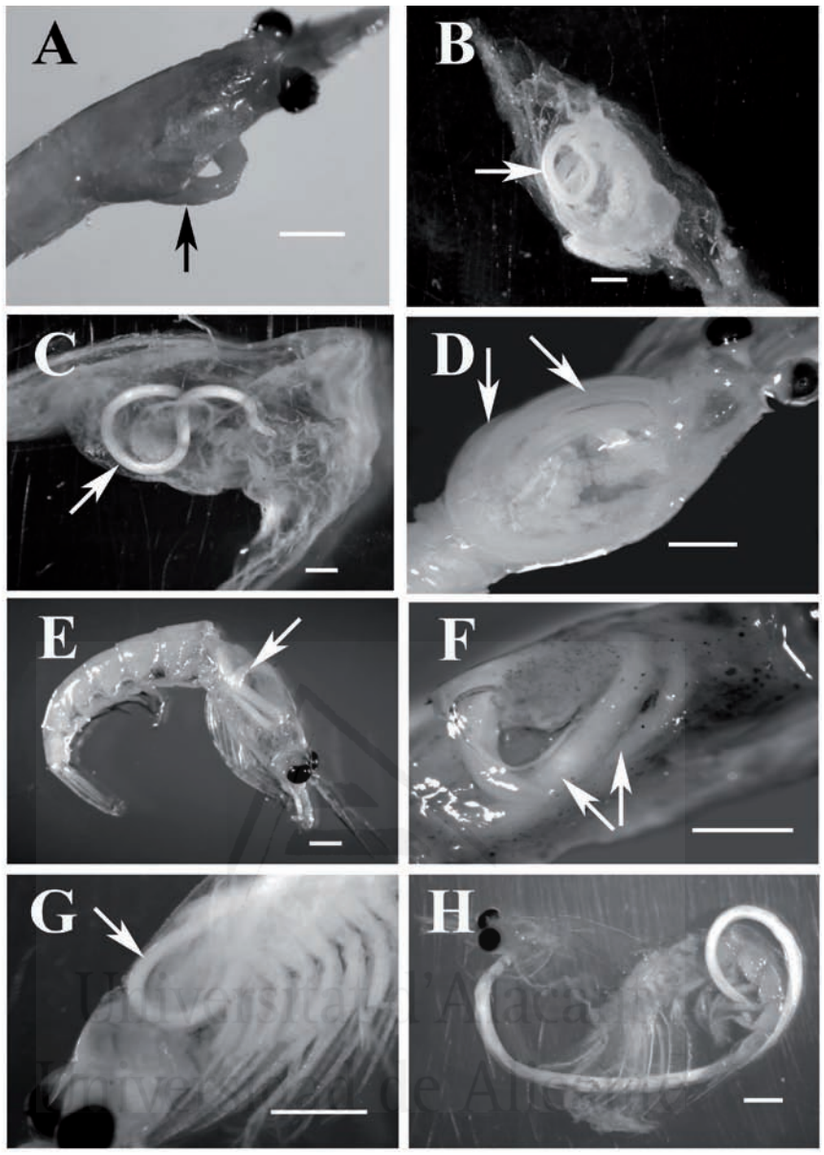


Fig. 5.I.1. Single infection of the *Anisakis simplex* complex L3. (A) *Nyctiphanes couchii* infected with A. simplex s.l.; (BC) *Salpa fusiformis* with *A. simplex s.l.*; (D) *N. couchii* infected with *A. simplex* s.s.; (E-F) *N. couchii* infected with *A. pegreffii*; (G) Unidentified mysid infected with *A. simplex* s.l.; (H) L_3 come from dead krill *N. couchii*.. Escale bar: 1000 µm.

5.I.1. Discussion

This study represents the first research on *Anisakis simplex* complex larvae from zooplankton communities collected on temperate NE Atlantic waters. The identification of *A. simplex s.s*. and *A. pegreffii* parasitizing zooplankton samples showed that both species are able to share the same intermediate euphausiid host *Nyctiphanes couchii*. This result reinforces their sympatric distribution in NE Atlantic (Rêgo et al. 1985, Abollo & Pascual 2001). This finding is not surprising considering that *N. couchii* is the main euphausiid in the European continental shelf (Lindley 1977) and that they have been previously described as intermediate host for anisakids (Smith 1983). Moreover, this krill species is one of the dominant taxa in the mesozooplankton communities over the shelf during summer and autumn off NW of the Iberian Peninsula waters (Gregori et al. 2013). *N. couchii* is also an important prey item of different cephalopods and fish species, which serve as paratenic hosts for transmitting *Anisakis simplex* complex (Abollo et al. 1998, Gestal et al. 1999, Valero et al. 2000, Abollo & Pascual 2001, Raga et al. 2009, Gregori et al. 2012, Llarena-Reino et al. 2012) towards their definitive host, the marine mammals, by means of predator-prey interactions (Marcogliese 1995). The overall prevalence values of anisakids from mesozooplankton communities off Galicia were low, however this feature is common at the zooplankton population level (Marcogliese 1995) and agree with previous epidemiological data (Table 5.I.1).

On the other hand, mysids are the most abundant crustaceans in the hyperbenthos, which are able to undertake vertical migrations and become part of the zooplankton (Jackson et al. 1997). This migration could allow *Anisakis simplex* to overstep habitats creating trophic linkages between benthic and pelagic environments, further increasing the ramifications for their transmission (Marcogliese 2002). In fact, mysids have been underlined to play an important role in the transmission of other anisakid nematodes as *A. simplex*, *Hysterothylacium aduncum*, *Pseudoterranova decipiens* and *P. adunca* (Makings 1981, Marcogliese 1992, Køie 1993, Marcogliese 1993, Jackson et al. 1997). Furthermore, Køie (1993) reported natural infections in soft bodied animals as second intermediate or transport host for anisakids. Nevertheless, we consider that the *A. simplex s.l*. found in *Salpa fusiformis* could be an artefact because July samplings were dominated by

salpids. Coupling this bloom of salps with the presence of free L_3 could have promoted their accidental introduction into the salps during the tows. Moreover, salps are considered herbivorous (Riedl 1986) thus it is unlikely that they could actively prey on infected zooplankton.

Previous records (Karasev 1993, Køie 2001) noticed that it is possible to find free parasitic larvae of anisakid species in the pelagic habitat, which are presumably coming from dead crustacean intermediate host or dead fish paratenic host. Additionally, in hot-spot fishing grounds it is common the discarding practices of L_3 at sea through by-catch or offal activities on-board.

Shimazu and Amano (2001) suggested that L_3 may be infective for younger euphausiids and/or the euphausiids become infected via copepods as paratenic hosts. In this work, we did not find *Anisakis simplex* L_3 infecting the larvae of *N. couchii* (calyptopis and furcilias with 0.8-2.20 and 2.2-5.5 mm in total length, respectively), or in copepods or other taxa except mysids and salpids. The absence of L_3 in copepods may be influenced by their small size, which is less than 3 mm (total length) for the biggest copepods in the studied area (*Calanus helgolandicus* Claus, 1863, *Calanoides carinatus* Kroyer, 1849 and *Paraeuchaeta hebes* Giesbrecht, 1888). The relative large size of the L_3 (~19.03 mm), suggests that only mysids and euphausiids are big enough to host nematode parasites in their body cavity. Consequently, it seems that in this area the smallest organisms cannot harbour large L_3 and neither act as intermediate nor paratenic host for *A. simplex* L_3. Furthermore, none of the examined infected animals showed more than one nematode per individual as found by Gómez-Gutiérrez et al. (2010), presumably because this intermediate hosts with multi-infections die (Smith 1983, Smith & Snyder 2005). This same pattern has been observed when *N. couchii* become infected with acanthocephalans' cystacanth larvae (Gregori et al. 2012, 2013).

The current analysis of nematode infection in different mesozooplankton communities shed light on the hypothesis that the parasite recruitment to these communities could be directly conditioned by the oceanography. Pascual et al. (2007) hypothesized that stability in water masses enhance parasite recruitment, especially for heteroxoneuous parasites with complex, multiple-host life cycles. As referred to by the authors, *Anisakis* spp. satisfies the conditions explained above and their recruitment is strongly affected by oceanographic conditions under

upwelling-downwelling events. In a global geographical scale they concluded that in upwelling systems parasite faunas are impoverished whereas downwelling events propitiate optimal conditions to successful recruitment. Our results suggest a similar pattern in a smaller geographical scale in the Ria of Vigo's upwelling-downwelling system. Herein, summer samplings were carried under downwelling-relaxation conditions that increased water stability. Within this context the recruitment of *Anisakis* spp. in summer communities was higher than in autumn, which occurred under upwelling conditions and strong water instability.

Additionally, summer frontal (SF) communities showed a higher amount of parasite larvae than oceanic communities (SO). This fact coincided simultaneously with a huge swarm of breeding adults of *N. couchii* located in SF (Gregori et al. 2013). Indeed, the L_3 found free in this community might come from dead krill (Fig. 5.I.1 H) as observed by Køie (2001). Moreover, the big euphausiid swarm observed in SF communities also hosted acanthocephalan larvae Gregori et al. (2012, 2013).

On the whole, this is the first record of *A. pegreffii* in *N. couchii*, which probably acts, through predator-prey interactions, as intermediate host in coastal waters of the NW Iberian Peninsula. As a result, the *Anisakis* sibling species are able to share the same intermediate host in a sympatric distribution among krill. The infected mysid suggests that *Anisakis* spp. are not specific at the mesozooplankton level and indicates that they use different hosts to cross habitats and enlarge the pathways in order to find their definitive mammal host. Further studies should be undertaken to identify the mysid host, as well as to test and evaluate variations of parasite prevalence and recruitment in the mesozooplankton and hyperbenthos under different upwelling/downwelling scenarios.

5.II. *Muggiaea* sp. (Siphonophora: Cnidaria) the second intermediate host of *Opechona bacillaris* (Digenea: Lepocreadiidae) from the Ria of Vigo NE Atlantic Ocean

5.II.1. Abstract

In the mesozooplanktonic community of the coastal upwelling system of the Ría of Vigo (NW Spain), the Siphonophora *Muggiaea* sp. has been identified for the first time in temperate waters of the NE Atlantic as the second intermediate host for metacercariae of *Opechona bacillaris*. As Digenean *O. bacillaris* is one of the most important endoparasites of fish with complex life cycles that uses a wide range of gelationous animals like medusa, ctenophore and chaetognata as secondary hosts. Their transmission to fish occurs under predator-prey interactions where fish predation on jellyfish is a widespread phenomenon. Parasites were identified using morphological characters described in 11 metacercariae. This is the first approach to understand the importance of the oceanographic conditions in the recruitment of parasites into the mesozooplankton communities. Prevalences found through the communities agreed with the coastal and estuarine character of the infection registered in trematodes. Moreover, the prevalence variation is coupled with both different oceanographic conditions and Siphonophora behaviour.

5.II.2. Introduction

Trematodes are parasitic worms that virtually infect all vertebrate groups including several fish families of significance to human health with considerable economic impact (Quinteiro 1990, Gibson et al. 2001). Metacercariae of the main groups of marine trematodes, Hemiuroidea, Lepocreadioidea and Didymozooidae have been found to infect zooplankton (Marcogliese 1995). Adult lepocreadioids typically inhabit the intestine or pyloric caeda of teleosts. Coelenterates and Ctenophores are well-documented intermediate hosts (Lebour 1916, Stunkard 1969, Reimer & Pierson 1971, Køie 1975, Yip 1984).

Opechona balillaris (Molin, 1859) Looss, 1907 is a cosmopolitan digenetic trematode in the Atlantic Ocean and adjacent seas that have as their final host the main commercial fish families such as the Scombridae, Carangidae, Gadidae,

Scorpaenidae, Clupeidae etc. It is known that in their free swimming stage the cercariae, actively finds and penetrates the epidermis of ctenophores or medusas where it develops in metacercariae using these animals as a second intermediate host as well as several species of Chaetognatha and Polychaeta (Køie 1975, 1984). In this particular case *O. bacillaris* cercariae does not depend on copepods for transfer as does somes other species of Lepocreadioidea (Køie 1975, Marcogliese 1995). The soft-bodies intermediate hosts indirectly transmit metacercariae when the definitive hosts prey on them (Marcogliese 1995).

Siphonophores are a group of pelagic colonial hydrozoans (Cnidaria) that have long been of general interest because of the division of labour between the polyps and medusae that make up these "superorganisms". Among them only in calicophores, two groups of parasites have been described. Totton (1954) reported fifty well-developed cercariae (with eyes) of *Lepocraedium album* Stossich, 1890 in *Hippopodius hippopus* Forsskål, 1776 and (Rose & Cachon 1951) described a peridinian *Diplomorpha paradoxa* Rose & Cachon, 1951 infecting them. Finally, an amphipod has been reported as a parasite of this kind of Siphonophora (Totton 1965). Thus, except these few reports nothing is known about parasites in calicophores or for trematodes. In North East of Atlantic Ocean *Muggiaea* sp. are generally small with a total length of <10cm and are also one of the most abundant non-crustacean zooplankton predators in the surface waters (Todd & Laverack 1991, Baxter et al. 2012, Gregori et al. 2013). As described in Gregori et al. (2013) *Muggiaea* sp. showed a marked seasonally related to the upwelling-downwelling events during 2008 in North East Atalntic Ocean. In this way, it is known that this kind of physical changes in the marine environment affects the composition and structure of plankton communities, including their parasites (Marcogliese 2004). *Muggiaea* sp. as member of the mesozooplankton community play an important role in marine ecosystem food webs, changing matter, energy and parasites from the lower to the higher trophic levels (Purcell 1982, Longhurst & Glen Harrison 1989, Marcogliese 1995, Purcell & Arai 2001, Marcogliese 2002, 2004).

According to Gregori et al. (2013) the mesozooplankton community in North East Atlantic Ocean is strongly controlled by the physical environment. Thus it is plausible to think that their parasites are also controlled by the same physical interactions in their free-living stage. Parasite transmission depends on the

presence of their intermediate or definitive host with which they have developed predator-prey interactions throughout of the co-evolution, ensuring a passive arrival to their definitive host. In the case of *Opechona bacillaris* this transmission takes place at the metacercariae larval stage which is infective for the definitive hosts Atlantic mackerel *Scomber scombrus* Linnaeus, 1758; Rudderfish *Centrolophus niger* Gmelin, 1789; Atlantic herring *Cuplea harengus* Linneaus, 1758; Atlantic cod *Gadus morhua* Linneaus, 1758; Red porgy *Pagrus pagrus* Linneaus 1758 etc. (http://www.nhm.ac.uk/research-curation/scientific-resources/taxonomy-systematics/host-parasites/ (Køie 1975, Marcogliese 1995, 2002, 2004, 2005).

Despite the crucial role of soft-body species as a component of the mesozooplankton in North East Atlantic Ocean trematode parasites remain both, without knowledge of their intermediate hosts and their life-cycle not completed. Moreover, nothing is known about how the oceanographic conditions affect their recruitment to the mesozooplankton as the lower level of the food chain. Due to this we would like to shed light on (i) the role of *Muggiaea* sp. on the life cycle of *Opechona bacillaris sensu stricto* metacercariae from the northwest of the Iberian Peninsula and (ii) provide demographic infection values of metacercariae among different communities of mesozooplankton through different oceanographic scenarios.

5.II.3. Material and Methods

5.II.3.1. Collection and processing of metacercariae

Zooplankton samples were caught in the Ría of Vigo in Galician waters (NW Spain) on board of the RV "*Mytilus*" (Fig.4.II.1). In the summer of 2008, four surveys were undertaken (2nd, 4th, 9th and 11th of July). Two samples were collected on each transect by double oblique towing, using a 750 mm diameter bongo net equipped with 375 µm mesh. At a ship's speed of 2 knots, the bongo net was first lowered and stabilized near the bottom for a period of 15 min, then hauled to the surface at 0.5 ms^{-1}. The bongo net was also equipped with a current meter to determine the filtered volume. Zooplankton samples were fixed on board with 70% ethanol and stored at -20 ºC to avoid DNA degradation (Passmore et al. 2006). Estimations for accompanying zooplankton taxa and abundance, Shannon index

and species evenness, where metacercariae were found, are recorded in Gregori et al. (2013). Moreover, Gregori et al. (2013) defined 3 characteristic mesozooplankton communities in the Ría of Vigo during the upwelling season following the bathymetric gradient, named as coastal, frontal and oceanic in early summer and in autumn. These 3 communities changed from summer to autumn due to a shift from relaxation-downwelled to upwelled conditions coupled with lifetime changes in the mesozooplankton.

For quantitative measures of parasites we focused our metacercariae study in samples collected in July. For determination of total abundances of the Siphonophora and metacercariae in the preserved samples, we used a sub-sample obtained a Folsom splitter (Omori & Ikeda 1984). At least 13 aliquots were counted using a Stempel pipette where infected and uninfected individuals were separated under a stereomicroscope (20x). When metacercaria were found, they were isolated from their host, and cleared using the protocol described by (Gregori et al. 2012). They were measured and photographed using a Nickon DS-Fi1 digital camera mounted on a Nikon ZMZ800 stereomicroscope. Scanning electron microscopy (SEM) preparations in a Philips XL 30 were used to clarify the morphological examination. Infection parameters were estimated following Bush et al. (1997) and Rósza et al. (2000). Sterne's exact 95% confidence interval (CI) was calculated for prevalence (Reiczigel 2003).

5.II.3.2. Statistical analysis

To test which variable better explained the observed infection we have used a logistic regression in a generalized linear model (GLM) with binomial error term and logit link. The best model was selected under the Akaike information criterion (Akaike 1974) as implemented in R 2,12.2 software (Bolker 2011). We also tested if the prevalence was significantly different among communities, station, strata or day as well as, how the abundance of the host was affecting it. The intensity was not analyzed. The metacercariae found free in the water column was not included in the statistical analysis and or in the prevalence calculation.

5.II.4. Results

In naturally infected gelatinous animals the metacercariae were found in the swimming bell (nectophore) especially near to the somatocyst and cormidia. The metacercariae along with its host was deposited at the Natural History Museum of London under collection number 2010.6.25.2.

The internal structures of the metacercariae are not different from those of mature cercariae. The pigment granules of the eyes are scattered and appear red-brown (Fig. 5.II.1 A-D). No growth of the testes or ovaries takes place in our specimens. The shape differs from being nearly oval and slightly dorso-ventrally flattened (Fig. 5.II.1 D-G) to being more cylindrical and with a narrowing about one third from the anterior end. We were able to appreciate some differences in shape due to the oral sucker sometimes appeared reverted. The ventral sucker occurs just posterior to the middle of the body (Fig. 5.II.1 D-G). Whole body is covered with tiny spines (Fig. 5.II.1 G-H). We also were able to distinguish the uroproct (the excretory aperture used also as a vent for gut contents) that allow us to identify the metacercariae as *Opechona bacillaris sensu stricto*.

Measurements in micrometres (mean ± SE) were based on 11 metacercariae taken from naturally infected *Muggiaea* sp. siphonophores (Table 5.II.1).

Table 5.II.1. Metacercariae measurements in micrometres. BL = body length, OS = oral sucker, VS = ventral sucker, Pharynx L = pharynx length and Pharynx W = pharynx weigh.

Label	BL	OS	VS (L x W)	Pharynx L	Pharynx W
270822Di1	555,36	95,55 x 83,55	54,52 x 61,90	46,43	45,104
270822Di2	677,91	123,90 x 111,95			
270821Di34	681,26	135,58 x 101,76	59,33 x 56,38	51,63	34,081
270822Di16		140,24 x 93,02	71,93 x 54,07	45,702	36,742
270822Di17	477,37	122,88 x 85,62	64,61 x 70,31	54,915	36,016
270822Di22	645,57	148,20 x 116,94	65,73 x 78,22		
270822Di23	573,61	119,89 x 91,75	66,81 x 42,51	49,863	37,022
970852Di19	613,03	105,17 x 72,51	69,55 x 66,85	43,379	33,793
970852Di128	515,70		69,37 x 27,86		
270822Di24	634,25				
970852Di76	612,31	88,75 x 59,76	64,26 x 46,55	42,043	31,138
Mean	598,64	120,02 x 90,76	65,12 x 56,07	47,70885714	36,27085714
SE	67,54	20,20 x 18,14	5,41 x15,48	4,627035387	4,399943728
Max	681,26	148,20 x 116,94	71,93 x78,22	54,915	45,104
Min	477,37	88,75 x 59,76	54,52 x 27,86	42,043	31,138

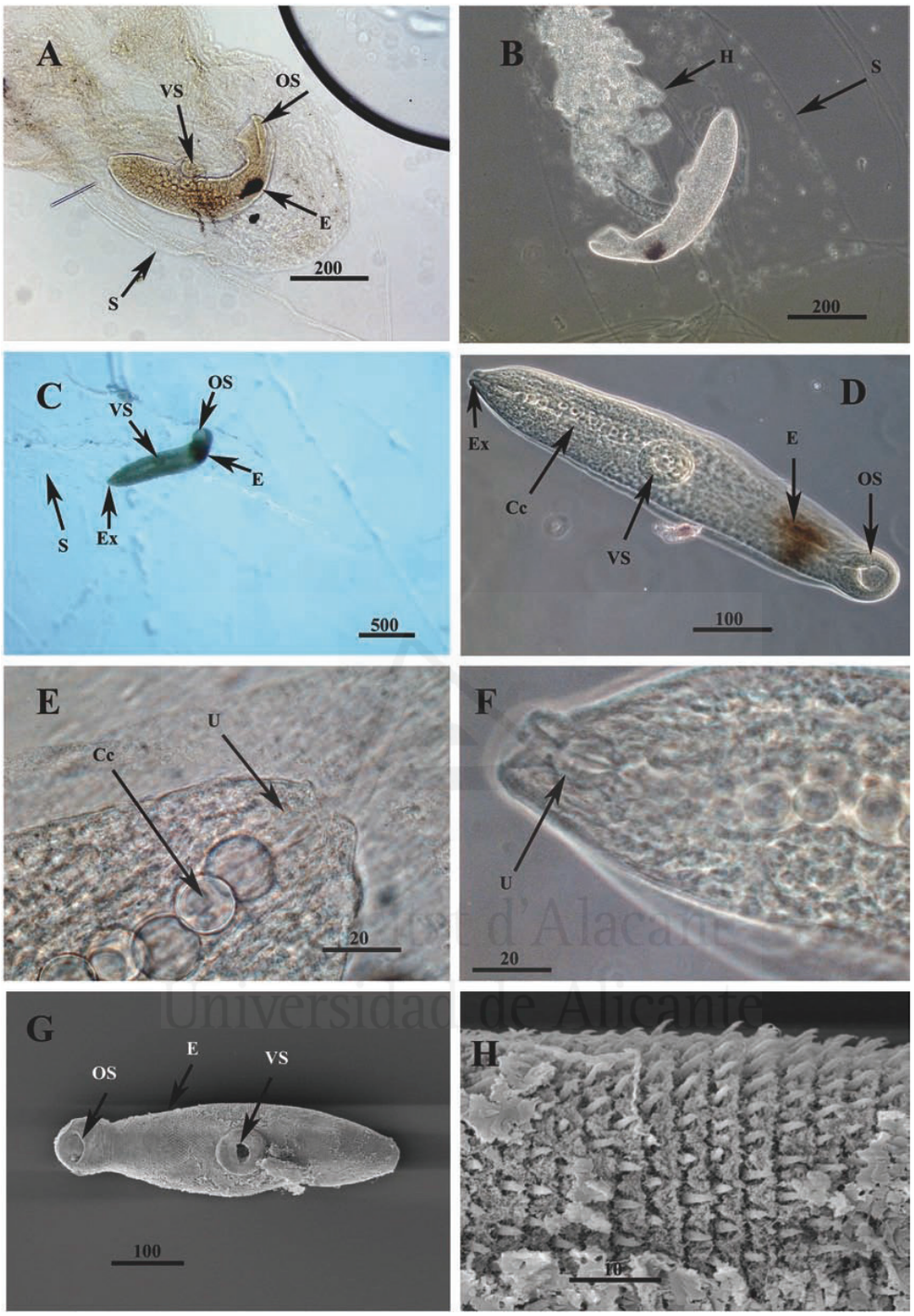


Fig. 5.II.1. *Opechona bacillaris* metacercaria A-C: metacercariae infecting *Muggiaea* sp. where OS = oral sucker, E = eyespots, VS = ventral sucker, Ex = Excretory pore, S = *Muggiaea* sp., H = hidroecium, Cc = calcarian concretions; (D) isolated metacercariae from host; (E-F) metacercariae body end where it is visible the U = uroproct;; (G-H) metacercaria surface detail SEM with E = body espination. Scale bar measured in micrometres. Scale picture H 10 μm.

5.II.4.1. Parasitological quantitative descriptors

A total of 945 *Muggiaea* sp. were examined for parasites divided in 106, 66 and 773 individuals in summer frontal (SF), summer ocean (SO) and summer coastal (SC) communities, respectively. The explanatory variables extracted from the analysis of deviance Table 5.II.2 were Day, Community, and Host abundance. Host abundance had a negative effect on prevalence with a p-value = 0.0071. Communities from highest to lowest prevalence were SC, SF, and SO (p-value = 0.0305). Days from highest to lowest prevalence were 11/7/08, 4/7/08, 2/7/08 and 9/7/08 with a p-value of 0.0024.

Infected, non-infected and abundance of the *Muggiaea* sp. as well as prevalence and confidence interval (95%) is shown at Table 5.II.3. The distribution of host abundances among communities and infected non-infected animals are shown in Fig. 5.II.2. Finally, 14 metacercariae were found in the water column without their intermediate host (free). Only one was fond in SF community whereas the rest (13) belonged to the SC.

Table 5.II.2. Deviance analysis table (type II test) where the response was infected. Day (Fac. day), Community (Comm), Host abundance (Hosttab).

	LR Chisq	Df	Pr (>Chisq)
Comm	6.9804	2	0.030495*
Fac. day	14.4136	3	0.002393**
Hosttab	7.2569	1	0.007063**

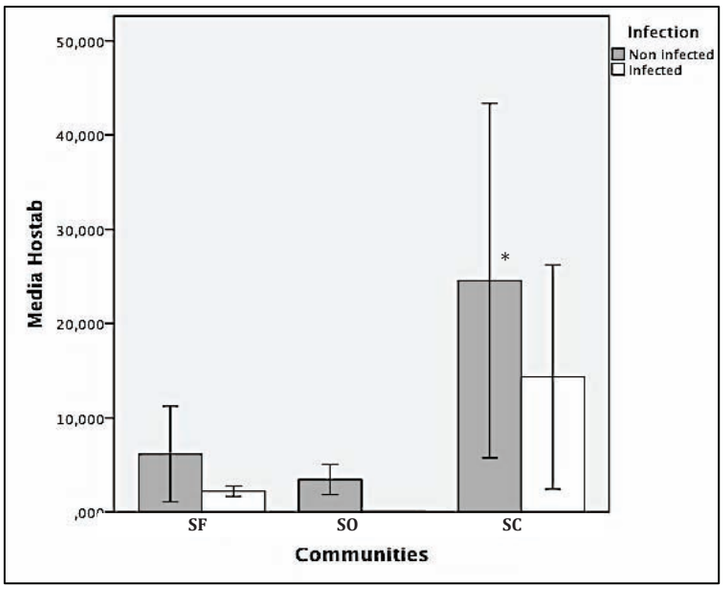


Fig. 5.II.2. Mean abundance of hosts distributed by community and infected non-infected animals. * = 0.00385 Significance of host abundance as explanatory variable.

Table 5.II.3. *Muggiaea* sp. population in summer communities from Ría of Vigo, Galicia, NW Atlantic, Spain during 2008, where metacercariae were found. Communities (comm) were: SF = Summer Frontal, SO = Summer Ocean, SC = Summer Coastal sampled on 2ond, 4th, 9th and 11th of July. I = Number of *Muggiaea* sp. infected with metacercariae *Opechona bacillaris*; NonI = Number of non-infected *Muggiaea* sp.; NHost = Total number of hosts; HotsAb = Abundance of *Muggiaea* sp. ($n*m^{-3}$); % [CI] = prevalence followed by the confidence interval (95%); P-values from statistical analyses.

Day/ Comm	SF					SO					RV					P-Value
	I	NonI	NHost	HostAb	% [CI]	I	NonI	NHost	HostAb	% [CI]	I	NonI	NHost	HostAb	% [CI]	Day
2/7/08	4	58	62	7,20	6,45 [1,79-15,70]	-	-	-	-	-	10	153	163	7,50	6,13 [2,98-10,99]	
4/7/08	1	31	32	2,03	3,12 [0,08-16,70]	-	-	36	4,73	-	11	129	140	16,21	7,86 [3,99-13,62]	0,1704
9/7/08	-	11	11	11,14	-	-	-	22	2,45	-	3	225	228	12,64	1,32 [0,27-3,80]	0,0204*
11/7/08	-	1	1	0,24	-	1	7	8	0,08	12,5 [0,32-52,65]	2	240	242	51,01	0,83 [0,10-2,98]	0,1427
Total	5	101	106	5,98	4,72 [1,55-10,66]	1	7	66	3,41	1,51 [0,04-8,15]	26	747	773	24,22	3,36 [2,21-4,89]	
P-value Comm					P<0.0001					0,1716					0,1011	

5.II.5. Discussion

The metacercariae of *O. bacillaris* has been found several times in zooplaktonic animals, but it is the first record of the Siphonophora *Muggiaea* sp. in NW Atlantic Ocean. Detailed description of *O. bacillaris*, measurements and location into their gelatinous hosts have been reported, thus they were described inside the umbrella, in the mesoglea or clinging to the manubrium or stomach wall

of medusae (Lebour 1916, Lebour 1917, Marshall 1925, Stunkard 1969, Reimer & Pierson 1971, Køie 1975). Other metacercariae were encountered located near the gastric cavity in *Pleurobranchia pileus* Müller, 1776 (Lebour 1916, Stunkard 1969) and often in the region of the ovaries in *Sagitta*, but sometimes also inside the alimentary canal (Lebour 1917), Martorelli (2001) also found *Opechona* sp. in the mesoglea near the circular canal in jellyfish and in the infundibulum in ctenophores. Finally, Totton (1954) discovered larval stages of *Lepocraedium album* infecting the *Hippopoius hippopus* (Calicophorae, Siphonophora) where trematodes made tubular tunnels through the mesoglea before losing their tails. Mapstone and Arai (2009) suggested that one possible function of the Siphonophora somatocysts, where metacercariae of *O bacillaris* were usually found, could be a food-storage reservoir for the host. Apparently, this locality could be preferred by the metacercariae because it represents a food supply into their habitat (host).

The uroproct has been widely considered as a characteristic feature in this group of digeneans, thus is used to taxonomically identify *Opechona bacillaris sensu stricto* and separate them from the *Prodistomum* whose representatives lack the uroproct (Bray & Gibson 1990).

It is noticeable the low prevalence found in the Siphonophora analysed. In general, prevalence of digeneans among zooplankton is very low, nevertheless this fact contrasts with the high infection rates registered in fishes (Marcogliese 1995). In other Atlantic areas prevalence of digeneans in Hydrozoa ranged from 1 to 100% depending on host species, parasite species, and sample size (Table 5.II.4). Moreover, the only prevalence data from *O. bacillaris* in their intermediate Ctenophore second host were given by Yip (1984) (Table 5.II.4). Regarding the intensity it was quite low (1) but not strange taking into account the intensities registered in Table 5.II.4. Despite the fact that none of the examined *Muggiaea* sp. showed more than one metacercariae per individual it is widely know that trematodes can reach a high number of in gelatinous zooplankton (Totton 1954). In this sense, possibly the free metacercariae found could come from *Muggiaea* sp. hosts, although we do not discard that they could come from other other second intermediate hosts in the studied area like medusae where we also observed this trematodes whose intensity was more higher (personal observation).

Table 5.II.4. Records of metacercariae Trematoda in different species of gelatinous zooplankton. IH = Intermediate host; % = prevalence; I = Intensity; Locality = part of the body where metacercariae were found.

Parasite	IH	%	I	Locality	Site	Reference
Monascus filiformis	*Liriope tetraphylla*	13.6; 80.2	1 - 9	Intersection between radial and circular channels; mesoglea	SW Atlantic	Giriola et al. 1992; Diaz et al. 2012
	Phialidium sp.	29.6	1 - 48	Intersection between radial and circular channels; mesoglea	SW Atlantic	Giriola et al. 1992
	Eucheilota ventricularis	64.7				
	Clytia simplex	33.3				
	Proboscidactyla mutabilis	97.6				
	C. simplex	33.3				
	Aequorea spp.	92.3		Mesoglea	SW Atlantic	Diaz et al. 2012
	Cosmetirella davisi	100*				
	Mitrocomella brownei	100*				
	Halopsis ocellata	100*				
	Leuckartiara octona	100*				
Opechona bacillaris	*Pleurobranchia pileus***	10			Galway Bay	Yip 1984
	Obelia sp.					
	Cosmetira pilosella					
	Turris pileata			Manubrium; stomach wall	Plymouth	Lebour 1916
	Phalidium hemisphericum					
	*P. pileus***			Inside of the stomach	Plymouth	Lebour 1916
	*P. pileus***				Øresund	Koie 1975
	Muggiaea sp.	3.2	1	Swimming bell (nectophore), near to the somatocyst and cormidia	NE Atlantic	Present study
Didymozoids	*P pileus***	5			Galway Bay	Yip 1984
Bacciger sp	*Phialidium* sp.	13	1 - 13	Mesoglea near the circular canal		
	*Mnemiopsis mccradyi***	2	1 - 2	Infundibulum	SW Atlantic	Martorelli 2001
	L. tetraphylla	0.1				
	Eucheilota ventricularis	1.7		Mesoglea	SW Atlantic	Diaz et al. 2012
	C. lomae	66.7				
Opechona pyriforme	*Eirene tenuis*	3.7	1.5±2.6	Umbrella, gonadal region, gastric peduncle, adjacent area to the mouth	W Gulf of Mexico	Martell-Hernández et al. 2011
	E. lactaea	0.39	1.5	Gastric peduncle and umbrella	W Gulf of Mexico	Gómez et al. 2000
Opechona sp.	*Phialidium sp.*	17	1 - 7	Mesoglea near the circular canal		
	L. tetraphylla	5	1 - 2	Mesoglea near the circular canal	SW Atlantic	Martorelli 2001
	*Mnemipsis mccradyi***	30	2 - 20	Infundibulum		
	Liriope tetraphylla	18.9		Mesoglea	SW Atlantic	Diaz et al. 2012
	E. ventricularis	46.8				
Opechona pyriforme	*Bougainvillia carolinensis*					
	Gonionemus vertens					Stunkard 1969
	C. quinquecirrha·					
	*Mnemiopsis leidyi***					
	M. bachei				Woods Hole USA	Stunkard 1974
	Aequorea forskalea				Woods Hole USA	Stunkard 1974
	Pelagia noctiluca				Woods Hole USA	Stunkard 1983
Opechona sp.	*C. lomae*	33.3				
	P. mutabilis	7.0				
	C. hemisphaerica	65.2				
	Aequorea spp.	76.9				
	C. hemisphaerica	52.2				
	Coryne eximia	100*				
	Bougainvillia sp.	100*		Mesoglea	SW Atlantic	Diaz et al. 2012
	Phialella falklandica	100*				
	Chrysaora lactea·	100*				
	Gossea brachymera	100*				
	Olindias sambaquiensis	100*				
Hemiuridae	*Phialidium sp.*	1.4	1 - 2	Mesoglea near the circular canal		
	*Mnemipsis mccradyi***	2	1 - 2	Infundibulum	SW Atlantic	Martorelli 2001
	L. tetraphylla	4.4				
	Eucheilota ventricularis	0.2			SW Atlantic	Diaz et al. 2012
	Proboscidactyla mutabilis	1.2				
Opechona cablei	*P. carnea*				Woods Hole USA	Stunkard 1980

In general and taking into account that *Opechona* have been recorded several times among different Hydromedusae and Ctenophora species we are able to confirm that this trematode is generalist at the mesozooplankton level being able to use a wide range of this gelatinous intermediate second host in the way toward their definitive host (Diaz Briz et al. 2012). In the area herein studied and regarding the generalist character of *Opechona* we would suggest *Muggiaea* sp. as a new second intermediate host which has not been previously described.

The idea of the *Muggiaea* sp. as a second IH is reinforced because of the importance of pelagic cnidarians in marine food webs which in turn is reflected in both, their use as a food source for fishes and their use as intermediate hosts for helminthes parasites. As intermediate hosts in the life cycles of marine digeneans, gelatinous zooplankton could play an important role in the dispersion and distribution of the metacercaria stage, as well as also linking this larval stage with their definitive host (Køie 1975, Purcell 1982, Marcogliese 1995, Jackson et al. 1997, Matsumoto et al. 1997, Martorelli 2001, Purcell & Arai 2001, Marcogliese 2002, 2004). Lots of reports reinforce the importance of the gelatinous zooplankton as part of the fish diet where adult stages of digeneans are present (Arai 1988, Ates 1988, Purcell & Arai 2001, Arnason 2007). Furthermore, Arai (1988) demonstrated under experimental feeding on atlantic mackerel that it preferred to eat jellyfish than copepods when they were offered together.

On the other hand, it is know that the amount and degree of jellyfish blooms are influenced by both the biology and behaviour of the animal, and the geographic setting and physical environment. Some species of *Muggiaea* have been used as indicators of the flow of certain types of waters into a particular region (Russell 1935, Southward et al. 2004). In this sense, hydrography is often enough to explain gelatinous zooplankton aggregations, however, it is clear that interactions between biology of the animal and physics of the water are very important sources of population variations, especially at local scales. Thus, the greatest prevalence in *Muggiaea* was found in SC community where we registered the highest abundance of these animals followed by SF and SO with their abundances also decreasing. As found in other works, it seems that highest prevalence has been recorded in estuarine or a bay environment where aggregations are usually recorded (Russell 1935, Girola & Martorelli 1992, Matsumoto et al. 1997, Gómez del Prado-Rosas et

al. 2000, Martorelli 2001, Costa et al. 2004, Martell-Hernández et al. 2011, Diaz Briz et al. 2012).

Summer communities were detected under downwelling-relaxation conditions where predominant southerly winds pushed onshore warm and salty surface waters of subtropical origin, in this context prevalence was changing with predominant oceanographic conditions registered during the sampling. Thus, the predominant northeastern winds registered before and during 2nd of July resulted in a strong upwelling with the entrance of deep, nutrient-rich water that enhanced primary production. The advective environment developed could have promoted the transport of siphonophores off-shore causing a little decrease in their abundance from SC to SF communities (dispersion effect). The fact that the prevalence remained similar could be related to the Siphonophora behaviour because through buoyancy control these animals are be able to avoid the advective force. On 4th of July the upwelling conditions still existed but an entrance of warm water started with the shifted wind from NE to SE. As a result we detected a downwelling-relaxation event where the abundance of Siphonophora increases from SF to SC as well as the prevalence. The in-shore current could have transported Siphonophora species increasing their abundance coupled with the coastal character of the infection pattern. On 9th of July the oceanographic conditions were as registered on 2nd of July but the previous upwelling was very weak. In that context the abundances between SC and SF were similar but we only found infected animals in SC community. Finally on 11th of July the effects of previous downwelling were registered during a transitional downwelling-relaxation-upwelling conducted by wind shift. In this oceanographic context both the abundance and prevalence were higher in SC than SF and SO.

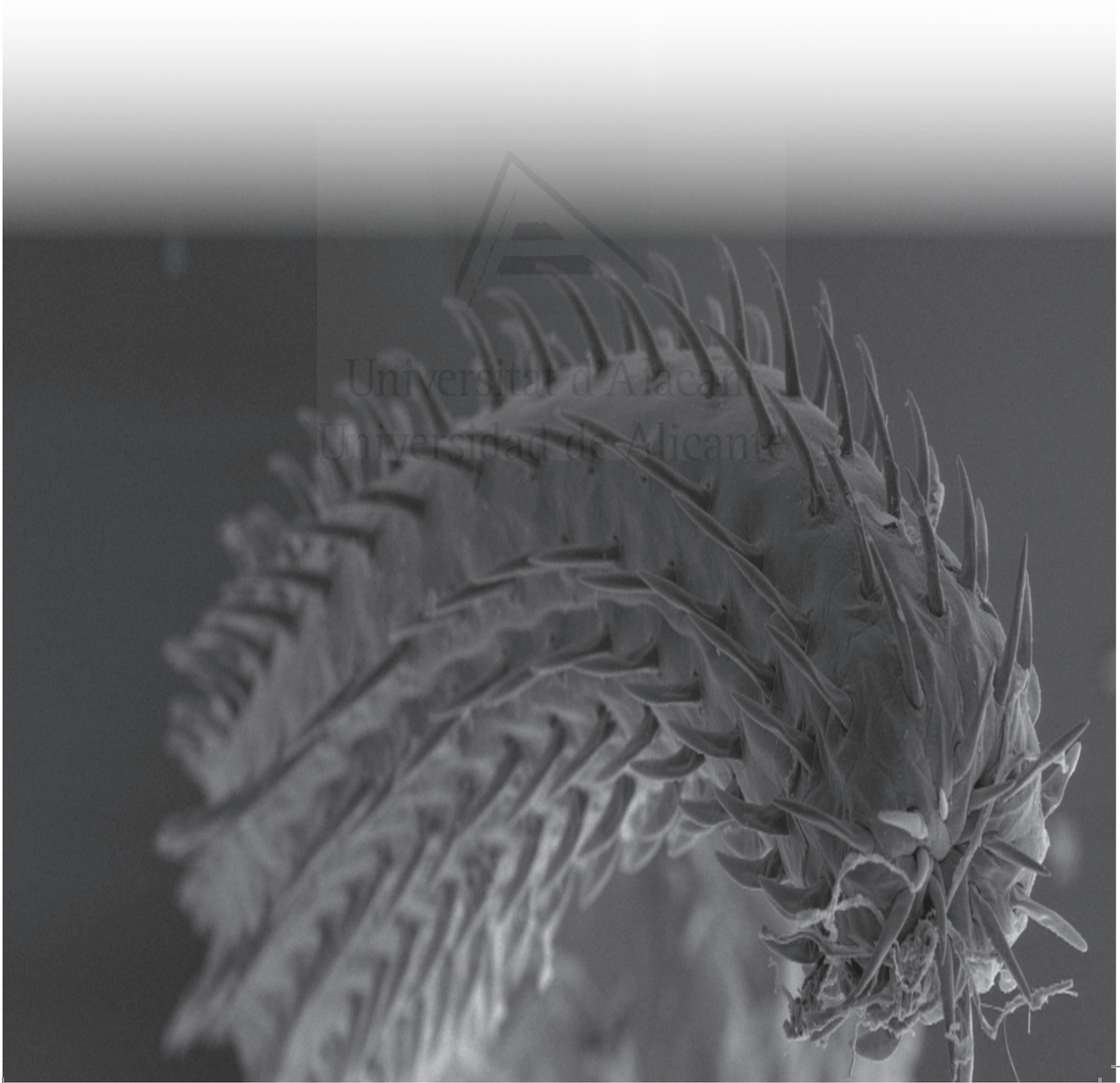
In summary, we would like to emphasize that this is the first record of *Opechona bacillaris in Muggiaea* sp. in coastal waters of the NE Atlantic Ocean (NW Iberian Peninsula) where *Muggiaea* sp. probably acts through predator-prey interactions as a second intermediate host.

This is the first approach to understand the importance of the oceanographic conditions in the recruitment of parasites into the mesozooplankton communities. Prevalences found through the communities

agreed with the coastal and estuarine character of the infection registered in trematodes and was very low.

In addition, it is remarkable the fact that the highest prevalence was registered in SC community under the strong downwelling event occurred on 11th of July. Under different oceanographic scenarios (upwelling-relaxation-downwelling-relaxation) we detected some *Muggiaea* sp. dispersion pattern through SC to SF communities and vice versa coincidently with prevalence changes. Thus it seems that prevalence and recruitment vary coupled with both different oceanographic conditions and *Muggiaea* sp. behaviour. The ecology and behaviour of gelatinous zooplankton remains poorly understood in many areas of the world due to difficulties in sampling and conserving the softbodies of most species (Purcell 1997). The variation in prevalence and recruitment registered through mesozooplankton population component recorded by previous works under different oceanographic scenarios agree with the results here discussed (Pascual et al. 2007, Diaz Briz et al. 2012, Gregori et al. 2012, 2013) Gregori et al. submitted). Moreover, this results shed light on the hypothesis that the parasite recruitment is influenced by the oceanography as Pascual et al. (2007) proposed. Finally, in spite of the low free metacercariae intensity found they could stem from the *Muggiaea* sp. or other gelatinous zooplankton that it should be studied in future studies.

Faunistic Research on Other Symbionts



6. FAUNISTIC RESEARCH ON OTHER SYMBIONTS

Other symbionts were found studying different samples taken from several places. In this chapter we would like to summarize the faunistic knowledge acquired from the study which encompasses nocturnal zooplankton samplings in the Ría de Vigo (2008-2009) as well as two oceanographic surveys over de shelf and continental slope off NW Iberian Peninsula (CAIBEX-I) and NW Africa (CAIBEX-III). Material and methods used in the surveys as well as oceanographic and biological contexts are available in Annex I from (Roura 2013).

Several Aegidae (Isopoda) family parasites were found free in the column water in Ría de Vigo (Fig. 6.1). These kind Isopods are facultative parasites of fish when adults. They feed on their hosts' blood before dropping off to digest the meal. Aegids resemble to cymothoids in the appendages structure. Because its adaptive life-style, they cling to living fishes therefore, their anterior pereopods (1-3) have an acute hook-like dactylus (). Contrary in cymothoids all seven pereopods have hook claws because they permanently live on their host.

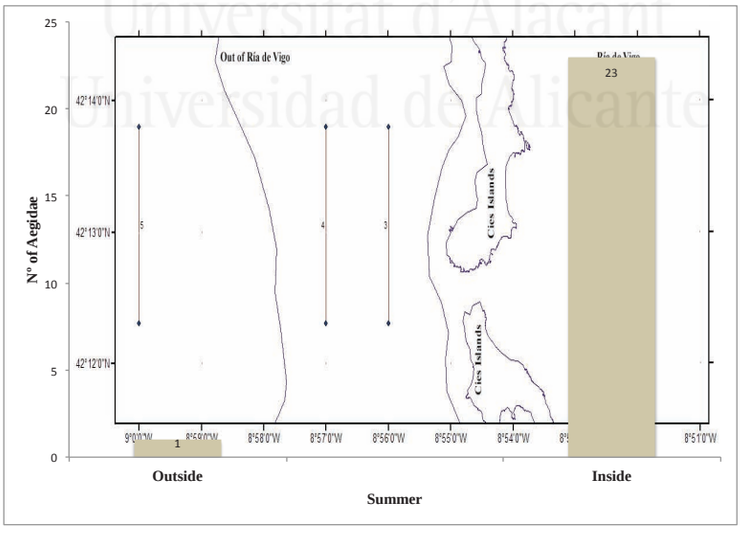


Fig. 6.1. Number of Aegidae found in summer 2008 inside and outside of Ría de Vigo in Galician waters.

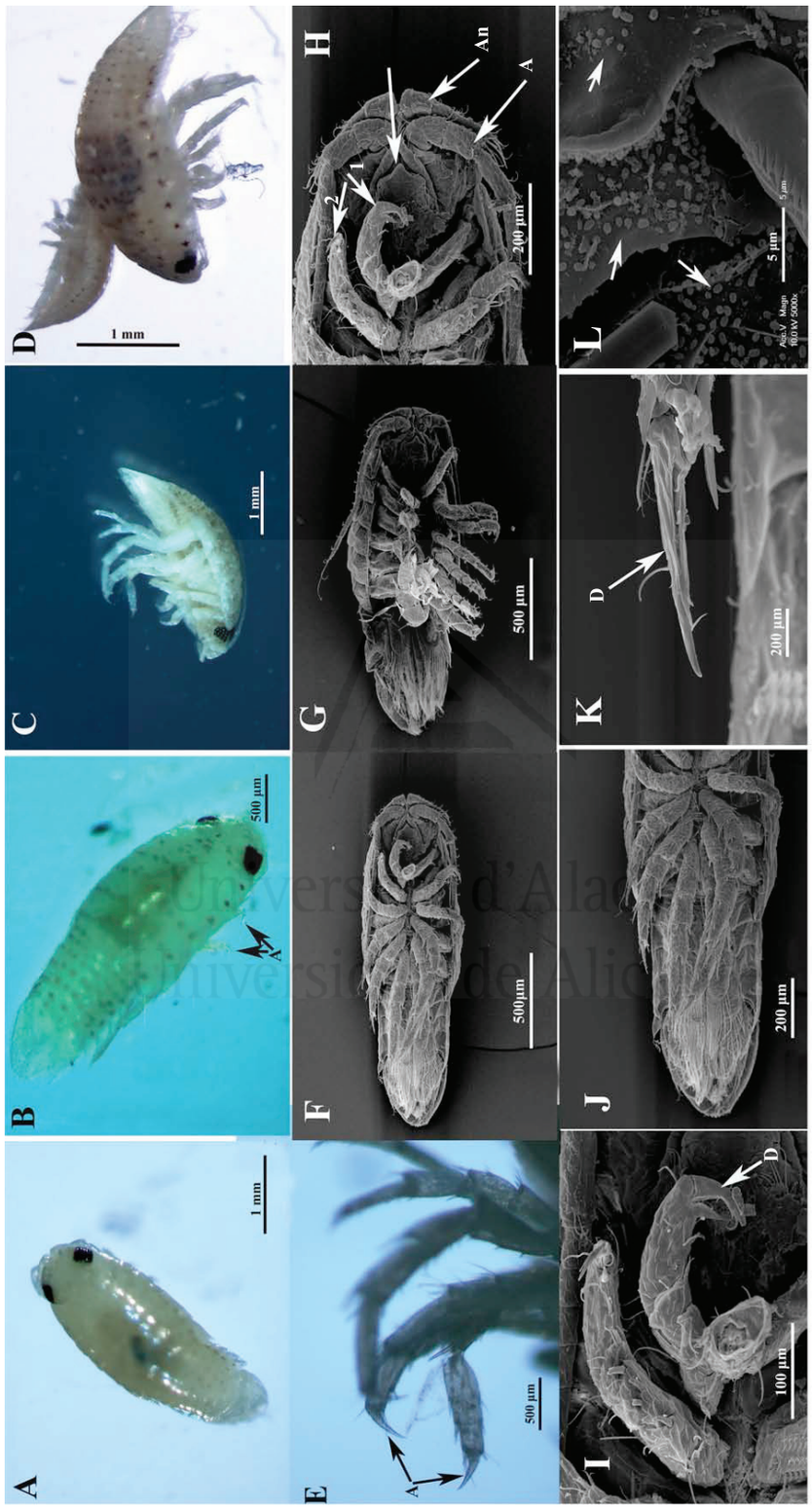
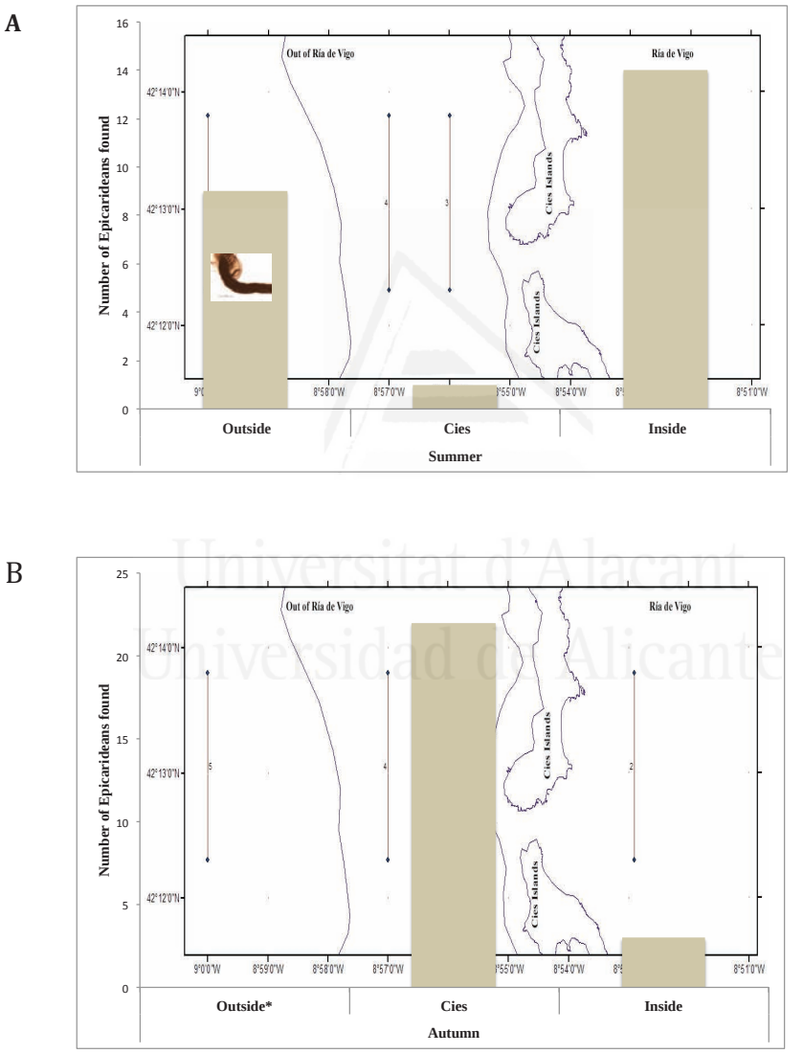


Fig. 6.2. Aegidae from column water. (A-D) General view of the animals. (E-N)Their seven pairs of pereiopods; (E) A = Detail fo dactylus on the 4th and 5th perepods; (F-G) General view of the pereopods from SEM; (H) An = Antennule; A = Antenna; Anterior pereopods (1-2) with an acute hook-like dactylus; (I) More detailed pereopod with its hook-like dactylus. (J) End body detail with the 4-7 pereopods. (K-L) Detailed dactylus of the lasted pereopods dactylus; (O) Associated symbiont bacterias

The Epicaridea is a grouping of isopods that are parasitic on other crustaceans as hosts. Bopyrids were found in samples from Ría de Vigo whereas Dajids were found in samples from CAIBEX-I and III. The number of parasites found in in Ría during summer and autumn of 2008 are showed in Fig. 6.3 A-B.

Fig. 6.3. (A) Number of Epicaridean found in summer 2008. (B) Number of Epicaridena found in autumn 2008. * = Data from outside of Ría could be different because they have not been studied yet.



The classification of Epicaridea is changing since new molecular evidence confirms that epicarideans are closely related to cymothoid isopods (Dreyer & Wagele 2001, Boxshall 2007). The hatching stage is the epicaridium (Fig. 6.4), it is

styliform and possesses suctorial mouthparts and six pairs of clawed peraeopods enabling it to attach to the primary host that is usually a copepod. On the primary host it undergoes a number of ecdyses from epicaridium to microniscus and to the free-swimming cryptoniscus that infects the final host where develops into adult. (Williams & Boyko 2012).

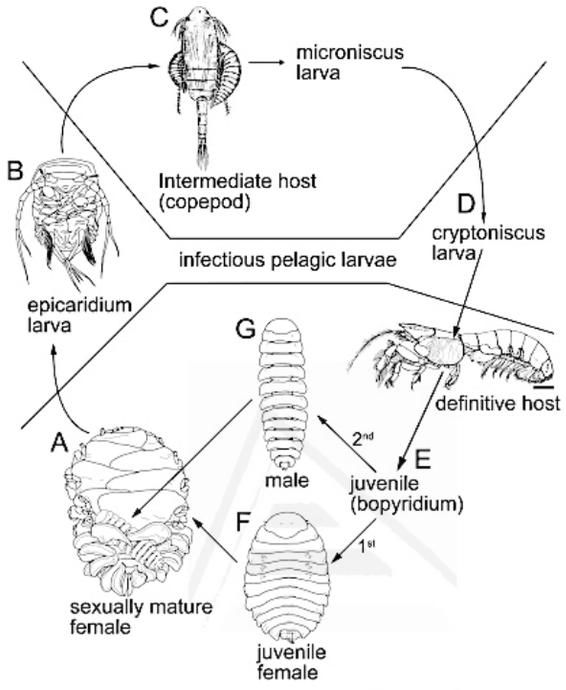


Fig. 6.4. Adapted from (Williams & Boyko 2012). Epicaridean general life-cycle. A sexually mature female and male in the gill chamber of the shrimp definitive host. The female releases epicaridium larvae that parasitize calanoid copepod intermediate hosts. The epicaridium larva metamorphoses into a microniscus larva and then a cryptoniscus larva that settles onto a definitive mud shrimp host. The first juvenile isopod (bopyridium) to parasitize a host becomes female; subsequent isopods become male(s) and live on the female. Scale bar: 1 cm for definitive host (rest not to scale).

The microniscus is the most common larval stage encountered in plankton samples, which can be found temporarily attached to planktonic copepods (Fig. 6.5). These parasitic isopods can be recognised by the presence of terminal claws on the tips of their walking legs (Fig. 6.5 F).

In these families the first cryptoniscus to settle on the final host becomes female and subsequent ones male. The female usually is large and often asymmetrical, while the male remains cryptoniscid in form and is usually attached to the female body near the genital openings (Fig. 6.6).

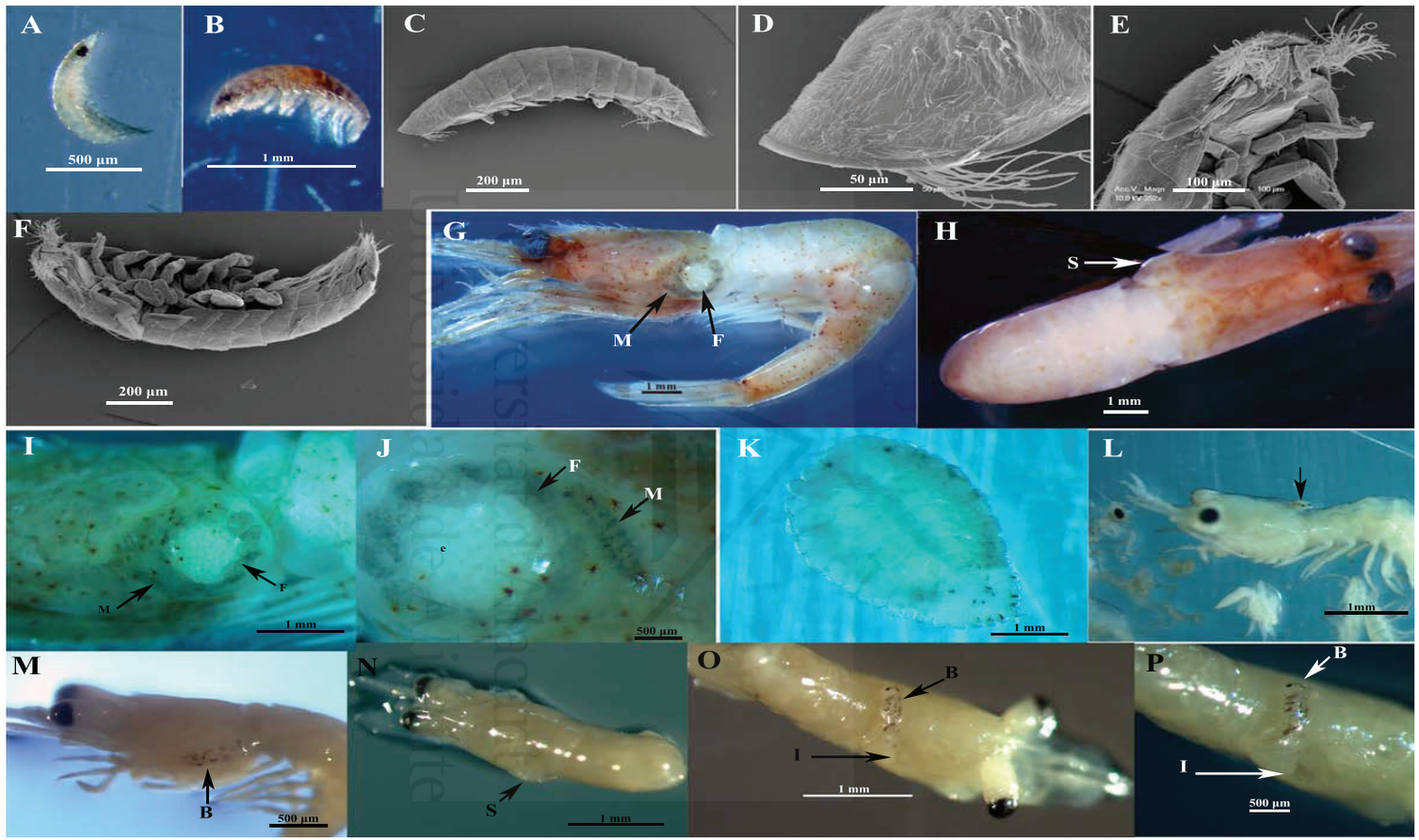


Fig. 6.5. Epicaridean from column water in Ría de Vigo. (A-C) General view of the animals. (D-E) Detailed of the anerior part of the body. (F) Their seven pairs of modified pereiopods; (G-H) Bopyrid female infecting a Decapoda; S = swelling injure; (I-K) General view of bopyrid female and male; F = female; M= male. (L-P) Cryptoniscus larva stage settling in the cephalothorax of Caridae larvae; B = Bopyrid Cryptoniscus larvae; S = swelling; I = Injury on the host.

Whereas bopyrids are parasitic in the branchial cavity or on the abdomen of decapods (Macrura and Anomura), dajids are parasitic in the incubatory chamber, dorsal surface of mysids and euphausiids or gills of this last aminals (Fig. 6.6 and Fig. 6.7 bopirids and dajids respectively) (Naylor 1972).

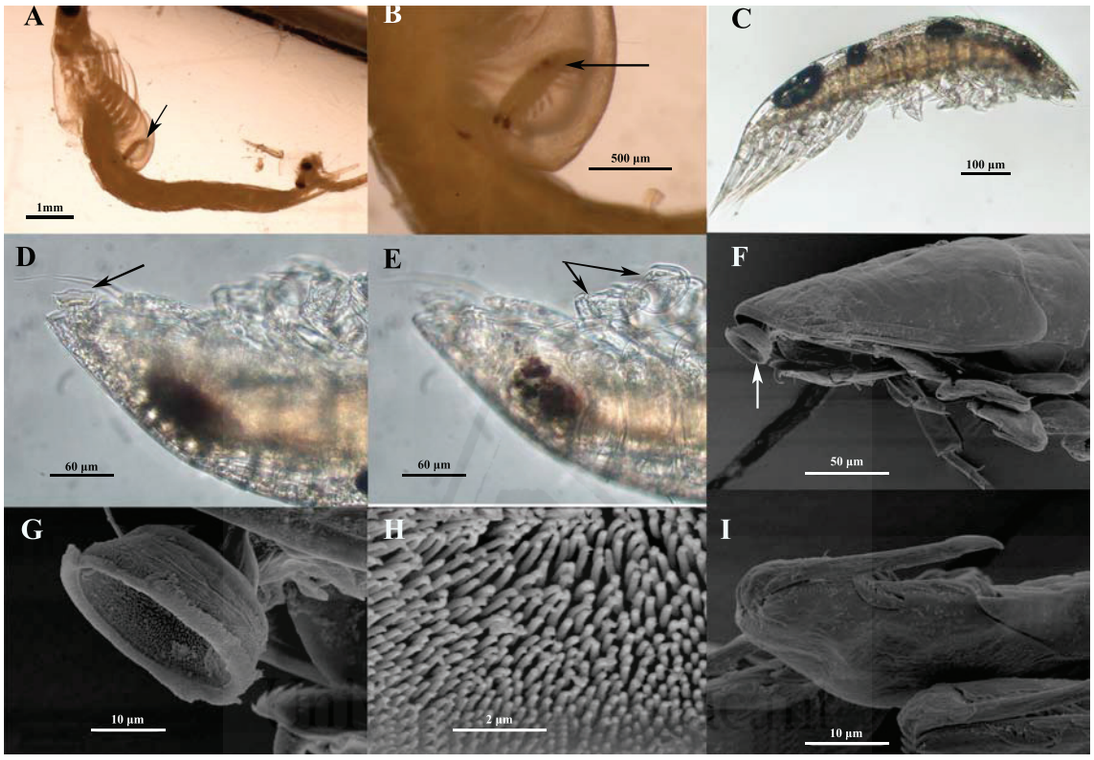


Fig. 6.6. Cryptoniscus larval stage of bopyrid infecting a mysid in Ría de Vigo (2008). (A) general view of the mysid with a parasite in their incubatory chamber (arrow); (B) detailed position of the parasite within the marsupi pouch; (C) general view of the cryptoniscus larvae; (D) the cryptoniscus larva usually attaches to the host, using an oral sucker (arrow); (E) propodus and dactylus of pereopod 2-3; (F-G) oral sucker, ventral; (H) interior surface of the oral sucker; (I) propodus and dactylus of pereopod 3.

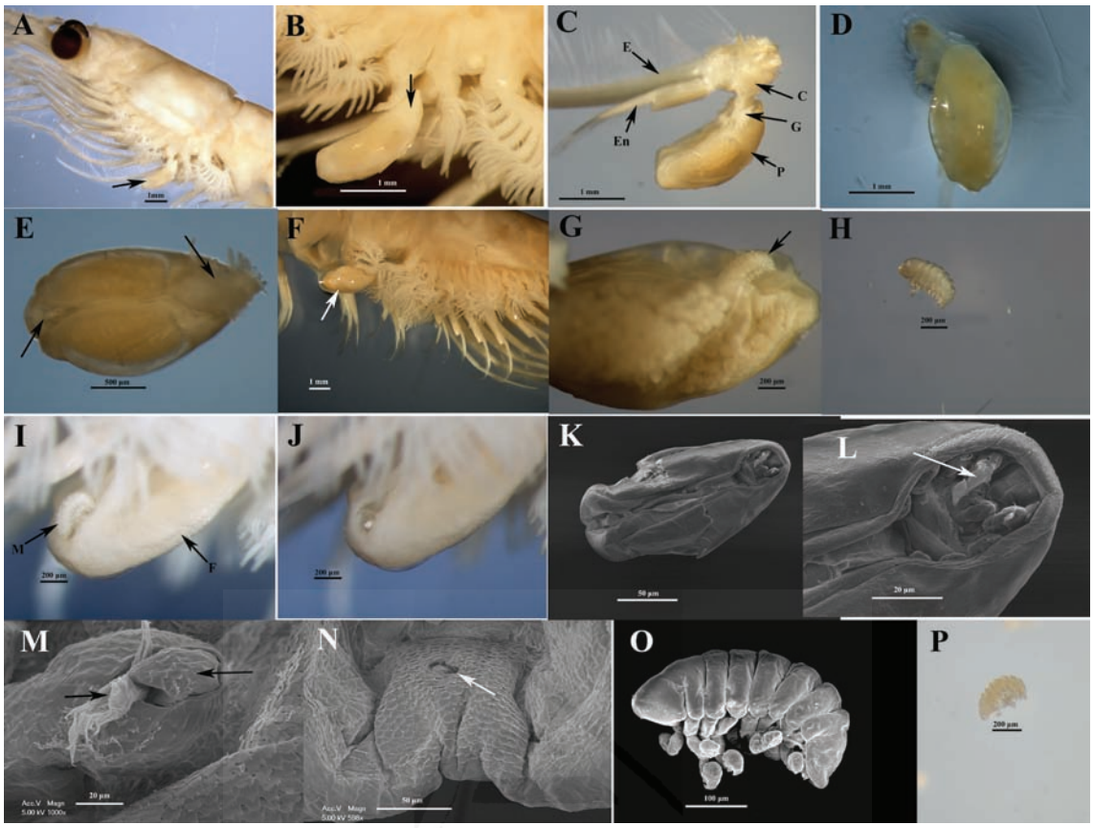


Fig. 6.7. Likely *Branchiophryxus* sp. infecting *Meganyctiphanes norvegica* from samples taken in CAIBEX-I; (A-B) general view of the Dajid attached to the left part of cephalothorax; (C) parasite anchored to the gill of the 6th thoracic leg; E = endopod; En = exopod; C = coxa; G = gill; p = ectoparasite; (D) dorsal view of the female parasite; (E) Ventral view of the female parasite; (F) *M. norvegica* infected with the same parasite in the right part of the cephalothorax anchored to the gill; (G and I) general view of the male on the female body end; (H) general view of the male isolated from female; (J) likely the spermatophore; (K) general view of the female from SEM; (L) ventral side of the oral area. The four pair of legs are small and closely crowded together. The arrow indicates a piece of the gill which was ripped when parasite was separated from their host; (M) clockwise the first arrow indicates the hook-like dactylus from the first pair of legs; second arrow indicates a piece of the ripped gill; (N) likely, injury caused for the attachment of male to the female body; (O-P) general view of male from SEM and microscope respectively.

The last isopods found in our samples belong to the family Gnathiidae. A total of 3 praniza larvae were found in summer of 2008, 2 of them inside of the Ría de Vigo and one off the Ría (Fig. 6.8). The adults of this family reproduce in benthic substrata after leaving their hosts thus, larvae are temporal ectoparasites (Fig. 6.9) of teleosts and elasmobranchs (Ota et al. 2012). They suck their host's body fluid with their needle-like mouthparts until their abdomens are remarkably swollen. These larvae are often taken in plankton nets (Naylor 1972).

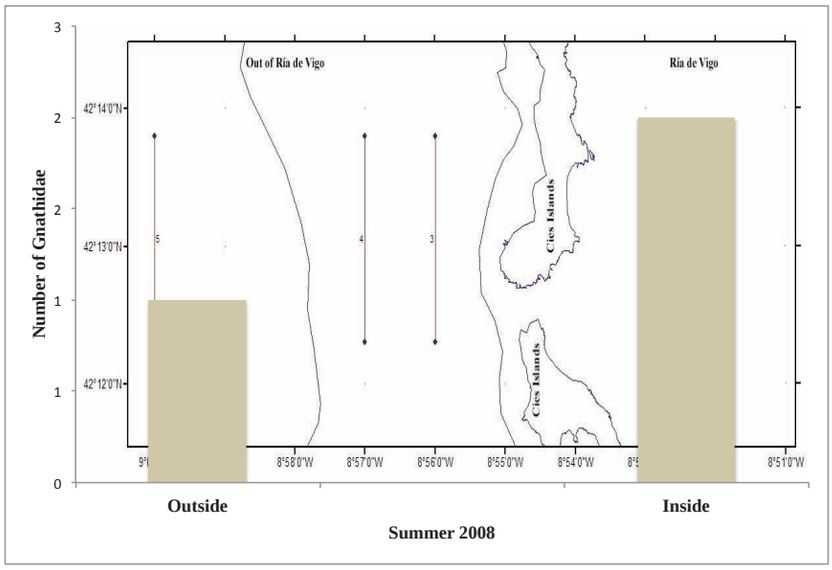


Fig. 6.8. Number of praniza (Gnathiidae) found in Ría of Vigo from summer 2008.

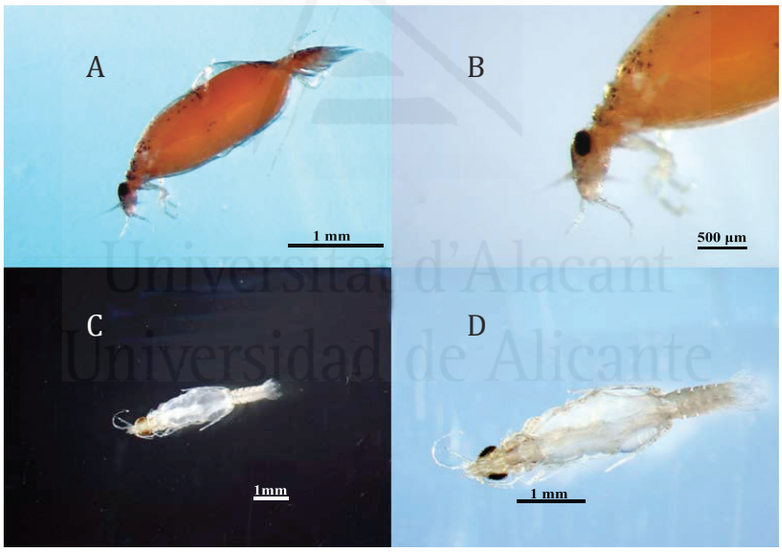


Fig. 6.9. General view of praniza larvae of Gnathiidae. (A-B) Individual well fed. (C-D) probably individual starvation.

Other crustacean parasites found were the Caligoid copepods. Sea lice are marine ectoparasites on the oral or gill cavities, operculum or body surface of fishes. Thus, they feed on the mucus, epidermal tissue, and blood of its host. Close to two dozen species of *Caligus* occur in the Atlantic and gulf coasts. Attachment is typically done during the copepodid stage. Despite the fact that adults are fish

parasites, several *Caligus* species are frequently caught in the plankton samples, especially males. In summer of 2008 we found four *Caligus*, 3 inside of the Ría de Vigo and one off of Ría near to the Cies Islands (Fig. 6.10 and Fig. 6.11). *Caligus elongatus* (one female and one females) and *Caligus* sp. were identified.

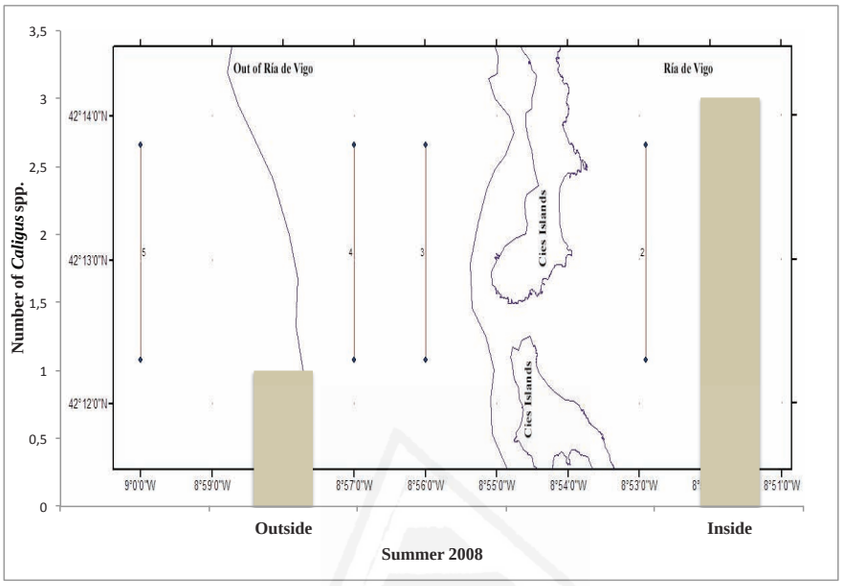


Fig. 6.10. Number of *Caligus* spp found in summer 2008.

As previously discussed throughout this thesis protozoan are the most common epibonts found on the copepod body surface. We found a diatom epibiont, likely *Licmophora* sp., on *Calanus helgolandicus* surface as well as a tumor-like anomalie probably caused by protozoan parasites. Moreover, this individual was infected with *Ellobiopsis chattoni* (Fig. 6.11 E-I).

More protozoan symbionts were found as for example apostome ciliates on the surface of *Meganyctiphanes norvegica* Sars, 1857, *Euphausia krohnii* Brandt, 1851 and *Pasiphaea sivado* Risso, 1816 (Fig. 6.12). Apostome ciliates are typically associated with many groups of marine Crustacea (Lynn 2008). The trophont encysts to form a tomont, which divides within the cyst to form tomites, these

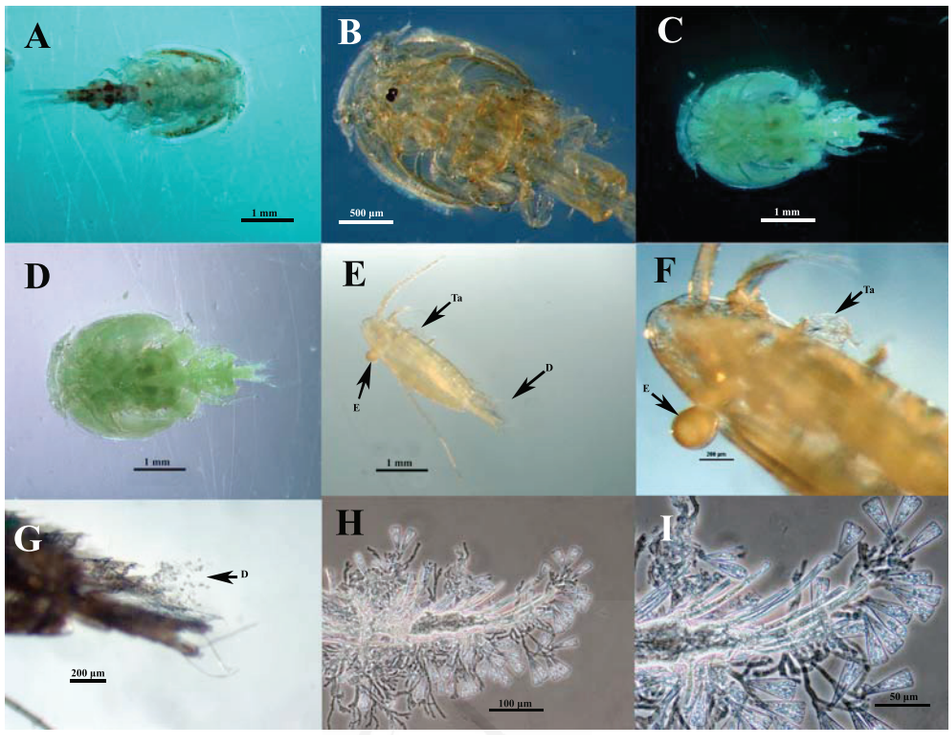


Fig. 6.11. (A-D) *Caligus* spp. from samples of the Ría de Vigo. (A) *Caligus elongatus* male; (B) *Caligus* sp. female. (C-D) *Caligus elongatus* immature female (E) *Calanus helgolandicus* carrying E = *Ellobiopsis chattoni*; Ta = tumor-like anomalies caused by protozoan parasites; D = epibiont diatom likely *Licmophora* sp.

emerge from the cyst and swim freely to find new hosts, on which they settle and become phoronts, the resting cysts. This stage (phoront) has been observed on euphausiids where are attached to different parts of the body especially on the buccal appendages, antennules, antennae, pereiopods, pleopods and to the setae of these appendages. Among euphausiid species it have been reported on *Euphausia hemigibba* Hansen, 1910, *E. krohnii*, *Meganyctiphanes norvegica*, *Nematoscelis megalops* G.O. Sars, 1883, *Nyctiphanes couchii*, *Thysanoessa gregaria* G.O. Sars, 1883, *T. inermis*, *T. longicaudata* and *T. raschi* (Lindley 1978).

Moreover we found *Ellobiocystis* Coutière, 1911 attached to different animals, from krill to Chaetognatha (Fig. 6.13) Ellobiopsidae family comprises 5 genera where we can found epibionts as *Ellobiocystis*, *Parallobiopsis*, *Thalassomyces* and ectoparasites as *Ellobiopsis* and *Rhizellobiopsis*. Except *Rhizellobiopsis* the rest of genera have been found infecting marine zooplanktonic

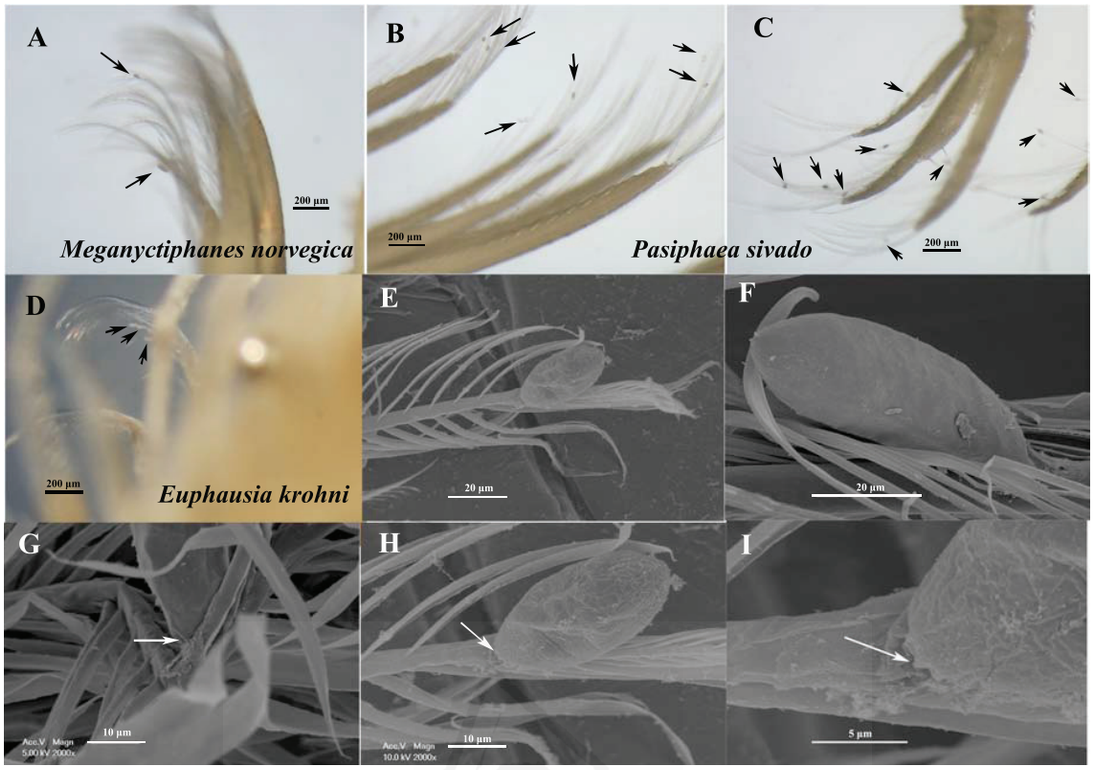


Fig. 6.12. Apostome ciliata found in different species from CAIBEX-I and CAIBEX-III. (A) *Meganyctiphanes norvegica*. (B-C) *Pasiphaea sivado*. (D) *Euphausia krohnii*. (E-I) Genral view and detailed inserction of the apostome to the seate of hosts.

crustaceans. Moreover, *Ellobiocystis* has been described primarily infecting shrimps and mysids (Shields 1994). In a recent checklist and classification of living dinoflagellates recorded in (Gómez 2012) we found least 7 species of *Ellobiocystis*. *E. caridarum* Coutière 1911 were found infecting several members shrimps of the family Acanthephyridae (*Acanthephyra purpurea* A. Milne-Edwards, 1881; *A. Eximia* Smith, 1884), Pasiphaeidae (*Pasiphaea pacifica* Rathbun, 1902; *P. semispinosa* Holthuis, 1951) Oplophoridae (*Systellaspis debilis* A.Milne-Edwards, 1881); Sergestidae (Sergestes spp.). *Ellobiocystis catenatus* and *E. tuberosus* Coutière 1911 were found on *Acanthephyra purpurea* whereas *E. tenuis* Coutière 1911 were encountered on *Pasiphaea sivado.* As far as we know this is the first time that *Ellobiocystis* have been found on *Meganyctiphanes norvegica*, *Euphausia krohnii* and Chaetognata. Finally several protozoans were found but we could not identify them (Fig. 6.14).

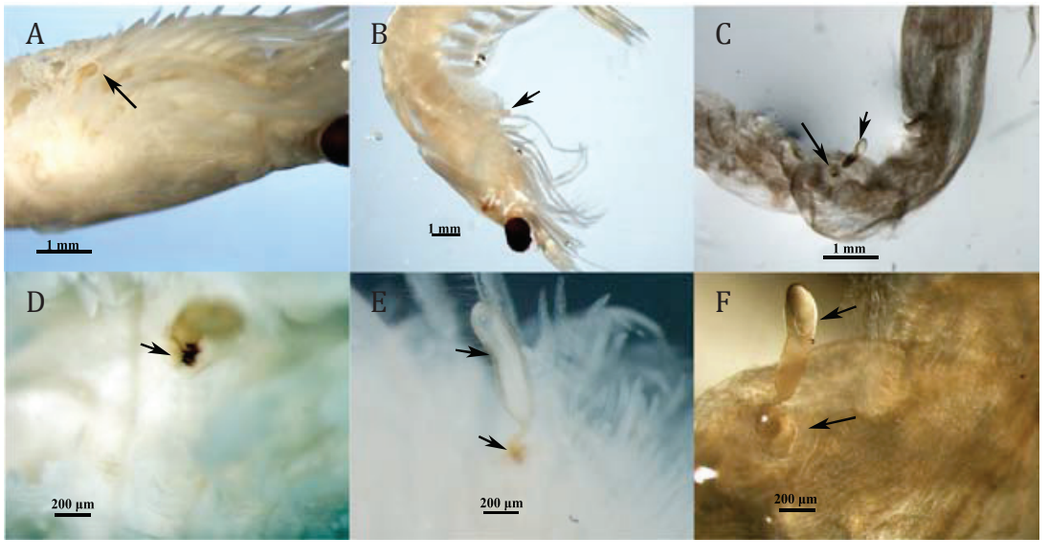


Fig. 6.13. *Ellobiocystis* on the surface of different hosts. (A-D) *Meganyctiphanes norvegica* carying *Ellobiocystis* on the ventral part of the cephalothorax from CAIBEX-I. (B-E) *Euphausia krohnii* carying *Ellobiocystis* on the ventral part of the cephalothorax from CAIBEX-III. (C-F) *Ellobiocystis* on the body surface of the Chaetognata from CAIBEX-III.

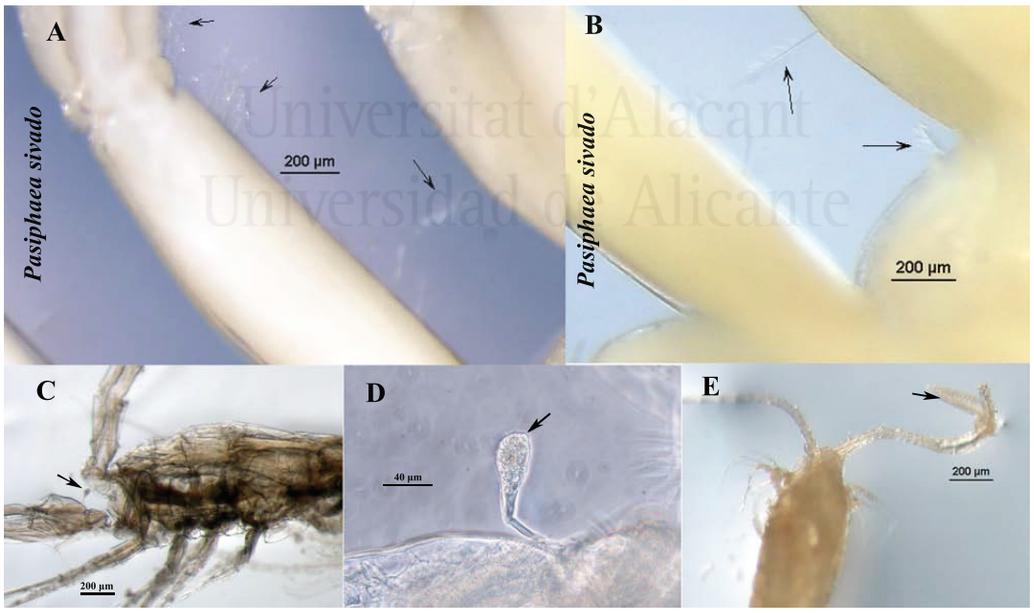


Fig. 6.14. Unidentified protozoa. (A-B) Protozoa on the surface appendages of *Pasiphaea sivado* from CAIBEX-I. (C-D) *Paraeuchaeta hebes* with a unidentified protozoa on its surface from Ría de Vigo. (E) *Centropages chierchiae* infected with unidenfied protozoan on its antenna from CAIBEX-I.

Trematode parasites are widespread among vertebrates and invertebrates in the marine enviroment. Generally, three host life histories are recorded (with some exception), with two intermediate invertebrate hosts and one definitive host, a vertebrate that harbors the adults. The metacercaria is the larval stage which infects the second intermediate host. In addition this larval stage is important resting stage that allows the parasite to survive until it can reach the definitve host. In this sense, the chaetohnath zooplankton act as host of different metacercariae. The Arrow worms are predators on zooplankton and at the same time prey for a variety of fish species. Because their central position in the food web and their vertical migratory habits facilitate their role as intermediate hosts. As predators, chaegognaths probably acquire trematode metacercariae by consiming infected copeods Dollfus 1960 in (Marcogliese 1995). Among arrow worms found in samples of CAIBEX-III we found at least four different metacercaria species Fig. 6.15).

Copepods are common parasites of marine fishes and have been reported from a great range of depths (Boxshall 1998). Very few families of parasitic copepods have successfully exploited deep-sea demersal fishes as hosts (Table 6.1) Among deep-sea demersal fishes, the family Mycophidae (lantern fishes) have been reported infected with *Sarcotretes* spp. Jungersen, 1911 (Pennellidae family Table 6.1). In fact, *Sarcotretes scopeli* Jungersen, 1911 occurs widely in the North Atlantic and the Pacific and infects a variety of meso- and bathypelagic fishes, including the myctophids *Benthosema glaciale* Reinhardt, 1837 *Benthosema mulleri* Gmelin, 1789 and *Myctophum evermanni* Gilbert, 1905 as well as the macrourid *Hymenocephalus gracilis* Gilbert & Hubbs 1920 (Boxshall 1998). In samples from CAIBEX-III we found one *Benthosema glaciale* apparently infected with *Sarcotretes* (Fig. 6.16 A-D). The inserction of the parasite to the host is different that usully described because our specimens inserted throughout gill of the host. They were located on the ventral part of the body host embebed between abdominal cavity and gill as described in Perkins (1983). Thus, it could be possible that our specimens belong to *Cardiodectes medusaeus.* As far as we know, it is the first record of *Lobianchia dofleini* Zugmayer, 1911 infected with this Pennellidae copepod but more studies from morphological to molecular are needed to elucidate their taxonomical position and identity.

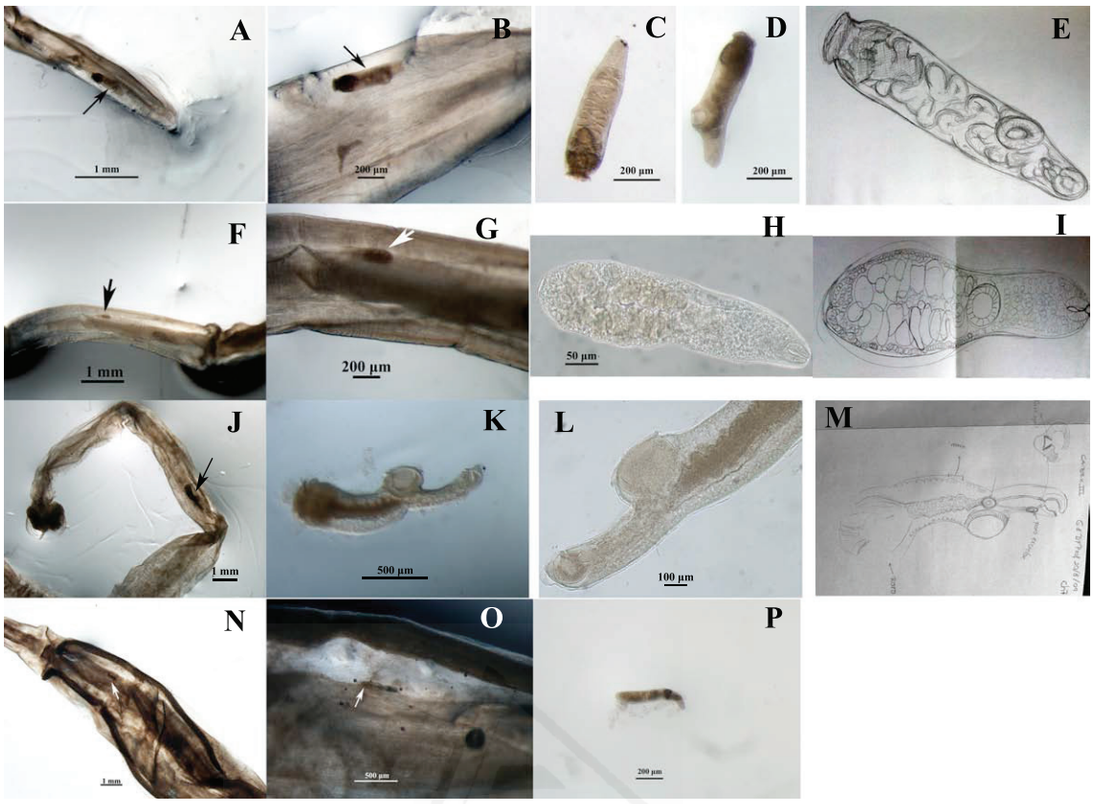


Fig. 6.15. Trematodes infecting Chaetognatha from CAIBEX-III.

Table 6.1. Number of copepods parasitic families that have been able to colonize and exploit deep-sea host species. Adapted from Boxhall 1998.

Family	Genus	No. deep-sea species
Order Poecilostomatoida		
Chondracanthidae	*Chondracanthodes* Wilson	3
	Lateracanthus Kabata and Gusev	2
	Jusheyhoea V. and F.	3
	Chelonichondria Ho	1
Philichthidae	*Sarcotaces* Olsson	2
Order Siphonostomatoida		
Sphyriidae	*Lophoura* Kollicker	15+
	Sphyrion Cuvier	2
	Paeonocanthus Kabata	1
Lernaeopodidae	*Clavella* Oken	10+
	Lernaeopodina Wilson	2
	Pseudolernaeopodina Hogans	1
Pennellidae	*Sarcotretes* Jungersen	2
	Exopenna Boxshall	1
Hyponeoidae	*Hyponeo* Heegaard	1
	Tautochondria Ho	1
Hatschekiidae	*Laminohatschekia* Boxshall	1

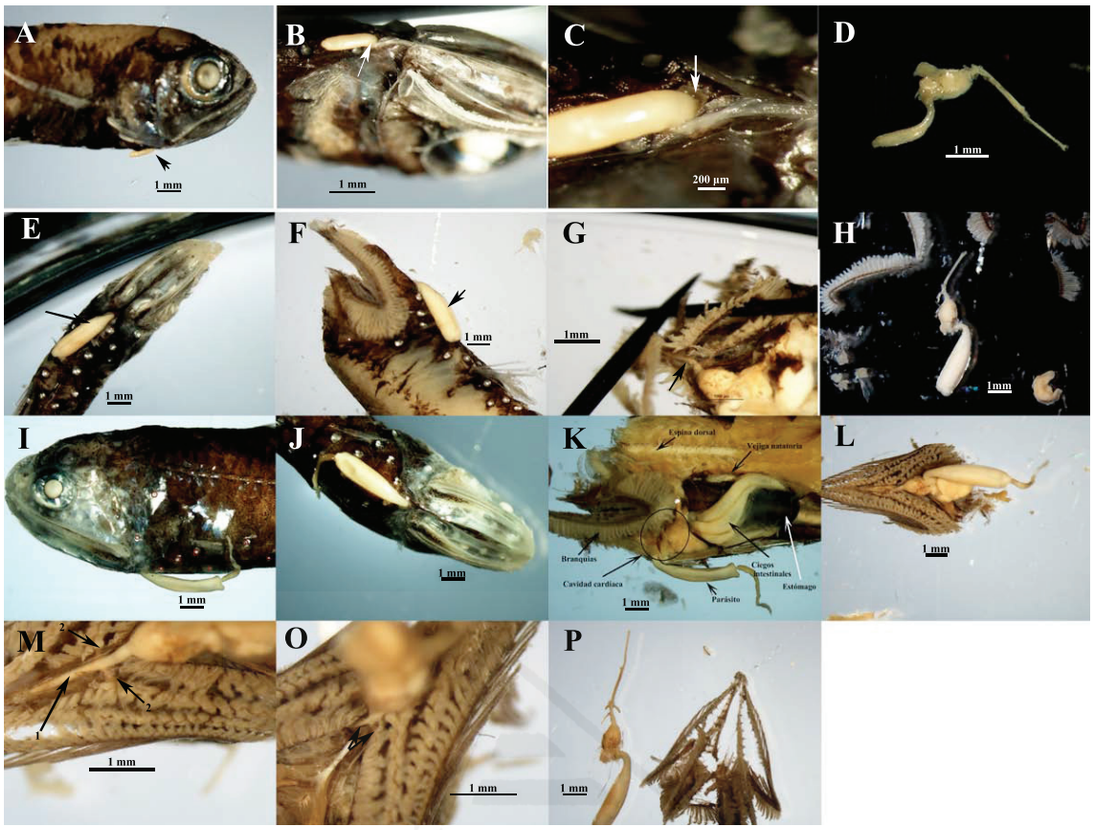
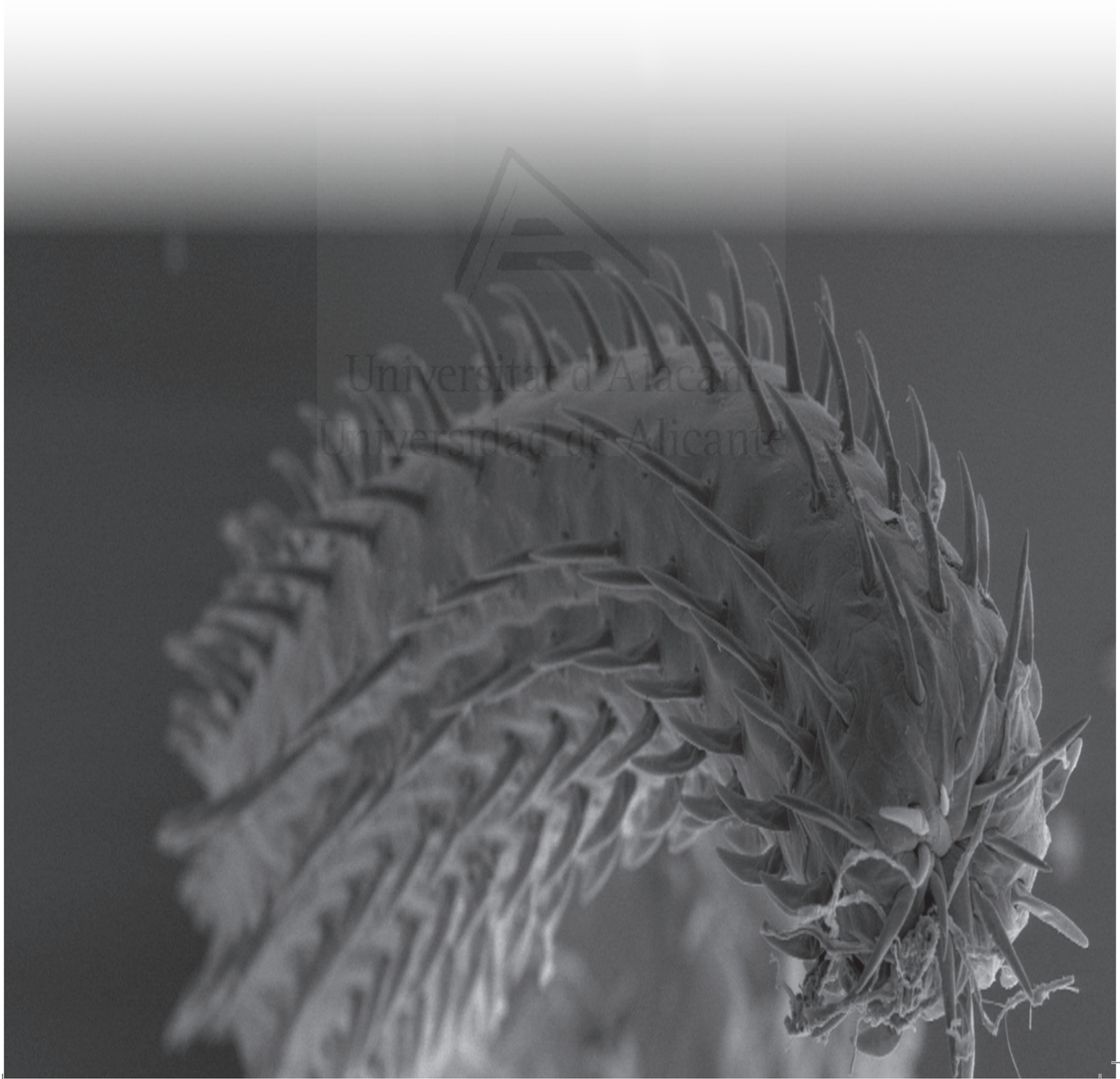


Fig. 6.16. Copepod Pennellidae from CAIBEX III infecting (A-D) *Bentosema suborbitale*; (E-J) *Lobianchia dofleini*; (K-P) detailed dissection of the parasite from the host.

General Discussion



7. GENERAL DISCUSSION

Climate change is addressed as cause of potential effects on parasitism in marine ecosystems (Lafferty et al. 2004, Lafferty et al. 2008, Burge et al. 2014). From an ecological perspective, any stressor factor may act synergistically with the effects of climate change. Habitat loss or fragmentation, overfishing, pollution, invasive alien species, hypoxia, acidification, altered hydrology etc. are some examples of these other stressors that play an additive role in the climate change effect. All these stressors interact at different levels with mesozooplankton community and eventually with human ecosystems (Fig. 7.1).

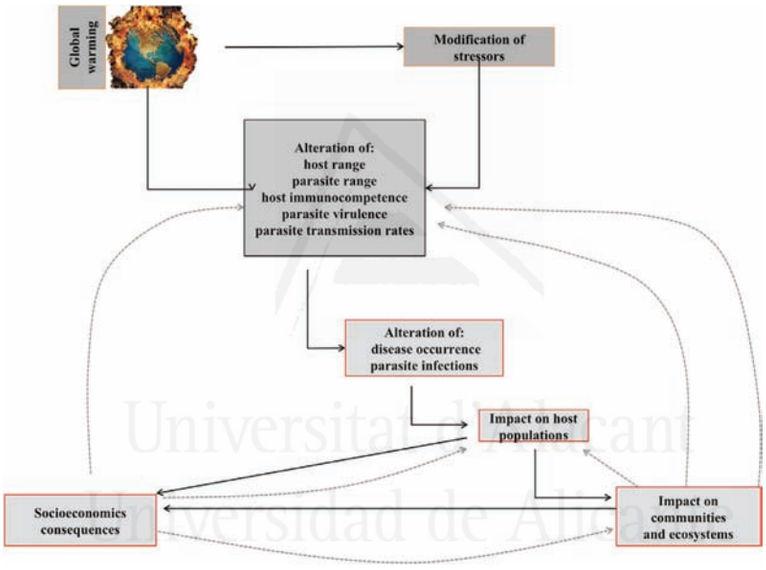


Fig. 7.1. Representation of effects of global warming on parasites and hosts. Effects will cascade up, leading to impacts on host population, communities and ecosystems. Effects will be modified by interactions with other stressors. Effects on populations, communities and ecosystems will "feed back" onto hosts and their parasites (dashed lines). Adapted from (Marcogliese 2008).

Mesozooplankton is thus considered a good indicator of climate change in the marine environment, as well as their parasites (Marcogliese 2002, Hays et al. 2005, Marcogliese 2005), which in fact represent an extended phenotype of their hosts. In this PhD thesis it addressed the key role that mesozooplankton communities play in parasite recruitment in waters off the NE Atlantic. Moreover, it was underlined the importance of the mesozooplankton communities in the epibiont diversity and survival, that under different biological or physical scenarios may affect from population to community of these important animals.

The understanding of effects of global warming on parasites and hosts requires monitoring studies on vectors and intermediate hosts among the mesozooplankton communities, the secondary producers of the marine realm. Innovative methods are needed to screen vast quantities of invertebrates for infection and reliably identify the infective form of parasites within. Application of immunological or molecular techniques such as ELISA or NGS (next generation sequencing) could very quickly yield a wealth of quantitative information (Nyholm & Graf 2012, Pompanon et al. 2012, Chu & Mazmanian 2013).

Many gaps that are necessary to cope with challenges raised by the climate change were identified throughout this PhD thesis (Fig. 7.2). This due to the lack of ecological knowledge that still exists about host-parasite systems (Fig. 7.2) population dinamics, community, quantitative data, georeferencing systems, transmission pathways, oceanographic changes response, etc. The progressive resolution to these gaps will help to improve effective risk assessment.

Overall, a complete study of parasites through their life history and location in the ocean ecosystems is limited to both economic and politic interests and the profitability of inspection-identification time (of parasite larvae) among each zooplankton species. Thus, much work remains to elucidate the basic life cycles of marine parasites, in face to provide responses to the colonization pathway into food webs, that affect the quality, sustainability and health of the animals from which human activities are dependent. Moreover, nothing is known about retention of parasite larval stages into an advective environment as the seasonal upwelling occurring in Galician waters (NE Atlantic Ocean). The results presented in this manuscript suggest that different oceanographic conditions determine the recruitment of parasites in the base of the food chain (mesozooplankton) following the pattern described in (Pascual et al. 2007). If, as predicted by climate change, water stability increases and the upwelling intensity decreases, we expect an increase of parasite larvae into the intermediate hosts with devastating consequences that can be transmitted throughout the entire ecosystem, for example the collapse of fishery resources due to the lack of food supply, diseases spread, etc.

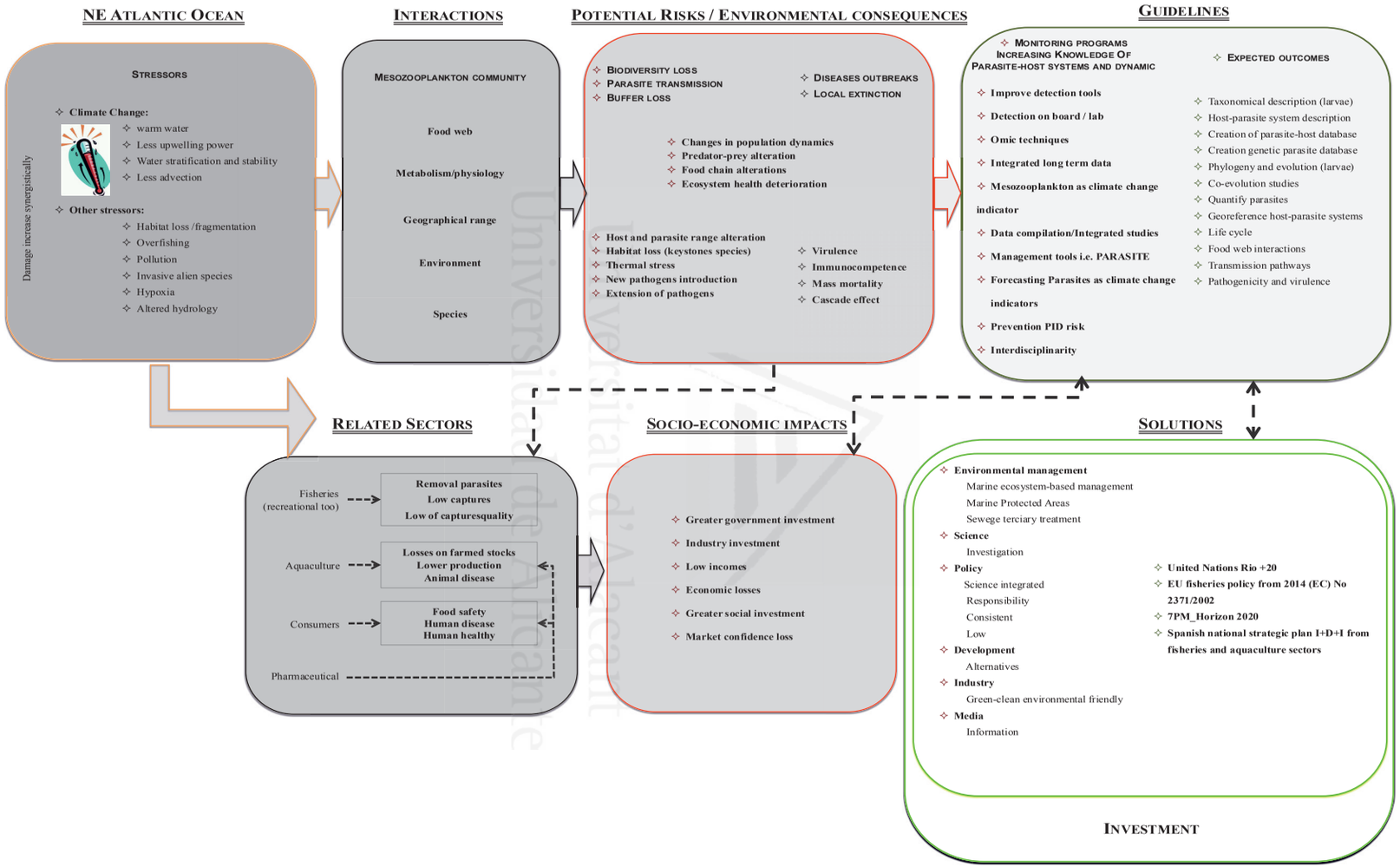


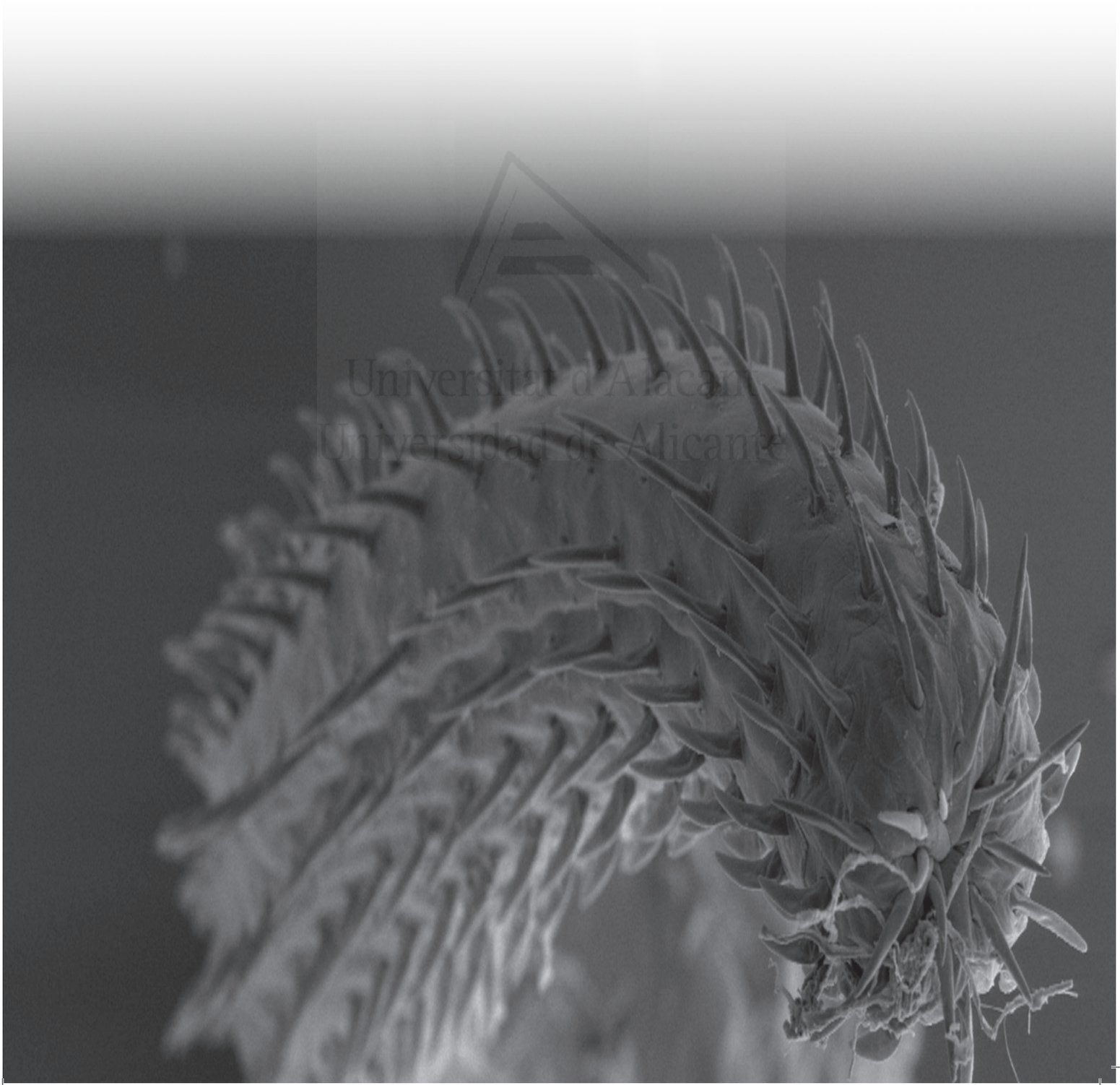
Fig. 7.2. Summary of interactions of climate variations and stressors in fisheries, aquaculture, health industry and consumers: consequences, guidelines and solutions.

This PhD thesis is the first approach that has been done trying to answer some of the above issues raised, where the significance of the results is evident in light of the numerous hypotheses that the achievement of the thesis project has been raised, especially in relation to two aspects of great scientific and socio-economic relevance

1. The knowledge about pathways colonization of parasitic infective forms in the food chain of marine ecosystems, particularly in the early stages of development in mesozooplankton communities.
2. The existence of relations between oceanographic water masses and recruitment of zoonotic parasites or pathogenic characteristics, which fit within the social challenge of Horizon 2020 "Climate Change and Food Safety".

It has to be underlined that interdisciplinary collaborative work is needed for addressing this huge task, especially in identifying keystone parasites that may cause a cascade effect through the communities altering the entire ecosystems. Furthermore, implications in public health will also occur since some fish diseases and infections can be transmitted from mesozooplankton to fish and the water to humans (anisakiasis, Swimmer's itch, schistosomiasis, diarrhoeal diseases, cryptosporidiosis, giardiasis etc.). Disease outbreaks are extremely cost effective in fisheries, aquaculture, related industries and public health (Marcogliese 2008). In this regard, this PhD thesis provides a strong scientific evidence for establishing monitoring programs, which help to improve understanding, early warning, and management of diseases and parasitism in marine systems under climate change. In the EU Horizon 2020 Research Framework several objectives are defined in face to guarantee the sustainability, exploitation and management of aquatic living resources to maximize the social and economic benefits from Europe's oceans and seas. The working hypothesis from this thesis fit very well with the calls launched under the societal challenge "forecasting and anticipating effects of climate change on fisheries and aquaculture".

Conclusions



CONCLUSIONS

1. Extensive faunistic investigation in mesozooplankton communities off the NE Atlantic waters has provided strong evidence of about 17 well-established symbiont systems representing a high diversity group of parasites and epibionts with low prevalences in zooplankton populations. Identified host-epibiont/parasite systems comprise:

1.1. New epibiont species ***Pelagacineta hebensis*** sp. n., found in adult forms of the copepod *Paraeuchaeta hebes*. It is the first time that this copepod is recorded as a basibiont for suctorian species. The new epibiont is described both upon morphological and molecular techniques, contributing to enlarge the genetic sequences available for the class Phyllopharyngea. Moreover, the taxonomic diagnosis of this epibiont is also provided.

1.2. **New host species of *Ellobiopsis chattoni*** found in adult forms of the copepods ***Calanoides carinatus*, *Centropages chierchiae*** **and** ***Metridia lucens*** extending the host range for this parasite. Prevalences among copepods were low showing different susceptibilities to the *Ellobiopsis* infection. Moreover, *Calanus helgolandicus* remains the preferred host for *E. chattoni*.

1.3. First record of the Acanthocephalan ***Bolbosoma balaenae*** has been recorded parasitizing the euphausiid *Nyctiphanes couchii*, which may play the role of intermediate host. Moreover, it was proposed the *B. balaenae* life cycle assuming that *Balaenoptera physalus* acts as definitive host of this acanthocephalan. These marine mammals would be responsible of eggs supply infrapopulation which support the infective forms in the euphausiids.

1.4. For the first time, the Acanthocephalan ***Rhadinorhynchus*** sp. has been identified infecting *Nyctiphanes couchii*. It is likely that this euphausiid acts through predator-prey interactions as the intermediate host. Insights from the morphological and phylogenetic study, along with the available epidemiological

information on *R. pristis* in scombrids and xiphids from the nearby Portuguese coast, the Madeira Islands and the North Atlantic Ocean, suggest that the cystacanths herein described probably belong to *R. pristis*. Nevertheless, strong discrepancies were found in the phylogeny herein presented with that on the state-of-the-art. Thus, we strongly recommend that a thorough review of the species, as well as the family Rhadinorhynchidae, should be carried out.

1.5. Regarding Nematode parasites, the faunistic investigation provided the first record of ***Anisakis pegreffii*** in *N. couchii*, which likely acts, through predator-prey interactions, as intermediate host in coastal waters of the NW Iberian Peninsula. Overall, the *Anisakis* sibling species are able to share the same intermediate host in a sympatric distribution among krill. The infected mysid suggests that *Anisakis* spp. are not specific at the mesozooplankton level. This finding is a strong evidence indicating that *Anisakis* spp. uses different hosts to cross habitats enlarging their recruitment pathways in order to find their definitive mammal host.

1.6. Concerning Trematodes, this PhD thesis has been provided the first record of ***Opechona bacillaris*** *sensu stricto* in the Siphonophora *Muggiaea* sp. In coastal waters of the NE Atlantic Ocean (NW Iberian Peninsula) *Muggiaea* sp. is probably acting through predator-prey interactions as a second intermediate host.

2. Ecological investigation on parasite recruitment at the mesozooplankton level on temperate water mass off the NE Atlantic has provided important findings on the niche dimensions of the identified symbiont systems. The most relevant one is the low degree of saturation of many potential niches available to parasites in zooplankton communities. Some other relevant issues have to be mentioned:

2.1. A different degree of host specificity among different parasites was remarkable: from those that infect a single host taxon to the generalists with a wide host range in mesozooplankton communities. Moreover, in the former exhibiting strong host

specificity even marked preference for certain microhabitats on or in the host body was evident. Host specificity at the mesozooplankton level represents a trade-off with those mechanisms ensuring dispersal (host range extension) and aggregation (distribution in space).

2.1. The influence of the oceanography (i.e., macrohabitat) upon the recruitment of infecting parasitic larvae, being different under upwelling or downwelling conditions. In upwelling systems parasite faunas with complex multiple-host life cycles are impoverished whereas relaxing downwelling events propitiate optimal conditions to successful parasite recruitment. The hypothesis that stability in water masses enhances parasite recruitment was proposed in a global scale by Pascual et al (2007). Our results suggest a similar effect of physical environment upon parasites in a local scale at Ría of Vigo.

3. Even only a small proportion of niches are filled (from a parasitological point of view), mesozooplankton communities play a major role in the life cycle of trophically-transmitted macroparasites and microparasites with well-known economic and public health concern. The results presented in this thesis reinforce the promising future role of zooplankton parasite surveillance in risk assessment studies integrated in fisheries, aquaculture and seafood safety management under National and Horizon 2020 frameworks.

CONCLUSIONES

1. La extensiva investigación faunística de las comunidades mesozooplanctónicas de las aguas del NE Atlántico, ha proporcionado una fuerte evidencia del establecimiento de cerca de unos 17 sistemas simbiontes que representan un grupo de alta la diversidad de parásitos y epibiontes con bajas prevalencias en poblaciones de mesozooplancton. Los sistemas hospedador-epibionte/parásito identificados comprenden:

1.1. Una nueva especie epibionte ***Pelagacineta hebensis* sp. n.**, que se encuentra en individuos adultos del copépodo *Paraeuchaeta hebes*. Es la primera vez que este copépodos se registra como basibionte para esta especie de protozoo epibionte. El nuevo epibionte se describe tanto morfológicas como molecularmente, lo que contribuye a ampliar las secuencias genéticas disponibles para la Clase Phyllopharyngea. Por otra parte, también se proporciona el diagnóstico taxonómico de esta nuevo epibionte descubierto.

1.2. Tres nuevas especies de **copépodos hospedadores del protozoo parásito *Ellobiopsis chattoni***. Éste encuentra en formas adultas de los copépodos ***Calanoides carinatus*, *Centropages chierchiae* y *Metridia lucens*** lo que extiende la gama de huéspedes para este parásito. Las prevalencias entre copépodos fueron bajas mostrando diferentes susceptibilidades a la infección de *Ellobiopsis*. Por otra parte, *E. Chattoni* mostró cierta predilección por el hospedador *Calanus helgolandicus*.

1.3. Por primera vez el acantocéfalo ***Bolbosoma balaenae*** se encontró parasitando el eufaisiáceo *Nyctiphanes couchii*, que puede desempeñar el papel de hospedador intermediario. Por otra parte, se propuso el ciclo de vida *B. balaenae* asumiendo que Balaenoptera *physalus* es el hospedador definitivo. Estos mamíferos marinos serían los responsables del suministro de la infra-población infectiva para los eufausiáceos.

1.4. Por primera vez el acantocéfalo ***Rhadinorhynchus* sp**. se ha identificado infectando *Nyctiphanes couchii*. Es probable que este

eufáusido actúe a través de las interacciones depredador-presa como huésped intermediario. Tanto el estudio morfológico como filogenético, junto con la información epidemiológica disponible sobre *R. pristis* en peces de las familias Scombridae y Xiphiidae de las costas de Portugal, las Islas de Madeira y el Océano Atlántico Norte, sugieren que los cistacantos aquí descritos pertenecen probablemente a *R. pristis*. Sin embargo, se encontraron discrepancias entre la filogenia que aquí se presenta y el "estado del arte". Por lo tanto, se recomienda una revisión exhaustiva tanto de las especies como de la familia Rhadinorhynchidae.

1.5. En cuanto a los Nematodos parásitos, la investigación faunística proporcionó el primer registro de ***Anisakis pegreffii*** infectando *N. couchii*, éste actuaría a través de las interacciones depredador-presa, como hospedador intermediario en las aguas costeras del noroeste peninsular. Además, estas especies hermanas de *Anisakis* (*A. simplex* y *A. pegrefii*) comparten el mismo hospedador intermediario en una distribución simpátrica. El misidáceo infectado indica que *Anisakis* spp. es generalista a nivel de mesozooplancton y que es capaz de utilizar diferentes hospedadores para cruzar hábitats (bentónico-pelágico) ampliando sus vías de reclutamiento con el fin de encontrar su hospedador definitivo (mamífero marino).

1.6. Esta tesis ha proporcionado el primer registro del trematodo <u>*Opechona bacillaris sensu stricto*</u> en el Sifonóforo *Muggiaea* sp. En las aguas costeras del NE Atlántico (NO Península Ibérica). *Muggiaea* sp. probablemente actúe, a través de interacciones depredador-presa como segundo hospedador intermediario.

2. <u>La investigación ecológica</u> en el reclutamiento de parásitos a nivel mesozooplanctónico realizado en las aguas templadas del NE Atlántico ha proporcionado importantes hallazgos sobre las dimensiones de los nichos de los sistemas de simbiontes identificados. El más relevante es el <u>bajo</u>

grado de saturación de los muchos nichos potencialmente disponibles para los parásitos en las comunidades de zooplancton. Además:

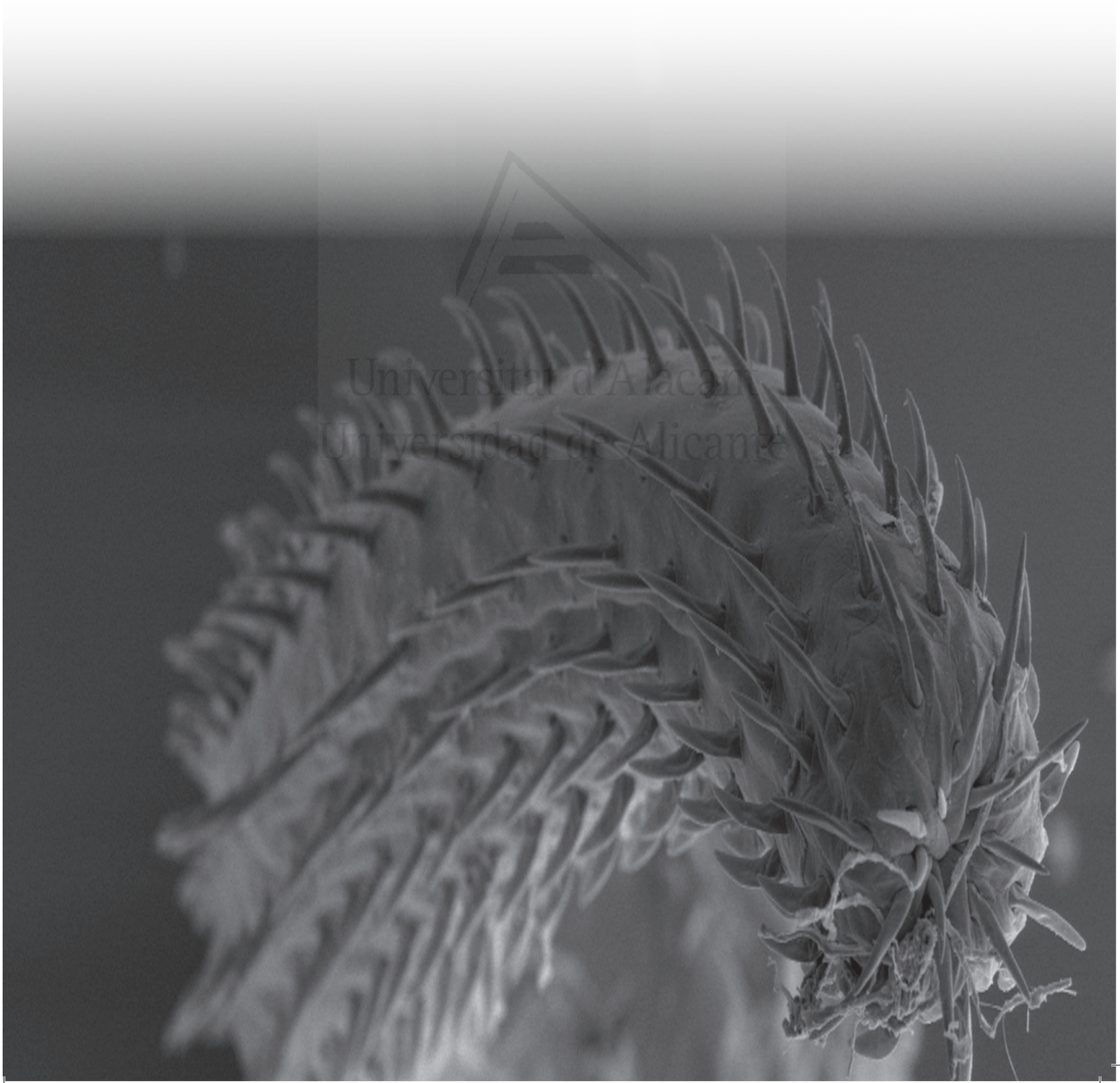
2.1. Los parásitos mostraron diferentes grados de especificidad por los hospedadores: desde los que infectan un solo taxón hospedador hasta los generalistas con una amplia gama de hospedadores entre las comunidades de mesozooplancton. Por otra parte, en los primeros (los que muestran especificidad) también muestraron una incluso marcada preferencia por determinados microhábitats sobre o en el cuerpo del hospedador. La especificidad a nivel mesozooplanctónico representa una solución de compromiso entren los mecanismos que garanticen la dispersión (ampliación del rango de hospedadores) y la agregación (distribución en el espacio).

2.2. Diferentes condiciones oceanográficas (macrohábitat) surgencia-hundimiento influencian el reclutamiento (colonización) de larvas infectivas. En los sistemas de surgencia las faunas de parásitos con ciclos de vida complejos con múltiples hospedadores intermediarios se empobrecen mientras que, los eventos de hundimiento con masas de agua más estables propician las condiciones óptimas para el éxito del reclutamiento parasitario. La hipótesis de que la estabilidad de las masas de agua aumenta el reclutamiento parásito fue propuesta en una escala global de Pascual et al. (2007). Los resultados obtenidos en esta tesis sugieren y apoyan un efecto similar, donde los procesos oceanográficos estables (hundimiento) favorecen la colonización de los parásitos al mesozooplankton a escala local en la Ría de Vigo.

3. Aunque sólo una pequeña proporción de los nichos están llenos (parasitológicamente hablando), las comunidades mesozooplanctonicas juegan un papel crucial en el ciclo de vida tanto de macroparásitos como microparásitos tróficamente transmitidos con el conocido problema de salud pública y económico que ello conlleva. Estos resultados refuerzan el prometedor futuro de la vigilancia y control de parásitos en el zooplancton en los estudios de evaluación de riesgos integrados en la pesca, la

acuicultura y la gestión de la seguridad alimentaria de los productos pesqueros dentro de los marcos Nacionales y del Horizonte 2020.

Annex



ANNEX

ANNEX 1. MATERIAL AND METHODS USED IN THE SURVEYS AS WELL AS OCEANOGRAPHIC AND BIOLOGICAL CONTEXTS ARE AVAILABLE IN (ROURA 2013)

Data acquisition for this study was carried out during cruises CAIBEX-I and III on board the RV Sarmiento de Gamboa: CAIBEX-I was carried out around Cape Silleiro (NW Spain; Fig. 1) from July 7 to 24 and CAIBEX-III around Cape Guir (NW África, Fig. 1c) from August 16 to September 5, 2009. The overall aim of these cruises was to define the physical, geochemical and biological three-dimensional meso-scale structure of the coastal transition zone off Cape Silleiro and Cape Guir and their exchange with the open ocean as a result of wind forcing. For this purpose high spatial and temporal mapping of the study área was carried to determine the oceanographic conditions (temperature, salinity and Chl-fluorescence) in situ using a towed vehicle (SeaSoar) that undulates between the surface and 400m depth. The information collected with the SeaSoar, together with real time satellite images of sea surface temperature (SST) and chlorophyll provided by the Plymouth Marine Laboratory (NEODAAS), helped to determine the location of the upwelled water and the filament. Once detected, the destiny of the upwelled water mass was studied carrying lagrangian experiments with an instrumented drifting buoy (IDB) that was deployed in the core of the upwelling/filament. The IDB was equipped with GPS and Iridium positioning system, an Acoustic Doppler Current Profiler (ADCP) at 2 m to determine current direction and velocities up to 100 m depth, and temperature sensors at 10, 20, 40, 60, 65, 80 and 100 m depth. CTD casts were carried before each plankton sampling. In order to follow the advected community, bongo samplings were carried close to the IDB. Further mesozooplankton samplings were carried apart in the upwelling area and the adjacent open ocean. Bongo nets were used to collect mesozooplankton at three depths (5, 100 and 500 m) doing double oblique towings. The deep scattering layer (DSL) was observed with the Echo sounder Simrad EK60, operating with five frequencies simultaneously ranging from 18 to 200 kHz, which allowed to detect animais ranging from 8.5 to 0.75 cm, respectively.

However, meteorological conditions during the first survey (CAIBEX-I) did not allowed to follow the fate of coastal waters offshore. Instead, two different

lagrangian experiment were carried: an upwelling relaxation over the slope (lagrangian 1, L1) and the onset of coastal upwelling with alongshore transpon (lagrangian 2, L2). Fortunately, favourable meteorological conditions during the second survey (CAIBEX-III) allowed us to carry the third lagrangian experiment (L3) following the onset of a wide upwelling (50 km width) that was exported off shelf by a long filament (150 km long). Following, a detailed description of the physical and zooplankton samplings carried out in both surveys is given separately.

CAIBEX-I

Although the survey started with strong northerly winds that should propel the upwelling, after three days the winds weakened without producing a filament. Nonetheless, real-time images facilitated by NEODAAS showed insights of upwelledwater around 41° 25'N. Thus, the IDB was deployed over the continental slope to carry the first lagrangian experiment (July 10-14 inclusive).

Strong northerly winds were expected during July 16 close to Cape Silleiro and the second lagrangian experiment (July 16-21 inclusive) was carried out to observe the onset of the upwelling. The IDB was deployed south-west of Cape Silleiro. From July 19 onwards the strong northerly winds weakened as well as the upwelled water. The morning of July 21 the IDB was recovered Between the end of the first and second lagrangian experiments 8 parallel cross-shelf CTD grids were performed from latitude 42°I8'N to 41°12'N, to understand the small-scale water properties throughout the coast.

After performing a CTD at the position of the IDB, zooplankton samplings were carried during the lagrangian experiments both at night (00:00 AM) and day (12:00 AM). Besides, some zooplankton samplings were carried during the cross-shelf CTD grids in order to know the mesozooplankton community outside the upwelled water. Mesozooplankton samples were collected with two 750 mm diameter bongo nets equipped with 375 m mesh and a mechanical flow-meter. At a ship speed of 2.5 knots three samples per station were collected: at the DSL level when the bottom depth allowed it (from 350-550 m), 100 and 5 meters. The bongo net was first lowered to the desired depth, towed for 30 minutes and subsequently hauled up at 0.5 m s-1. Then, it was cleaned onboard and back to the sea for the next towing. Plankton

samples were fíxed with 96% ethanol and stored at -20°C to allow DNA preservation for dietary analysis.

CAlBEX-lll

Satellite images indicated an upwelled mass at Cape Guir on August 17. A SeaSoar mapping consisting of 6 sections parallel to the coast, from the ocean to the coast, was carried out to obtain a synoptic image of the study area (August 17-20), plus a coastal section with 10 CTDs. SeaSoar section number 6 (the closest to the coast) was repeated on 23 August to follow the structure of the filament. Once the core of the filament was identifíed, the first of the two 3-day lagrangian experiments were set up (August 23-26, Fig. 1). The IDB was deployed in the core of the filament following the advected water following the same procedure as in CAIBEX-I cruise.

After the first lagrangian experiment a longitudinal section of 13 CTDs through the filament were performed at the same longitude of the Seasoar section number 6. At the end of the section the second 3-day lagrangian experiment was performed (August 28-31), deploying the BB close to the PB that followed its way transponed by the filament. Afterwards, we have to interrupt the survey due to technical problems and return to Las Palmas de Gran Canaria, loosing two sampling days. Then, the SeaSoar section number 6 was repeated northward and southward because the rough meteorological conditions did not allowed the towing of the SeaSoar at 8 knots during the northward section (September 2). The rough conditions kept until the end of the survey prevented further CTD casts and the replication of the SeaSoar sections. At the end five ARGOS surface drifters, plus a 300-m ARGOS drifter were deployed in the core of the filament to track the upwelled water for long-time. Mesozooplankton samplings were carried out as in CAIBEX-I during day and night following the PB in the core of the filament, at the same three depths (DSL, 100 and 5 meter). Furthermore, CTD casts together with satellite images allowed identifying the periphery of the filament and the warm oceanic waters to carry out, whenever possible, mesozooplankton samplings outside the filament. These samplings together with one sampling carried at night close to the coast, were essential to understand the plankton community present outside the filament.

Cephalopod and macrozooplankton identification

...The dominant groups in the macrozooplankton fraction based on trawl catches, both in abundance and biomass, are fishes and shrimps from 2 to 10 cm together with small pelagic cephalopods. Thus, special effort was allocated to the identification of these two groups... Results from macrozooplankton present in the different stations among CAIBEX-I and III are available in table 1 and 2 extracted from Roura 2013.

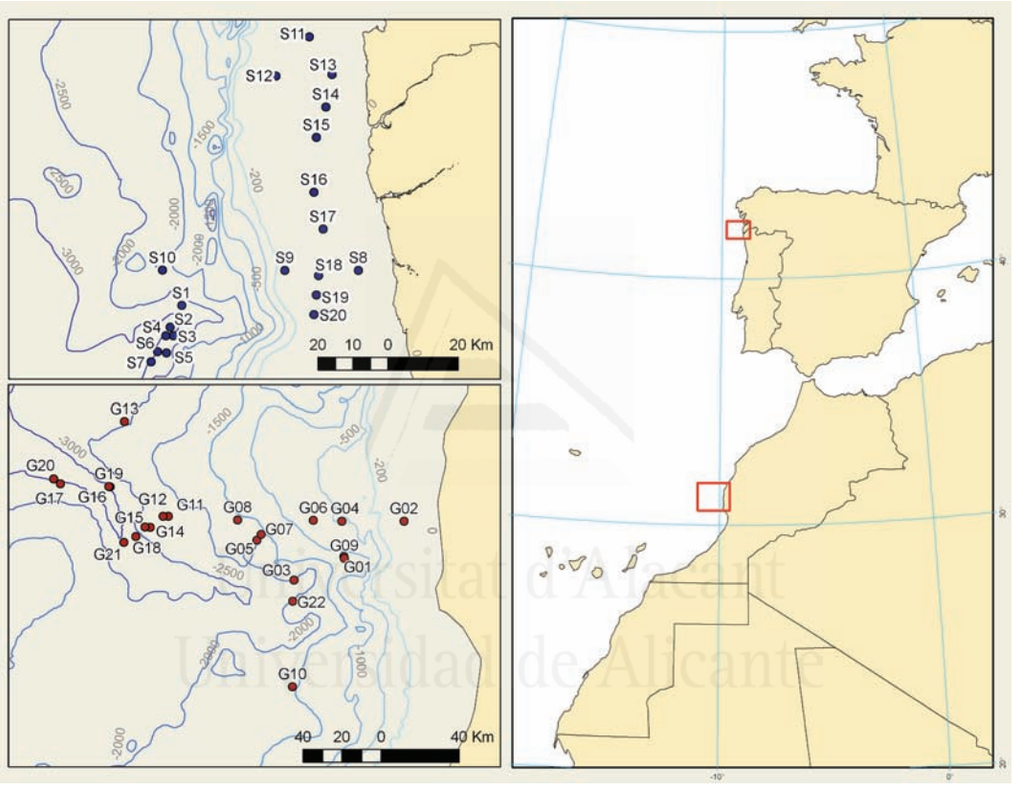


Fig. 1. a) Schematic map of the Canary Basin showing the áreas sampled (red boxes) and the main currents (light blue: surface currents; dark blue: slope current), major capes, freshwater (blue arrows) and dust inputs (>10 g m"2 y , shaded yellow), retention (orange) and dispersión (green) zones on the shelf, frontal zone between water masses {dashed blue lines) and mesoscale eddies (blue: cyclones; red: anticyclones) south of the Canary Islands. NACW: North Atlantic Central Water; SACW: South Atlantic Central Water; AC: Azores Current; CanC: Canary Current; MC: Mauritanian Current; NEC: North equatorial Current; NECC: North equatorial Countercurrent; PC: Portuguese Current; SC: Slope Current. Modified from Arístegui eí ai, (2009). b) Zooplankton samples collected during the cruise CAIBEX-I off the NW coast of the Iberian Península, around Cape Silleiro (42°N). Samples SI to S7 correspond to the first lagrangian experiment (L l) and S13 to S20 tothe second lagrangian experiment (L2). c) Zooplankton samples collected during the cruise CAIBEX-III off the NW África coast, around Cape Guir (30°N). See table 2 for details.

Table 1 Macrozooplankton present in different stations carried out during CAIBEX-I.

	Taxa	Coast				Ocean					
		day		night		day			night		
		100	5	100	5	500	100	5	500	100	5
Cephalopoda	*Octopus vulgaris*	7	4	12	27	5	4		14	10	15
	Alloteuthis media	2		1	1						
	A. subulata			7	3						
	Loligo vulgaris			2							
	Sepiola tridens			5							
	Illex coindetii				1						
	Ommastrephidae	8		3	1		1			1	
Euphausiacea	*Meganyctiphanes norvegica*					23			45	162	4
	Nematoscelis megalops								4	2	
	Euphausia krohnii								1		
	Nematobrachion boopis					1			1	1	
Decapoda	*Systellaspis debilis*					3			16	2	
	Acantephyra purpurea								3		
	Gennadas brevirostris								1		
	Sergestes robustus								2		
	Pasiphaea sivado					26			24	69	10
	Phyllosoma larvae								1	2	16
	Polybius henslowii	2	7	1	8						
Mysidacea	*Eucopia sculpticauda*								19	2	
Fish	Myctophidae					1			8	18	3
	Gonostomatidae					131			147		
	Stomiidae								2		
	Sternoptychidae								2	1	
	Syngnathid larvae	1		6	2						
	Pleuronectiform larvae	2		6	9						
	Larvae indet.			16	1				1		
	Hyperiids						1		2	2	2
	Cnidaria	1							3		
	Siphonophora								1		
	Ctenophora						3		6	1	
	Urochordata						1		2	4	
	Pteropoda									2	

Table.2. Macrozooplankton present in different stations carried out during CAIBEX-III.

		Coast		Upwelling						Filament						Ocean					
		Night		Day			Night			Day			Night			Day			Night		
		100	5	500	100	5	500	100	5	500	100	5	500	100	5	500	100	5	500	100	5
Cephalopoda	*Octopus vulgaris*	1	8		1		2	2	4	2	1			1	1	1	1		1	2	7
	Alloteuthis media	91	16																		
	A. subulata	8																			
	Sepiola atlantica	1																			
	S. ligulata	1																			
	Rondeletiola minor	4	1																		
	Heteroteuthis dispar													2							
	Sepiolidae				1																
	Ommastrephidae								1		2		1								
	Abraliopsis morisii												1	1	1	3	1		1		1
	Brachioteuthis riisei												1	2		4	1			4	6
	Enoploteuthidae									1					1						
	Liocranchia reinhardtii															1	1			1	
	Mastigoteuthis hjortii																		1		
	Ancistroteuthis lichtensteinii									1	1		2	2	4		1		2	5	
	Onychoteuthidae																			3	
	Oegopsida														4	3	4				2
	Pyrotheuthis margaritifera										1		1				4	1	2	2	
	Pyrotheutidae									1										1	1
Euphausiacea	*Meganyctiphanes norvegica*			3			1			1											
	Nematoscelis megalops			1						7			23	1					1		
	Euphausia krohnii							1	1	2			7	2					4	1	
	Nematobrachion boopis									2			2						1		
	Stylocheiron maximun																		1		
	Thyssanopoda microphtalma									1			2						13	1	
Decapoda	*Systellaspis debilis*			9			55	12		7			8	13					10	3	
	Acantephyra purpurea						1						11	3					19		
	Oplophorus spinosus						2			1				2					1		
	Sergestes arcticus			61			14			1			2						1	1	
	Sergestes robustus						12	4					13	4					6	2	
	Sergestes sargassi												1			2					
	Sergestes vigilax			1			1			2			1	7		2			3	4	
	Sergestes corniculum											1								1	
	Sergestes atlanticus												1								
	Gennadas brevirostris						7	3					17	8					8	6	
	Parapandalus richardii															1			3	2	
	Pasiphaea multidentata						1						1								
	Pasiphaea sivado			3				2		5			2								
	Phyllosoma larvae			2	1		7	4	11	12	13		43	33	8	20	22	10	21	19	1
	Scyllarides latus juvenil						1						1	2	0				1	2	
	Stenopus hispidus														1						
Mysidacea	*Gnatophausia zoea*						2						1								
	Lophogaster spinosus																			1	
	Eucopia sculpticauda												2								

Table 2. Continuation.

Fish	Gonostomatidae			48			351			333			289			496			365	5	
	Myctophidae			3			17	14		21			16	21		33			26	29	
	Stomiidae			2			2			5			1	1		1			1		
	Sternoptychidae			1			5			3			9			19			16		
	Phosichthyidae			4			25			31			18	2		38			49	3	
	Opisthoproctidae									1			1			1			2	2	
	Pleuronectiform larvae												1			1	1				
	Clupeiform larvae													1	20						
	Leptocephala larvae													1							
	Larvae indet.						1			1			5			1	1		10	4	
	Stomatopod larvae	38	8				44	1	13	7	3		4	2	2	14	5	58	5	12	4
	Hyperiid									3	2		3	7	2	6	4	2	20	19	2
	Chaetognatha			112	27	11	141	42	8	230	160		476	229	605	309	135	33	372	137	53
	Cnidaria								1				3						1		
	Pteropoda							1		4	1								1	3	
	Pterotrachea			1					1				1	1	2	2	2	3	5	2	3
	Tomopteris spp									1				2						1	
	Macrocypridina castanea						1						1							1	

ANNEX 2. PUBLISHED MATERIAL

Gregori M, Fernández-Leborans G, Roura A, Abollo E, González AF, Pascual S. 2014. Description of a New Epibiontic Relationship (Suctoria - Copepoda) in NE Atlantic waters: from Morphological to Phylogenetic Analyses. Acta Zoologica. (Submitted).

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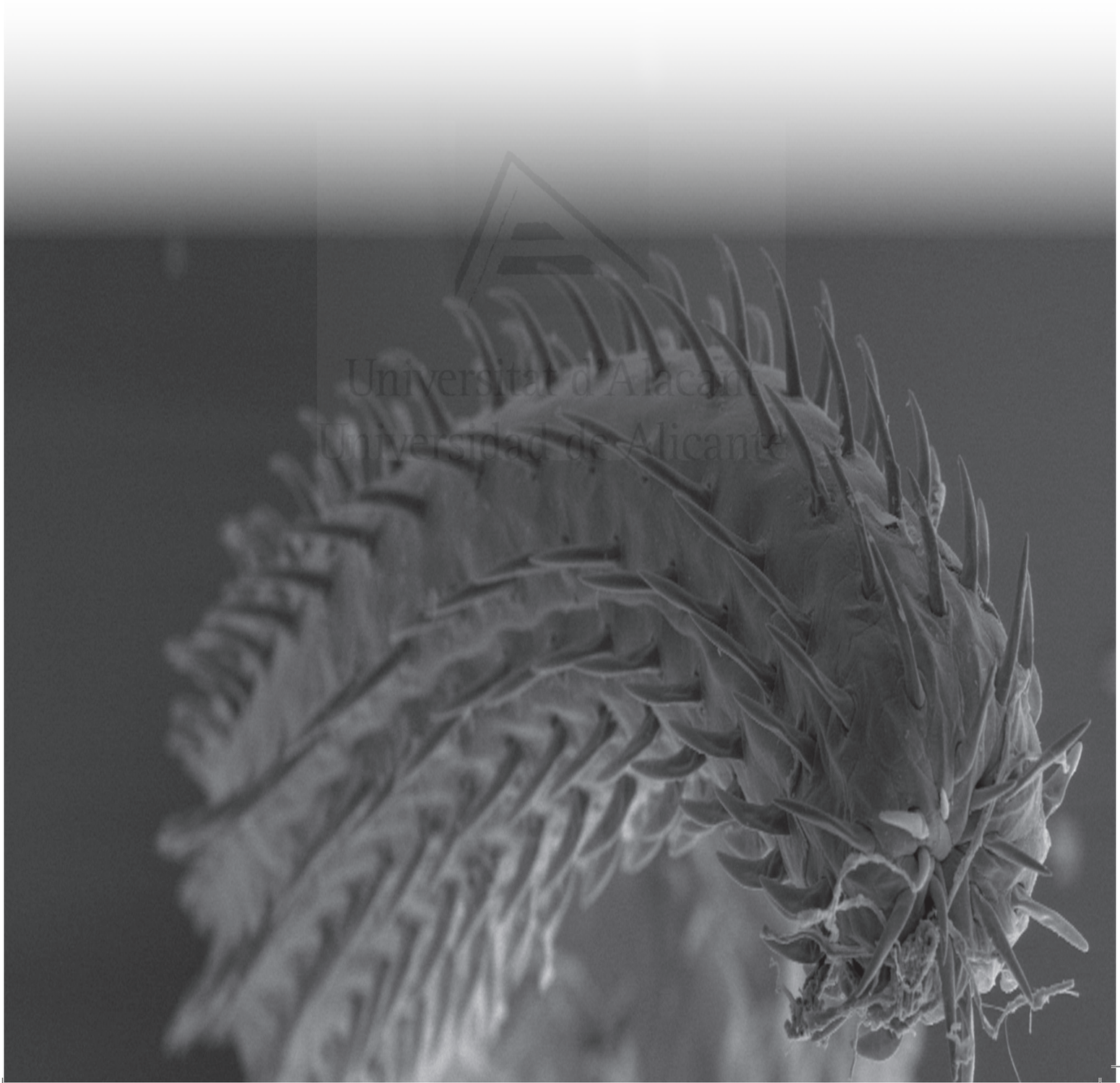

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