Universitat Autònoma de Barcelona

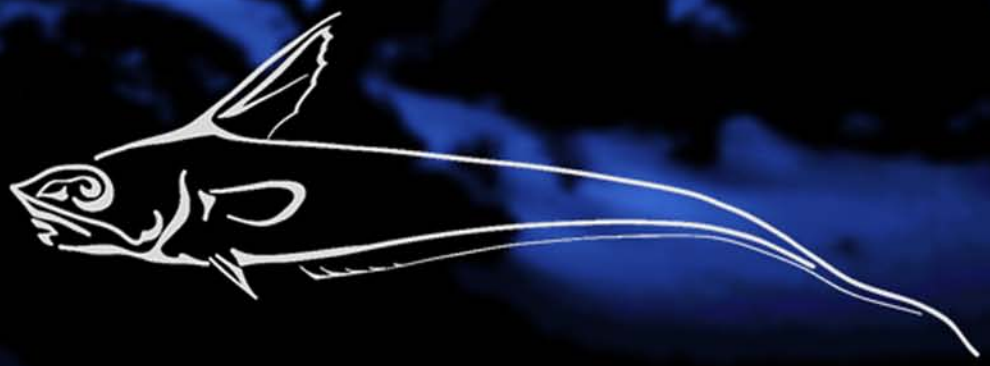
A Journey to the Deeps of the Sea: Parasite communities of the Alepocephalidae and the Macrouridae in the Balearic Sea (NW Mediterranean)

David Pérez García

A Journey to the Deeps of the Sea: Parasite communities of the Alepocephalidae and the Macrouridae in the Balearic Sea (NW Mediterranean)

David Pérez García

PhD Thesis 2017



Universitat Autònoma de Barcelona
Facultat de Biociències
Departament de Biologia Animal, de Biologia Vegetal i d'Ecologia
Unitat de Zoologia

PhD Thesis

A Journey to the Deeps of the Sea: Parasite communities of the Alepocephalidae and the Macrouridae in the Balearic Sea (NW Mediterranean)

A thesis submitted by David Pérez García for the degree of Doctor in Aquaculture under the direction of Dr. Maite Carrassón López de Letona, Dr. Maria Constenla Matalobos, Dr. Anna Soler Membrives and the supervision of Dr. Maite Carrassón López de Letona.

This thesis has been inscribed in the Aquaculture PhD program from the Universitat Autònoma de Barcelona.

Director and supervisor
Maite Carrassón López de Letona
Universitat Autònoma de Barcelona

Director
Maria Constenla Matalobos
Universitat Autònoma de Barcelona

Director
Anna Soler Membrives
Universitat Autònoma de Barcelona

PhD student
David Pérez García
Universitat Autònoma de Barcelona

Bellaterra, March 2017

Acknowledgements

When I started to write this section I was pondering how to do it. It's obviously a place to thank all the different persons involved somehow in the present thesis. Then I realized that this section is probably the only one where I'll be using the first person form to express myself. That's right, this section it's not only about those persons involved with the thesis, but also it's about me and how I'm linked to them. Being partially an emotional script, English will be tossed aside in favour of Catalan. But no worries, English will be recovered for acknowledgements directed to those out of Spain and then, for the rest of the thesis. Now it's time to start:

Aquesta tesi no hauria sigut possible sense l'ajut i amistat de diverses persones. Segurament el lector pensarà en algun moment al llegir aquest apartat "què té a veure tot això amb la present tesi? I aquesta persona?". La resposta es ben senzilla, "tot i res". L'autor d'aquest escrit estarà ple de contradiccions, però sap molt bé el que ell vol dir; i si aquest segment de la tesi li serveix, encara que sigui una mica, com una petita catarsi, espero que el lector li perdoni cert punt de divagació.

Primer de tot, agrair a mons pares tot el suport no només durant la tesi, sinó també durant el transcurs de la meva infància. Sent tots dos de formació econòmica, van fomentar i/o aguantar la passió per la natura del seu fill. Encara de tant en tant esmenten a nous coneguts històries sobre un nen que es quedava davant la televisió embadalit amb els documentals de natura que es retransmetien; i que per més que un cregui que ja no retransmeten aquests documentals antics, els segueixen emetent i l'autor de la present tesi diu en veu alta "repetit!" mentre segueix mirant sense canviar de canal. També sobre visites continues al zoològic de Barcelona i un nen que coneixia el nom (almenys els noms comuns) de tots els animals que allà s'hi trobaven i que els va aprendre en una pantalla. Per tot això, gràcies.

Tot i que mons pares promoguessin que veiés tots els documentals que volgués, aquest mèrit també correspon a personalitats que mai he conegut i que tantes hores hem compartit. D'entre ells cal destacar a Sir David Frederick Attenborough (espero que em disculpi per no dirigir-me en anglès en aquest cas). Tot i que no hagi fet res específicament pel desenvolupament de la present tesi, ha fet una gran contribució transmetent el seu amor per la natura i els éssers vius a diverses generacions d'actuals i futurs naturalistes. Ens ha mostrat les meravelles d'aquest món i ens ha fet somniar amb elles. Gràcies a tot això, Sir David Attenborough és parcialment el culpable de que m'hagi dedicat a la biologia i no pas a l'astronomia.

Seguint la tendència de la meva entrada i assentament en el món de la biologia, hauria d'agrair a tots els professors relacionats amb la matèria. Evidentment tenen la seva importància, però queda en part eclipsada per les següents personalitats. La primera d'elles, i segurament la més important en la pròpia realització d'aquesta tesi doctoral, és la Doctora Maite Carrassón (els més observadors ja hauran vist que és el mateix nom que a la primera plana és acompanyat per "Directora de tesi"). Abans de tot, ella va ser professora de Biologia Marina, que vaig cursar allà cap a l'any 2011. Havent sigut ara jo també professor, suposo que ben poc em recorda d'aquesta època. A l'any següent vaig tindre el meu primer contacte amb parasitologia marina durant les "pràctiques en empreses i institucions" de la carrera. És en aquest moment en el que la Maite va començar a veure què significava tutelar-me. Al termini de la meva estança, em va oferir la opció de realitzar els estudis de doctorat sota el present tema. Suposo que la resta és història. Només m'agradaria afegir el meu agraïment per la teva paciència i la teva comprensió en els moments més durs, Maite. Un cop més, gràcies.

Les dues persones següents també són molt importants en la tesi doctoral. Com no, són les dues codirectores de tesi, les Doctores Maria Constenla i Anna Soler. A tu Maria, per començar et va tocar fer la revisió dels meus peixos (santa paciència!). En aquell moment no eres codirectora, però treballar en aquesta tesi et va tocar igual. Després va ser pitjor, vas passar de revisar peixos a revisar els meus escrits abans d'enviar-los a revista! L'únic que puc dir es gràcies. Ara ja fa un temps que no et tenim acompanyant-nos per dinar amb nosaltres, però segurament estaràs completament satisfeta amb la companyia de les teves filles. Felicitats!

A tu Anna, que també vas començar patint sense ser codirectora. Quants dies vam estar fent molecular? Un munt! I gràcies a tu vaig entendre que això de fer molecular és fer màgia negra (no li tingueu en compte, la percepció que tinc i el nom són de la meva pròpia collita). Ara seqüenciem uns cucus; ara uns hyads; ara uns Scolex i...vaja, no surt res. No queda més que tornar a començar. Després, per igualar condicions amb la Maria, també et va tocar revisar els articles. Igual que amb ella, l'únic que puc dir és gràcies.

Tota la gent del laboratori també heu tingut el vostre paper. Particularment el Doctor Francesc Padrós, que revisava la meva feina d'histologia quan la Maria no podia. I per haver-me presentat els títols de pel·lícules de les "bones". Encara n'he de veure algunes. Ah! I per haver-me descobert que el treball de laboratori sembla heroic sota el ritme de la música èpica. També als companys de feina que han anat passant: Sandra, Aitzar, Davinia i Oriol. Conjuntament amb els alumnes de pràctiques heu fet que les

estones de laboratori fossin més entretingudes. I a en Wolf! Que durant les seves petites estades vaig agrair la seva companyia, i durant l'estança a la República Txeca també.

A la Doctora Montserrat Solé, gràcies per acollir-me no un, sinó dos cops al teu laboratori a l'Institut de Ciències del Mar. Crec que mai he pipetejat tant en la meva vida com en el teu laboratori. Aprofitant que estem pel ICM, gràcies al Doctor Joan Cartes, que ha tingut una implicació molt important (i paciència!) cap al final de la tesi amb les dades de dieta dels macrúrids.

Ara és temps d'una mica d'anglès. Thanks to all the people I've met in České Budějovice. First of all, to Aneta Kostadinova, who was responsible of my stays in the Institute of Parasitology of the Academy of Sciences of the Czech Republic. I swear I've never eaten half a dish of another person of the table before meeting Aneta. Nice try, but I didn't gain any gram (maybe it's true your theory that I have some intestinal parasite). Also thanks to David González, who taught me everything about nematodes. To Roman Kuchta, for his stories during those long dinning sessions. And then, Simona, who was always helpful in the laboratory and friendly outside. But there are more! Those who, like me, were passing by this place. Soengjun, who made my last two weeks of the stay really enjoyable. And Olena, a good friend that now is in South Africa, but I hope I will be able to meet again. We visited lots of places together, like "her castle" in Hluboká, and an express visit to Austria on one weekend. To all of you, thanks.

Pels últims agraïments tornaré al català. Aquests quedaran escrits aquí, però segurament els destinataris mai se n'assabentaran; en part perquè suficient vergonya em fa escriure'ls aquí. Potser algun dia aquest paràgraf acabarà accidentalment a les seves mans. A vosaltres, els amics de japonès que portem tant de temps fent quedades, Andrea, Jordi i Lorena. I a tu, Alba, que ens coneixem des de fa tant temps. Igual que la Maite és responsable de l'inici d'aquesta tesi, vosaltres sou sens dubte, juntament amb mons pares, els majors responsables de que l'hagi acabat. Tots vosaltres sabeu que més d'un cop he estat a punt de deixar-ho; i Jordi, però sobretot Alba, heu pogut entreveure com d'enfonsat he arribat a estar. No és una cosa que m'agradi deixar per escrit, però no em semblaria correcte no plasmar-ho aquí, en el resultat final que s'ha obtingut gràcies al vostre suport, us en adonéssiu o no. Per això, les últimes i més sentides paraules d'aquests agraïments són per a tots vosaltres. Família i amics, moltes gràcies per tot.

David Pérez García

Abstract / Resum

ABSTRACT

Despite the great number of studies on the Mediterranean, its deep sea remains largely unknown, especially in its parasite fauna. Parasites are important components of ecosystems. Being ubiquitous throughout all food webs, they can provide significant information of their hosts. Since most parasites are trophically transmitted, all hosts required for the completion of their life cycle must be present within the geographic region. Therefore, parasites can be used as biological tags of stock and ontogenetic or seasonal migrations. In addition, they can be used to infer the trophic links between host, preys and predators and to detect ontogenetic shifts in the diet, niche shifts due to competition or other factors, individual feeding specializations within a population and seasonal changes in diet.

Parasitism causes a stress on the hosts by definition. It is expected that host respond to the harmful effects of parasites at different levels. Histo-cytological alterations and/or variations on the levels of certain biochemical markers can be related to the presence of parasites and are means to evaluate fish health.

Parasitism can provide important information about the biology of fishes, the distribution of their populations and their trophic links within the food web. Since most of the important deep Mediterranean fishes, particularly the *Alepocephalus rostratus* and the macrourids *Coelorinchus caelorhincus*, *Coelorinchus mediterraneus*, *Coryphaenoides guentheri* and *Coryphaenoides mediterraneus*, are still largely unknown, their parasites can prove to be valuable. For this reason, the objectives of the present thesis are to characterize the parasite communities of *A. rostratus, C. caelorhincus*, *Coe. mediterraneus*, *C. guentheri* and *Cor. mediterraneus*, their natural variability and their effects on fish health. In addition, the relationship between the trophic profile of hosts and parasite communities will be discussed.

In the chapter 3 of the present thesis, the parasite communities of *Alepocephalus rostratus* and its influence on some fish biochemical markers and histological alterations were examined. *A. rostratus* constitutes the second most important fish species, in terms of biomass, inhabiting the deep slope of the Balearic Sea (NW Mediterranean). The study revealed eight different parasite species in this host: one coccidian, one digenean, one monogenean, one cestode and four nematodes. The parasite fauna of *A. rostratus* was partially dominated by larval forms (four of the seven metazoan taxa found), which combined with low species richness correspond to a parasite fauna pattern more typical of bathypelagic fish species rather than demersal ones. The larval tetraphyllideans and cucullanid nematodes were the predominant

species. In relation to depth, differences in abundance of the nematodes Cucullaninae gen. sp. and *Hysterothylacium aduncum* were found, probably due to the dietary shift in the fish host at greater depth. Thus, they could be regarded as indicators for discriminating populations of *A. rostratus* in relation to depth in NW Mediterranean waters. Of the biochemical markers examined, acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) activities and lipid peroxidation (LP) levels, only LP showed significant differences between depths. A positive relationship was found between AChE activity and Tetraphyllidea fam. gen. sp., *Anisakis physeteris* and *H. aduncum* abundance and a negative one with the abundance of Cucullaninae gen. sp. LDH showed a positive relationship with the abundance of the parasites *Paracyclocotyla cherbonnieri* and Tetraphyllidea fam. gen. sp. At cyto-histological level, coccidians were detected in the pyloric caeca with a prevalence of 90% in Barcelona, but in the rest of organs almost no alterations were detected. The restricted macro-planktonic diet of *A. rostratus*, that maintains it distant from the sea-floor for longer periods than other demersal species, probably makes this species less susceptible to sediment-associated impacts including parasitism.

Chapter 4 analysed the parasite communities of *Coelorinchus caelorhincus*, *Coelorinchus mediterraneus*, *Coryphaenoides guentheri* and *Coryphaenoides mediterraneus* of the middle and lower slope of the Mediterranean Sea. Histopathological, enzymatic activity (AChE and LDH), dietary and environmental (oxygen, salinity, temperature and turbidity) information were also obtained. A total of 11 parasite taxa were found among all four fish species, being the copepod *Hamaticolax resupinus* the only parasite shared by all of them. *Coelorinchus mediterraneus*, *Coryphaenoides guentheri* and *Cor. mediterraneus* exhibited rather homogeneous parasite communities, especially in the case of the latter two. *Coelorinchus mediterraneus* showed the highest richness of parasite taxa (eight species), whereas *C. guentheri* and *Cor. mediterraneus* harboured up to five and six, respectively, and *C. caelorhincus* up to three. Several of the encountered parasites occurred at very low prevalence (<10%), while only three species were exceptionally prevalent and abundant: Cucullanidae fam. gen. sp. larvae in *C. caelorhincus*; *Lepidapedon desclersae* in *Coe. mediterraneus* and *Hysterothylacium aduncum* in both *Coryphaenoides* spp. The abundance of the nematode *H. aduncum*, present in all host species except for *C. caelorhincus*, increased with water temperature and depth and became the dominant parasite below 2000 m. Salinity might be an important on the distribution of *H. resupinus*. The diet was generally homogeneous between the studied species, being *C. guentheri* more specialized on suprabenthic/benthic prey. The

parasites *H. aduncum* and Tetraphylidea, and to lesser extent *Rhaphidascaris* sp., were related with the most mobile (swimming) prey consumed by macrourids (chaetognaths, decapod larvae, and *Boreomysis arctica*). The parasites *L. desclersae, Capillostrongyloides morae* and *Otodistomum* sp. were related in *Coe. mediterraneus* with epibenthic prey (ophiuroids, isopods and tanaids). *Coryphaenoides guentheri* was the smallest macrourid analysed, with the poorest parasite fauna and higher proportion of larval stages. Few histopathological alterations were found, being epitheliocystis the most extended and prevalent one. Few parasite effects on fish health were reflected at enzymatic and histological level, probably due to the low parasite burden in their hosts. It is possible that the role of small macrourids, especially of *C. guentheri*, is closer to act as intermediate host in deep-Mediterranean trophic webs.

Hamaticolax resupinus n. sp. is described in chapter 5 from specimens collected from the gill cavities of *Coelorinchus mediterraneus* and *Coryphaenoides mediterraneus* at depths between 1,236-1,626 m. *Hamaticolax resupinus* n. sp. closely resembles *H. maleus* Oldewage, 1994, but differs from the latter by its smaller body size and in having a genital double-somite in the female that is markedly wider than the free abdominal somites and has strongly convex lateral margins. The new species is only the second bomolochid found on a macrourid host and is the first from depths greater than 1,200 m. *Hamaticolax resupinus* n. sp. also represents the first parasitic copepod recorded from *Coe. mediterraneus* and the third from *Cor. mediterraneus* worldwide.

A new nematode species, *Raphidascaris* (*Raphidascaris*) *macrouri* n. sp. (Anisakidae), is described in chapter 6 from male and female specimens found in the intestine, and occasionally in stomach and pyloric caeca, of two deep-water macrourid fishes (Gadiformes) off Barcelona, Mediterranean Sea: *Nezumia aequalis* (Günther) (type-host) and *Trachyrincus scabrus* (Rafinesque). Based on light and scanning electron microscopy examination, the new species shows similar morphological features as the other four valid species of the subgenus *Raphidascaris* Railliet and Henry, 1915, but it differs from *Raphidascaris* (*Raphidascaris*) *acus* (Bloch, 1779), *Raphidascaris* (*Raphidascaris*) *lutjani* Olsen, 1952 and *Raphidascaris* (*Raphidascaris*) *mediterraneus* Lèbre and Petter, 1983 in the high number of precloacal papillae (23–32) and from *Raphidascaris* (*Raphidascaris*) *gigi* Fujita, 1928 in the length of the spicules. Morevover, *Raphidascaris* (*Raphidascaris*) *macrouri* n. sp. exhibits a high variability on the number and distribution of caudal papillae, which was not recorded in the other four mentioned species. This is the first species of this subgenus reported from the family Macrouridae. Sequences of ITS1–5.8S–ITS2 region are analyzed and compared with

closely related nematode species confirming the uniformity of the *R.* (*R.*) *macrouri* n. sp. between hosts.

RESUM

Tot i el gran nombre d'estudis del mar Mediterrani, el seu mar profund segueix sent en gran part desconegut, especialment en relació a la seva fauna parasitària. Els paràsits són components importants dels ecosistemes. Podent ser trobats al llarg de la xarxa tròfica, aquests poden aportar informació sobre el seu hoste. Com la majoria de paràsits són transmesos de forma tròfica, tots els hostes necessaris per a la finalització del seu cicle vital han d'estar presents en una determinada regió geogràfica. Per aquest motiu, els paràsits poden ser utilitzats com a marcadors biològics de les poblacions de l'hoste i de les seves migracions ontogèniques i estacionals. A més a més, poden ser utilitzats per deduir relacions tròfiques entre l'hoste, preses i predadors i detectar canvis ontogènics en la dieta, canvis de nínxol degut a la competència o a altres factors, especialitzacions en l'alimentació dins d'una població i variacions estacionals en la dieta.

Per definició el parasitisme causa un estrès sobre l'hoste. És d'esperar que l'hoste respongui als efectes perjudicials dels paràsits a diferents nivells. Les alteracions cito-histològiques i/o les variacions de certs marcadors bioquímics poden ser relacionats amb la presència de paràsits i ser útils per avaluar la salut dels peixos.

Degut a l'escàs coneixement dels ecosistemes de mar profund, tota aquesta informació sobre l'hoste pot ser extremadament valuosa. El parasitisme pot aportar informació important sobre la biologia dels peixos, la distribució de les seves poblacions i les relacions tròfiques d'una xarxa tròfica, entre d'altres. Com la majoria dels peixos del Mediterrani profund, i en concret *Alepocephalus rostratus* i els macrúrids *Coelorinchus caelorhincus*, *Coelorinchus mediterraneus*, *Coryphaenoides guentheri* i *Coryphaenoides mediterraneus*, són encara en gran part desconeguts, el coneixement dels seus paràsits poden ser valuosos. Per aquesta raó, l'objectiu de la present tesi és caracteritzar les comunitats de paràsits de *A. rostratus, C. caelorhincus*, *Coe. mediterraneus*, *C. guentheri* i *Cor. Mediterraneus*, la seva variabilitat natural i el seu efecte sobre la salut dels peixos. A més a més, es discutiran la relacion entre el perfil tròfic dels hostes i les comunitats parasítiques.

En el capítol 3 de la present tesi s'examinen les comunitats de paràsits *d'Alepocephalus rostratus* i la seva influència sobre certs marcadors bioquímics i alteracions histològiques. *Alepocephalus rostratus* constitueix el segon peix més important, en termes de biomassa, del talús inferior del mar Balear (Mediterrani nord occidental). L'estudi va revelar vuit espècies de paràsits diferents en aquest hoste: un coccidi, un digeni, un monogeni, un cestode i quatre nematodes. La fauna parasitària

d'*A. rostratus* es troba parcialment dominada per formes larvàries (quatre dels set taxons metazous trobats), que combinats amb una riquesa d'espècies baixa correspon a una fauna parasitària més típica de peixos batipelàgics que demersals. Tetraphyllidea fam. gen. sp. i els nematodes cucullànids, ambdós en estadis larvaris, van ser les espècies predominants. En relació amb la profunditat, es van trobar diferències en les abundàncies dels nematodes Cucullaninae gen. sp. i *Hysterothylacium aduncum*, probablement degudes al canvi de dieta de l'hospedador a major profunditat. Per aquest motiu, aquests podrien ser indicadors per discriminar poblacions d'*A. rostratus* en relació amb la profunditat al Mediterrani nord occidental. Dels marcadors bioquímics examinats, l'activitat acetilcolinesterassa (AChE) i lactat deshidrogenassa (LDH) i la peroxidació de lípids (LP), només LP va mostrar diferències significatives entre profunditats. L'activitat de AChE estava positivament relacionada amb l'abundància de Tetraphyllidea fam. gen. sp., *Anisakis physeteris* i *H. aduncum* i negativament amb la de Cucullaninae gen. sp. L'activitat de LDH va mostrar una relació positiva amb l'abundància dels paràsits *Paracyclocotyla cherbonnieri* i Tetraphyllidea fam. gen. sp. A nivell cito-histològic, es van detectar coccidis als cecs pilòrics amb una prevalença del 90% a Barcelona, però pel que fa a la resta d'òrgans, quasi no es van detectar alteracions. La limitada dieta macroplanctònica d'*A. rostratus*, que manté aquest peix allunyat del fons marí (més que en altres espècies demersals), probablement fa que aquesta espècie sigui menys susceptible als impactes associats amb el sediment, parasitisme inclòs.

En el capítol 4 s'examinen les comunitats de paràsits de *Coelorinchus caelorhincus*, *Coelorinchus mediterraneus*, *Coryphaenoides guentheri* i *Coryphaenoides mediterraneus* del talús mig i inferior del mar Mediterrani. També es van analitzar les alteracions histològiques, les activitats enzimàtiques AChE i LDH, la dieta de les quatre espècies de macrúrids i es van obtenir les variables ambientals (oxigen, salinitat, temperatura i terbolesa). Un total de 11 taxons van ser trobats en les quatre espècies de peixos, sent el copèpode *Hamaticolax resupinus* l'únic paràsit comú entre totes elles. *Coelorinchus mediterraneus*, *Coryphaenoides guentheri* i *Coryphaenoides mediterraneus* van exhibir unes comunitats de paràsits bastant homogènies, especialment en els últims dos peixos. *Coelorinchus mediterraneus* va tenir la major riquesa de taxons paràsits (vuit espècies), mentre que *C. guentheri* i *Cor. Mediterraneus* van tenir cinc i sis, respectivament, i *C. caelorhincus* tres. Gran part dels paràsits van ocórrer en baixa prevalença (<10%), mentre que només tres espècies van ser excepcionalment prevalents i abundants: les larves de Cucullanidae fam. gen. sp. en *C. caelorhincus*; *Lepidapedon desclersae* en *Coe. mediterraneus* i

Hysterothylacium aduncum en ambdues espècies de *Coryphaenoides.* L'abundància del nematode *H. aduncum*, que estava present en tots els hostes excepte *C. caelorhincus*, va incrementar conjuntament amb la temperatura i la profunditat i va esdevenir el paràsit dominant per sota dels 2000 m. La salinitat podria ser un factor important en la distribució de *H. resupinus*. La dieta va ser generalment homogènia entre les espècies estudiades, sent *C. guentheri* més especialitzat en preses bentòniques i suprabentòniques. Els paràsits *H. aduncum* i Tetraphyllidea, i en menor mesura *Raphidascaris* sp., van ser relacionats per les preses més mòbils (nedadores) consumides pels macrúrids (quetògnats, larves de decàpodes i *Boreomysis arctica*). Els paràsits *L. desclersae, Capillostrongyloides morae* i *Otodistomum* sp. van ser relacionats amb preses epibentòniques (ofiúrids, isòpodes i tanaidacis) en *Coe. mediterraneus*. *Coryphaenoides guentheri* era el macrúrid analitzat més petit, amb la fauna parasitària més pobre i major proporció d'estadis larvaris. Es van trobar poques alteracions histopatològiques, sent epiteliocistis la més prevalent. Pocs efectes en la salut dels peixos deguts als paràsits van quedar reflectits a nivell enzimàtic i histopatològic, probablement degut a la poca càrrega parasitària en els hostes. És possible que el paper dels petits macrúrids, especialment de *C. guentheri*, sigui el d'actuar com hostes intermediaris en les xarxes tròfiques del Mediterrani profund.

En el capítol 5 de la present tesi es descriu *Hamaticolax resupinus* n. sp. Els espècimens d'aquesta nova espècie van ser recol·lectats de la cavitat branquial de *Coelorinchus mediterraneus* i *Coryphaenoides mediterraneus* capturatsal Mediterrani occidental a profunditats entre els 1236 i els 1626 m. *Hamaticolax resupinus* n. sp. és molt similar a *H. maleus* Oldewage, 1994, però es diferencia d'aquest per tenir una mida del cos més petita, per tenir una doble somita genital en femelles que és marcadament més ample que les tres somites abdominals i per tenir els marges laterals marcadament convexos. La nova espècie és el segon Bomolochidae trobat en un hoste macrúrid i és el primer en ser trobat per sota dels 1200 m de profunditat. *Hamaticolax resupinus* n. sp. també representa el primer copèpode paràsit trobat en *Coe. mediterraneus* i el tercer en *Cor. mediterraneus* a nivell mundial.

En el capítol 6 es descriu una nova espècie de nematode, *Raphidascaris* (*Raphidascaris*) *macrouri* n. sp. (Anisakidae) a partir de mascles i femelles trobats a l'intestí, ocasionalment també a estómac i cecs pilòrics, de dos peixos macrúrids d'aigües profundes de Barcelona, Mar Mediterrani: *Nezumia aequalis* (Günther) (hoste tipus) and *Trachyrincus scabrus* (Rafinesque). En base a l'examen sota els microscopis òptic i electrònic d'escombratge, la nova espècie mostra característiques morfològiques similars a les de les altres quatre espècies vàlides del subgènere

Raphidascaris Railliet & Henry, 1915, però es diferencia de *Raphidascaris* (*Raphidascaris*) *acus* (Bloch, 1779), *Raphidascaris* (*Raphidascaris*) *lutjani* Olsen, 1952 i *Raphidascaris* (*Raphidascaris*) *mediterraneus* Lèbre & Petter, 1983 en el gran nombre de papil·les precloacals (23–32) i de *Raphidascaris* (*Raphidascaris*) *gigi* Fujita, 1928 en la llargada de les espícules. A més a més, *Raphidascaris* (*Raphidascaris*) *macrouri* n. sp. exhibeix una gran variabilitat en el nombre i distribució de papil·les caudals, fet no reportat en les quatre altres espècies mencionades. Aquesta és la primera espècie d'aquest subgènere trobat en membres de la família Macrouridae. Seqüències de la regió ITS1–5.8S–ITS2 són analitzades i comparades amb espècies de nematodes emparentades i es confirma la uniformitat de *R.* (*R.*) *macrouri* n. sp. entre hostes.

Introduction

INTRODUCTION

1. A sea surrounded by land

The Mediterranean Sea is an enclosed water mass bounded by the coasts of Europe, Africa and Asia. It opens naturally to the Atlantic Ocean at West through the Strait of Gibraltar and to the Sea of Marmara through the Dardanelles at East (IHO, 1953). Since 1869 it is also artificially connected to the Red Sea through the Suez Canal, constructed in the Isthmus of Suez (Egypt). It covers an area of 2,967,000 km^2, which represents the 0.82% of the ocean surface (Eakins and Sharman, 2010). It has an average depth of 1450 m, much lower than the average depth of world oceans (~3850 m) (Danovaro et al., 2010) and a maximum depth of 5121 m in the Matapan-Vavilov Deep (Cartes et al., 2004). It is considered an oligotrophic sea, with low levels of nutrients and productivity (Estrada, 1996).

Two main basins compose the Mediterranean Sea, the Western Basin and the Eastern Basin (Figure 1), separated by de Sicily Channel (max. depth of 400 m). The Eastern Basin presents more geological activity than the western one, whilst the Western Basin has higher primary production, effectively making it richer in species (Cartes et al., 2004). Among the many subdivisions of the Western Basin, the Balearic Sea is enclosed between the Iberian Peninsula and the Balearic Islands.

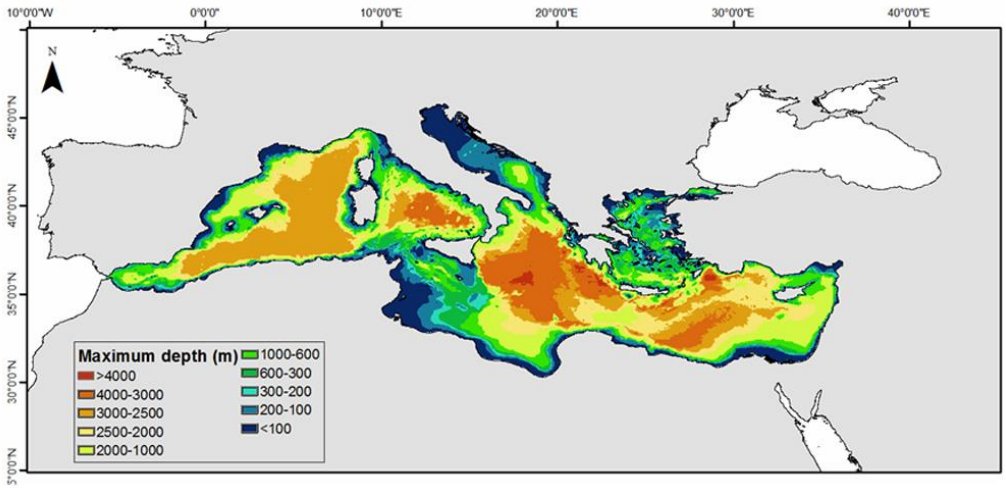


Figure 1: Bathymetric map of the Mediterranean Sea extracted from Coll et al. (2010).

The Mediterranean Basin has been recognized as a global biodiversity 'hotspot' and one of the recommended areas for conservation priority (Coll et al. 2010; Myers et al., 2000). The Mediterranean Sea accounts for an estimated 17000 or more species of marine organisms, corresponding about the 4 to 25% of world marine species (Coll et al. 2010). It is also worth to note that around 25 to 30% of these marine species are

considered endemic, which remarks the rich biodiversity for such a small area (Cuttelod et al., 2008). With a total number of at least 693 vertebrate species, fishes are represented by 650 species, being Teleosteans the best represented group (about 531 species). However, vertebrates are not the richest group in the Mediterranean Sea, this tag belongs to the marine invertebrates, with more than 10902 species of invertebrates (Coll et al. 2010).

The natural resources of the Mediterranean Sea have been exploited on the continental shelf since ancient times by different cultures. The exploitation of the bathyal domain started in the early decades of the 20th century, and have increased in importance since the 1950's, especially on the Ligurian, Catalan and Balearic coasts (Sardà et al., 2004).

1.1. The dark side of the Mediterranean

For a long time, deep sea remained out of reach for exploration. Before the 19th century, humans could only imagine and fear the mysteries lying below the surface of the seas. As Jules Verne wrote in his *Twenty thousand leagues under the sea*, published in 1869:

'*The great depths of the ocean are totally unknown to us. Sounding lines have been unable to reach them. What transpires in those remote abysses? What beings live or can live twelve or fifteen miles below the surface of the sea? What is the make-up of these animals? We can scarcely even guess.*'

Those lines were written by Jules Verne under the light of the beginning of deep sea exploration. There were increasing evidences refuting the 'Azoic Theory' (Forbes, 1844), which depicted depths desolated of life. There were low evidences of life, such as the solitary coral *Caryophyllia smithii* Stokes & Broderip, 1828 sampled by F. Jenkin on a submarine cable from 2184 m or the publication of a deep-water species list by M. Sars and his son G.O. Sars from below 600 m depth (Ramírez-Llodra et al. 2010). The increasing interest on the deep sea produced new discoveries, which ultimately leaded to the *Challenger* expedition and settled the base for an era of deep sea exploration.

Deep sea conforms roughly about the 90% of the oceans and includes the waters and sediments beneath approximately 200 m depth. They represent the largest biome in the world, covering more than 65% of the surface of the earth and including more than 95% of the global biosphere (Danovaro et al., 2010). About the 50% of the deep sea occurs below 3000 m depth (Ramírez-Llodra et al., 2010), with an average depth of

3850 m. Despite the great extension of this habitat, only the surface waters have traditionally been the target of marine research due to their relatively easy access. It has not been until recently that the development of new marine technologies has allowed getting down to the depths of the oceans, exploring, investigating and exploiting its resources.

Deep-sea ecosystems are formed by different kinds of habitats that support unique microbiological and faunal communities. The abiotic characteristics of deep sea are often considered fairly constant, being depth, temperature and light penetration the major affecting factors in most oceans (Angel, 2003; Bucklin et al., 2010). Deep sea is often defined as marine environment below the penetration of sunlight sufficient for photosynthesis, which is often located around 200 m depth. From this point to around 1000 m depth, temperature gradually decreases until it stabilizes at approximately 4^{o}C. Usually, bacterial activity along the water column reduces the amount of organic matter and dissolved oxygen in it with increasing depths. A few tens of meters into the water column immediately above the seafloor the Benthic Boundary Layer (BBL) opens out. This zone has strong gradients in physicochemical properties that develop due to the presence of the seabed itself (Boudreau and Jørgensen, 2001). The intense transport of solutes and suspended particles, both vertical and lateral, produces an increase of planktonic and nektonic biomass near the bottom within this layer (Angel, 1990).

One of the main features of the deep Mediterranean waters is the high stability in its physical parameters compared to the other areas, with largely uniform temperatures at around 12.5–14.5^{o}C from approximately 300 m to the bottom in the Western Basin and 13.5–15.5^{o}C in the Eastern Basin, high salinity (38.4–39.0 PSU) and high oxygen levels (4.0–5.0 mL L^{-1}) (Cartes et al., 2004; Emig and Geistdoerfer, 2004; Hopkins, 1985; Miller et al., 1970). This contrasts with the neighbouring Atlantic Ocean, where temperature decreases until around 4^{o}C and salinity increases with depth (Cartes et al., 2004). In addition, the Mediterranean Sea has limited freshwater inputs (the freshwater deficit is around 0.5 to 0.7 m $year^{-1}$). The Atlantic surface water enters into the Mediterranean through the Strait of Gibraltar (Mariotti et al., 2002) while deep Mediterranean water is forced out into the Atlantic Ocean. The Mediterranean Sea is characterised by its oligotrophic conditions (especially in the Eastern Basin), partially due to the escape of the deep water rich in nutrients into the Atlantic, but mainly because the high thermal stability of the deep Mediterranean waters contributes to a rapid degradation of particulate organic matter. This, in conjunction with a low primary production on the surface, causes low taxes of organic matter accessible to the deep seabed (Danovaro et al., 1999).

Compared to other oceans, the Mediterranean deep-sea fauna is relatively young. During the Messinian (Upper Miocene, 7.246–5.333 Ma), as a result of tectonic movement of the European and African plates, the water flow between the Atlantic Ocean and the Mediterranean Sea was cut off. The resulted isolation of the Mediterranean ended in an almost complete drying of the Mediterranean, what is called the Messinian salinity crisis. It was not until the Lower Pliocene that the water exchange between the two water masses re-started (5.333–3.600 Ma) and introduced Atlantic bathyal fauna to the Mediterranean. From this last event, the Mediterranean Sea obtained its characteristic fauna and started to differentiate from the Atlantic one due to the lack of current exchange of deep water and fauna from the Atlantic Ocean (Cartes et al., 2004).

Deep Mediterranean fauna knowledge was limited until 1980's to the bathymetric range exploited by fishing (down to approximately 800 m). First explorations of the Catalano-Balearic Basin focussed mainly in ecological aspects of the megafaunal assemblages between 1000 and 2300 m, paying special attention to the dominant megafaunal groups (Carrassón and Cartes, 2002; Cartes 1994, 1998; Cartes and Carrassón, 2004; Cartes and Sardà, 1992, 1993; Maynou and Cartes, 2000; Moranta et al., 1998; Stefanescu et al., 1992a, 1992b, 1993). The Mediterranean deep-water assemblages are dominated by decapod crustaceans (about 70 species) and fishes (around 90 species only in the Western Basin) (Cartes et al., 2004). Decapods are a key component of those assemblages in terms of abundance and biomass; their values recorded appear to be higher in the Catalan Sea than the values reported for eutrophic regions in the North Atlantic (Cartes and Sardà, 1992).

2. Importance of the Alepocephalidae and Macrouridae in the Mediterranean Sea

The Alepocephalidae is one of the most important families of deep sea fishes in terms of diversity (Markle and Quéro, 1984). In the Mediterranean Sea, *Alepocephalus rostratus* Risso, 1820 is the only representative of the family Alepocephalidae (Markle and Quéro, 1984) and it is one of the most abundant species between 1000 and 1600 m (Stefanescu et al., 1993).

The Macrouridae, commonly referred as grenadiers or rattails, comprise a diverse group of deep-sea gadiform fishes extended world-wide (Cohen et al., 1990). Eight species of grenadier inhabit in the Mediterranean Sea and are important components of the fish community along the continental margin (D'Onghia et al., 2008; Massutí et al., 1995; Moranta et al., 2007; Stefanescu et al., 1992a, 1993; Tecchio et al., 2011): *Coelorinchus caelorhincus* (Risso, 1810), *Coelorinchus mediterraneus* Iwamoto &

Ungaro, 2002, *Coryphaenoides guentheri* (Vaillant, 1888), *Coryphaenoides mediterraneus* (Giglioli, 1893), *Hymenocephalus italicus* Giglioli, 1884, *Nezumia aequalis* (Günther, 1878), *Nezumia sclerorhynchus* (Valenciennes, 1838) and *Trachyrincus scabrus* (Rafinesque, 1810). Even though they are important in Mediterranean assemblages, biological data of macrourids in this sea is still limited in some aspects, especially in the deepest dwellers.

2.1. *Alepocephalus rostratus*

Alepocephalus rostratus is distributed throughout the Eastern Atlantic Ocean and the Mediterranean Sea (Markle and Quéro, 1984). In the Balearic Sea, it can be found from 984 to 2209 m depth (Stefanescu et al., 1992a). It is the fourth most abundant fish species between 1000 and 1600 and the main one in terms of biomass between 1400 and 1800 m (Stefanescu et al. 1993). At higher depths, both the abundance and biomass of *A. rostratus* is turned down in favor of the deepest macrourids.

The total length of *A. rostratus* ranges from around 4 to 48 cm long in the Mediterranean Sea (Figure 2). Smaller individuals tend to stay at shallower waters, never being found below 1400 m while older individuals are distributed uniformly along the bathymetric distribution of the species (Stefanescu et al., 1992b). Mature individuals can be found throughout the year and, among females, they represent more than 30% of the whole population (Morales-Nin et al., 1996). *Alepocephalus rostratus* is, as most of the rest of the species of the family, a stenophagic predator characterized by a specialized diet on pelagic organisms (Carrassón and Matallanas, 1998; Golovan and Packhorukov, 1980; Marshall and Merrett, 1977; Mauchline and Gordon, 1983). Important components of its diet in the Balearic Sea are the macroplanktonic organisms *Pyrosoma atlanticum* Péron, 1804 and *Chelophyes appendiculata* (Eschscholtz, 1829) that dwell near the bottom (Carrassón and Matallanas, 1998).

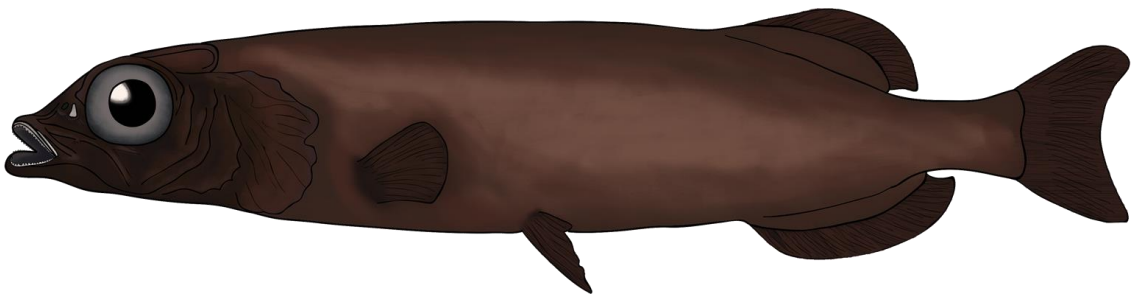


Figure 2. *Alepocephalus rostratus*.

2.2. *Coelorinchus caelorhincus*

Coelorinchus caelorhincus is a non-commercial benthopelagic fish found in the NE Atlantic and the Mediterranean Sea between 90 and 1485 m (Froese and Pauly, 2016; Goren and Garil, 2015). In the Balearic Sea, it inhabits between 300 and 700 m depth (Fernández-Arcaya et al., 2013). It is a small-medium sized fish (Figure 3), with a preanal length (PAL) ranging from 2.1 to 12.3 cm, being males smaller than females (Isajlović et al., 2009; Morey et at., 2003). Specimens of 1–2 year old are concentrated between 300 and 400 m, whilst older ones (up to 10 years) are located at greater depths (Massutí et al., 1995). This fish present spawning females all the year around, except in spring. Concerning the alimentation of *C. caelorhincus*, it preys mainly on polychaetes, followed by amphipods and copepods (Madurell and Cartes, 2006). The inferior position of the mouth of this macrourid may be helpful on foraging slow moving prey, such the polychaetes, with the snout orientated to the substrate (Macpherson, 1979).

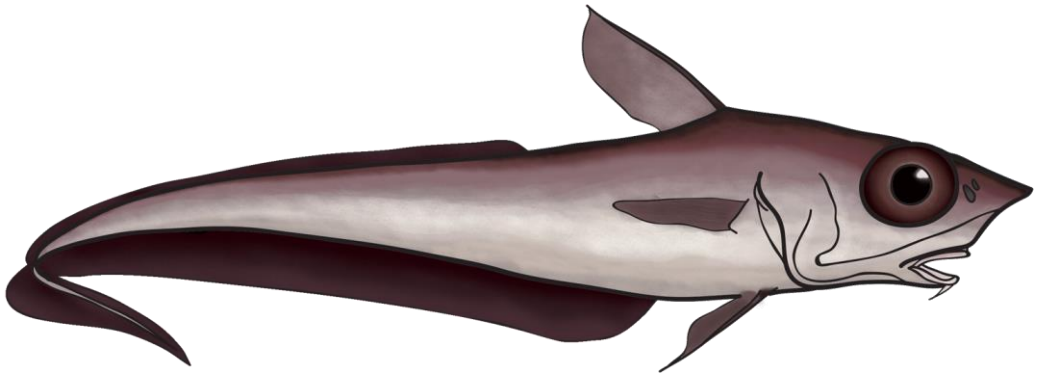


Figure 3. *Coelorinchus caelorhincus*.

2.3. *Coelorinchus mediterraneus*

Originally named *Coelorinchus labiatus* (Köhler, 1896) or *Coelorinchus occa* (Goode & Bean, 1885), the specimens of the Mediterranean were re-described and assigned to a new species, *Coelorinchus mediterraneus*, at the beginning of the 21th century (Iwamoto and Ungaro, 2002). It is the only endemic macrourid of the Mediterranean Sea.

Coelorinchus mediterraneus bathymetric distribution ranges from 1046–2201 m depth, although it is only really abundant between 1200 and 1600 m, being exceptionally rare outside this range (Stefanescu et al., 1992a). Individuals of all sizes are found in the rank of maximum abundance. This fish species PAL ranges from 2.5 to 9 cm, being males especially abundant between 5.5 and 8 cm (Figure 4). In the NW Mediterranean,

Coe. mediterraneus follows a “smaller-deeper” trend (Massutí et al., 1995). This species feeds mostly on polychaetes and gammarid amphipods, and also on copepods as secondary prey (Carrassón and Matallanas, 2002). At reproductive level, there are spawning females mainly all year around except in spring (Fernández-Arcaya et al., 2013).

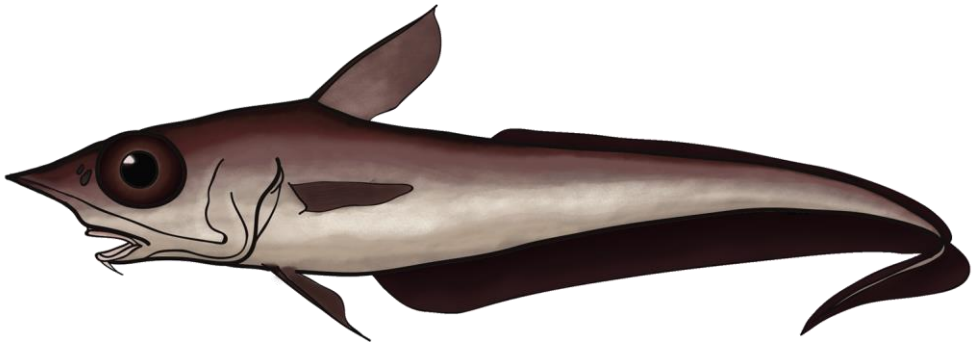


Figure 4. *Coelorinchus mediterraneus*.

2.4. *Coryphaenoides guentheri*

Coryphaenoides guentheri is a bathydemersal fish that inhabits in the North Atlantic waters (Coad and Reist, 2004; Geistdoerfer, 1990) and the Mediterranean Sea (Stefanescu, 1992a, 1993) between 831 and 2830 m depth (Cohen et al., 1990). In the Balearic Sea, *C. guentheri* can be found between 1308 and 2251 m depth (Stefanescu et al., 1992a). It is the second most abundant species and the third in biomass between 2000 and 2200 m depth (Stefanescu et al., 1993). It has a fish PAL ranging from 1.3 to 7.0 cm, being the smallest macrourid found below 1000 m in the area (Figure 5). This species does not show any distribution of individuals of different sizes along depth (Stefanescu et al., 1992b). *Coryphaenoides guentheri* mostly feeds on gammarid amphipods, being cumaceans its secondary prey. Occasionally it also preys on polychaetes, reaching high importance in the diet of this fish between 1425 and 1800 m (Carrassón and Matallanas, 2002).

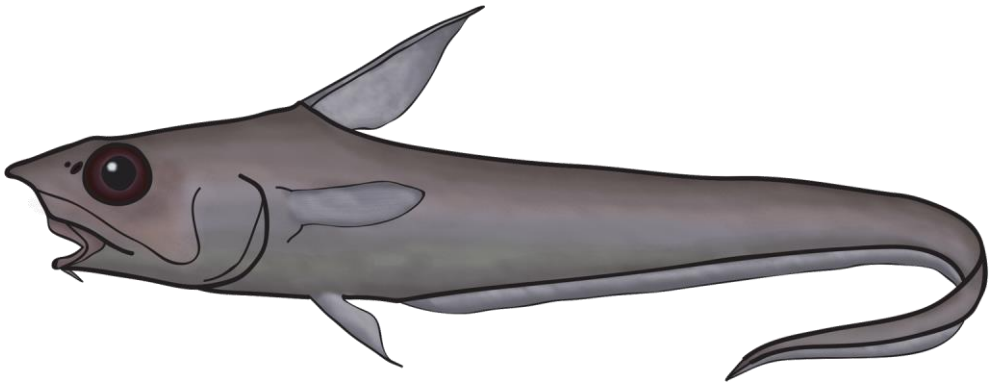


Figure 5. *Coryphaenoides guentheri*.

2.5. *Coryphaenoides mediterraneus*

Previously named *Chalinura mediterranea* and actually accepted as senior synonym, *Cor. mediterraneus* is a bathypelagic species dwelling between 1000 and 4262 m depth (Goren and Garil, 2015) and it is distributed throughout most of the North Atlantic and the Mediterranean Sea (Froese and Pauly, 2016). In the Balearic Sea, it is found between 1308 and 2251 m depth (Stefanescu, 1992a). It is the third most abundant species and the fifth in biomass between 2000 and 2200 m depth (Stefanescu et al., 1993). In the Balearic Sea, *Cor. mediterraneus* is slightly larger than the other co-inhabiting macrourid, *C. guentheri* (Figure 6). Its PAL ranges from 1.5 to 10.2 cm and individuals of different sizes do not show any trend in its bathymetric distribution (Stefanescu et al., 1992b). Mysids and copepods constitute the main prey of *Cor. mediterraneus*, while gammarid amphipods correspond to its secondary prey. Other groups that are occasionally predated by this fish are Natantia decapods and polychaetes (Carrassón and Matallanas, 2002).

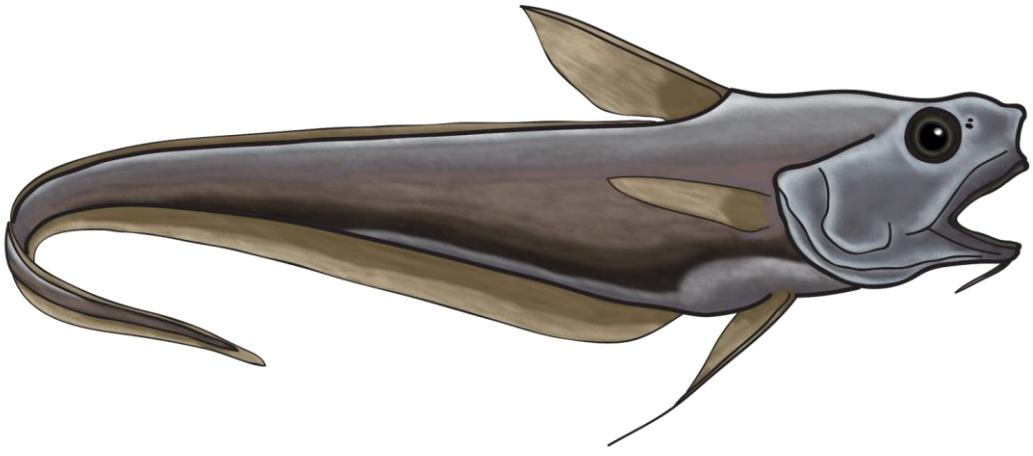


Figure 6. *Coryphaenoides mediterraneus*.

3. Parasites: the unwanted passengers (?)

There are several types of associations between organisms, ranging from beneficial interactions between them (mutualism) to parasitism. In the current text, parasitism is understood as a close interaction between two organisms, the parasite and the host. The former obtains a benefit (usually food) from the latter, which is harmed in some degree. Usually neglected, parasitism is a highly successful life history strategy from the point of view of the parasite. Virtually all species are host to at least one parasite species, probably outnumbering free-living organisms (Poulin and Morand, 2000; Price et al., 1986). Therefore, parasites comprise an important component of the ecosystems of the world, including the marine environment (Marcogliese, 2004).

3.1. Deep sea parasites

Parasitological studies on deep-sea fishes are relatively scarce even nowadays, compared to studies on commercially important species and fishes from coastal areas. The difficulty of access to this environment was and it still is the main obstacle to the research. The first comprehensive survey on deep-sea parasites was conducted by Manter (1934) in Tortugas, Florida. After that, Noble et al. (1972) studied the parasites of *Coryphaenoides rupestris* Gunnerus, 1765 in Karsfjorden (Norway). Campbell et al. (1980) conducted in New York Bight probably one of the most cited researches in deep-sea parasitology. Zubchenko (1981) investigated three macrourid species from the North Atlantic Ocean and Houston and Haedrich (1986) studied in the parasites of the digestive tract of 471 fishes from Newfoundland, Canada. Those studies represent part of the beginning of parasite research in deep sea and the background for the current steadily increasing numbers of research in this field.

Klimpel et al. (2009) summarized the available literature within a deep parasite checklist, reporting 789 parasite taxa from 511 fish species, which represents an average of 1.5 parasite species per fish species. Of these, 8% belonged to Myxozoa, 39% to Digenea, 9% to Monogenea, 14% to Cestoda, 7% to Nematoda, 5% to Acanthocephala, 1% Hirudinea and 17% to the Copepoda. It should be noted that most parasite records from the checklist originate from commercially important fishes. In addition, some areas, such as the North Atlantic Ocean, have been subject of a major number of studies than other ones.

The most predominant groups, the digeneans, cestodes and crustaceans, are especially diverse close to the sea floor and less abundant along the water column. This coincides with the patterns observed by Campbell et al. (1980) and Marcogliese (2002) related to the general scarcity of resources in the meso- and bathypelagic realm.

It is also in accordance with a dominance of adult digeneans and nematodes on the benthodemersal fishes, whereas those fish species from the pelagic realm tend to harbor parasite larval forms (Campbell, 1983; Campbell et al. 1980; Marcogliese, 2002).

Not all parasite families are able to inhabit the deep sea; their parasite fauna is often represented by few of them. Among the approximately 150 known families of Digenea only 17 have been reported into deep sea (Bray, 2004; Bray et al., 1999). Fellodistomidae, Hemiuridae, Lepocreadiidae, Opecoelidae and Accacoeliidae represent some of these encountered families. The genera *Lepidapedon* and *Steringophorus* are particularly frequent in the deep sea (Campbell, 1983). Monogeneans are basically represented by the family Diclidophoridae, although other families such the Microcotylidae have also been recorded. Monogeneans have their range distribution limited down to the lower slope, where fish densities tend to be too low for successful transmission (Campbell et al. 1980). Trypanorhyncha and Tetraphyllidea are prominent orders of the Cestoda, and are encountered as adults in deep-sea elasmobranchs and their larvae can be encountered in teleosts. All reports of adult cestodes in deep-sea teleosts correspond to the Pseudophyllidea (Bray, 2005). Despite the great diversity of nematodes, relatively few are known from deep sea. The families Anisakidae, Capillariidae, Cucullanidae, Cystidicolidae, Philometridae and Rhabdochonidae have been reported from fishes. In the case of anisakids, most of them are encountered as larval forms, which are likely found as adults in cetaceans and pinnipeds (Bray, 2005). Among Acantocephala, adults of the families Echinorhynchidae and Rhadinorhynchidae are the most commonly reported (Bray, 2005). Finally, considering the number of taxa that conform the Copepoda, few families reach the deep sea. According to Boxshall (1998) two families of the order Poecilostomatoidea and five from the Siphonostomatoidea have been reported.

It should be considered after reviewing this little summary that a huge proportion of the deep sea still remains unknown. Virtually, everything known about deep-sea parasites comes from the stages infesting fishes, especially from the largest gadiform families in deep sea such the Macrouridae (Bray, 2005).

3.2. Parasites starring in the ecological film

Historically, parasites received little to no attention in ecological studies. Although they cause certain pathologies and diseases, they only attracted researchers and public interest when human health was compromised or they degraded biological products, thus reducing the production yield and the economic benefits. Moreover, they are often considered by modern society as a nuisance that should be eradicated. Nowadays

society may maintain this point of view. However, since the pioneering works of Holmes (1961, 1962) on the quantitative approach on parasite communities, there are an increasing number of publications on the ecological aspects of these organisms.

First and foremost, the effect of parasites has already been mentioned partially above these lines; parasites have an impact on their hosts. These impacts range from altering the behavior of the host, impose energetic demands, affect the morphology and appearance, reduce fecundity and growth and to cause mortality (Marcogliese, 2004). Those impacts can be important in modeling the ecological structure of the habitat of their hosts. Behavioral alterations may lead to increased vulnerability to predation and enhance transmission to the next host in the life-cycle of the parasite. Therefore, parasites can affect the diet of predators, influencing predator-prey dynamics and competitive interactions between that host and other organisms (Lafferty et al., 2000). Parasites such *Ligula intestinalis* (L.) are known to cause endocrine disruption and effectively castrating its host (Trubiroha et al., 2010). Therefore, parasites can affect sex ratio and mate choice (Minchella and Scott, 1991). These aspects reflect the impacts that parasites exert on host fitness, and thus, play a role in the natural selection of host characteristics. In addition, host population can be significantly impacted if prevalence and abundance of parasites are high (Anderson and May, 1979; May and Anderson, 1979). Moreover, when the host population is dominant in a certain ecosystem, the impacts of parasites may have repercussions through all the food web and ecosystem structure (Dobson and Hudson, 1986; Hagen, 1996; Minchella and Scott, 1991).

Parasites may influence the ecosystems where they inhabit, but this is also true in the opposite way. The marine environment may model certain parasite characteristics. Parasites of marine fish tend to be generalists for intermediate and definitive hosts, probably an adaptation to ensure transmission in a dilute environment (Bush, 1990). Marine parasites tend to be long lived (Campbell, 1983); many marine parasites indiscriminately infect paratenic and transport hosts, possibly as an adaptation to the long food chains and also due to the dilute oceanic environment (Marcogliese, 1995). In addition, marine vertebrates tend to have generalized broad diets that allow prey switching; thus, dietary overlap favors the transmission of parasites. These factors, however, do not contribute to a homogenized distribution of parasites. Fish and invertebrate partition the physical habitat and prey resources in space and time; this contributes to the formation of distinct parasite assemblages among host species (Marcogliese, 2002). All factors together point to a possible advantage for generalist parasites versus those who have high ecological and host restrictions in

disadvantageous environments. Moreover, the costs of generalist species seem to be sufficiently small to maintain it over evolutionary time (Palm and Klimpel, 2007).

The abundance of free-living fauna is also a factor to take into account since the abundance and prevalence of most parasites with complex life-cycles is dependent on it. Therefore, species richness and intensity of infection are highest in epipelagic and benthic zones, where there number of organisms is high; they decrease in vertically-migrating mesopelagics and are lowest in deep non migratory mesopelagic and bathypelagic fishes (Marcogliese, 2002). Mesopelagic and bathypelagic fishes have impoverished parasite communities compared to those of benthic fishes, which possess more diverse adult and larval helminths. The high diversity, density and longevity of benthic invertebrates compared to pelagic ones promote parasite transmission (Campbell, 1983; Campbell et al., 1980). These factors account for the relative high diversity of parasites in benthic fishes, comparable to those found in epipelagic areas (Marcogliese, 2002).

3.3. Parasites as tools in ecological studies

Parasites have proven to be more than enough interesting for themselves, but they keep more information under their sleeves than one can expect at first sight. Due to the nature of parasitism, important data from the host and the environment can be extracted from them.

Different studies in aquatic systems have effectively used parasites as indicators of stocks and their ontogenetic or seasonal migrations (MacKenzie, 2002; MacKenzie et al., 2005 and references therein). The basis behind using parasites as tags is that fish become infected of certain parasites only when they are within the geographic region in which the transmission of those parasites can occur. Therefore it is possible to infer the area where the fish dwelled at some time in its past history (MacKenzie et al., 2005). In a similar way, parasites with complex life-cycles that are transmitted via predator-prey interactions result in excellent indicators of host diet (Lafferty et al., 2008; Marcogliese, 2003). In comparison to stomach contents, trophically transmitted parasite assemblages are the accumulated consequence of long-term feeding by their hosts, reflecting interactions that occurred over weeks or even months (Lafferty et al., 2008; Marcogliese, 2004). In addition, they can reveal the existence of predation on soft-bodied organisms or other quickly digested food items that can be underestimated when only the gut content is taken into account (Lafferty et al., 2008). Moreover, they can indicate ontogenetic shifts in diet, whether hosts feed on more than one trophic level, niche shifts due to competition or other factors, individual feeding specializations

within a population, seasonal changes in diet, and temporary links in a food web such as periodic migrants into a system (Marcogliese, 2003). All these data from the host can be extremely valuable when it is not possible to carry out extensive studies on the biology of fishes or other organisms. This is particularly true in the deep-sea environment, where parasites shine in providing information otherwise difficult to obtain.

In an analogous way to inferring information of the parasite host, they excel as indicators of biodiversity, trophic webs and ecosystem health. Parasites with complex life-cycles require the presence of all necessary hosts to complete their life-cycles and therefore the occurrence of a certain parasite in a certain place implies the presence of all its hosts. The trophic web is suddenly exposed under the light of trophic transmitted parasites since they not only indicate the prey of their host, but also point to its predators (Marcogliese, 2002, 2003). Those same characteristics make parasites valuable as sentinel of ecosystem disturbances, often from anthropogenic origin. Usually, ectoparasites have direct life-cycles and are constantly in contact with the environment; in the course of their evolution they have developed flexibility and resistant to certain natural changes. In front of certain types of environmental stress, many ectoparasites have shown that they can be more tolerant even than their host, usually increasing in abundance and/or prevalence front a debilitated host (MacKenzie et al., 1995 and references therein). However, the type of responses of parasites in front of stress varies depending on the parasite taxa involved and the kind of stress facing against (Lafferty, 1997; Overstreet, 1997; Sures, 2005, 2008). This variability is even more pronounced in parasites with complex life-cycles, most of them endoparasites. There are several factors that influence the response of these parasites, since the effects of stress can be exerted on the parasite itself during its free-living stages (if it has them), or as an indirect impact on the different hosts present throughout the life-cycle (Marcogliese, 2005 and references therein).

The parasite usefulness exposed above is futile without an extensive knowledge of parasites themselves, their biology and their ecology. Without a proper identification of parasites and knowledge about their life-cycles, little information can be extracted. Thus, science basis like parasite taxonomy and ecology would remain as a keystone for a proper application of parasites on an ecosystem.

4. Fish health evaluation

Based on the nature of parasitism itself, parasites can be considered as natural stressors that have detrimental effects on their hosts (Combes, 1996). Therefore, the adverse effects should be reflected at different levels in the fish. Usually the interest

lies in the interactions between parasites and the stress from the environment (reviewed in Marcogliese and Pietrock, 2011) and not in solely the effects of parasites on the markers. In this case, the knowledge on this field still remains very limited (Marcogliese and Pietrock, 2011 and references therein).

Among the tools that can be used in the assessment of fish health, two of them will be briefly introduced: histopathology and the use of biochemical markers.

4.1. Hystopathology

Histo-cytological responses are relatively easy to determine and can be related to health and fitness of the individuals, which made them ideal as biomarkers (Au, 2004). Since parasites can be found in different organs, the responses of the host to the infections may greatly vary, but they are usually localized in the attachment region of the parasite. Among the typical responses of the host it can be found the benign encapsulation of the parasite by host cells, chronic inflammation of the affected area sometimes leading to the formation of granulomes, hyperplasia and necrosis (Feist and Longshaw, 2008). The possible alterations due to parasitism are plenty and it is out of the scope of this thesis to review them, but there is no doubt that histopathology is a useful tool in the assessment of parasitism effects on fish health.

4.2. Biochemical markers

Relatively recent and innovative approaches to fish and ecosystem assessments evaluated the effects of parasites on the biochemical markers (Belló et al., 2000; Dallarés et al., 2014, 2016; Espinal-Carrión and López-López, 2010). Although research in this field is scarce, the basic principle is the same as in the general responses at histological level: since parasites can be considered as natural stressors (Combes, 1996), the effects of parasitism should consequently be reflected at biochemical level. The few works that exist make use of general stress markers such as lipid peroxidation (Belló et al., 2000; Espinal-Carrión and López-López, 2010) and enzymes such as acetylcholinesterase (Gupta and Agarwal, 1985). The results are promising, but there are still few researchers implied in this area.

Never before, researchers have currently so many tools at their disposal to evaluate fish and ecosystem health. Whilst some fields still lack development, researchers should pursue the progress and be encouraged to integrate all different areas for an expanded and more accurate assessment of the environment. This will allow better management and protection of the different ecosystems.

Objectives

OBJECTIVES

The general target of the present thesis is twofold: the primary purpose is to provide information for the first time on the composition and structure of the parasite communities of the alepocephalid *Alepocephalus rostratus* Risso, 1820; and the macrourids *Coelorinchus caelorhincus* Risso, 1810; *Coelorinchus mediterraneus* Iwamoto and Ungaro, 2002; *Coryphaenoides guentheri* (Vaillant, 1888); and *Coryphaenoides mediterraneus* (Giglioli, 1893) in the NW Mediterranean Sea. In addition, we aimed to test whether variations in parasite community structure can be related to fish health and to natural variability (environmental factors, geographically and temporally). To achieve these aims, we shall develop the following objectives:

1) To revise in detail the parasite communities of *Alepocephalus rostratus* along depth in two localities (Barcelona and Balearic Islands).

2) To exhaustively revise the parasite communities of *Coelorinchus caelorhincus, Coelorinchus mediterraneus*, *Coryphaenoides guentheri* and *Coryphaenoides mediterraneus* along depth in two localities (Barcelona and Balearic Islands) and three seasons (spring, summer and autumn).

3) To provide accurate morphological descriptions of those parasites encountered during the revisions of the parasite communities that were poorly described or encountered for the first time in science.

4) To assess the variability of the parasite community structure geographically and in time for all target species and to discuss about their possible use as biological tags.

5) To assess the variability of the parasite community structure due to the effects of temperature, salinity, oxygen concentration and turbidity.

6) To provide new data on the diet composition in all four macrourid species and to relate the different parasite species encountered in those macrourids to the prey items of their hosts in order to uncover trophic links and transmission pathways.

7) To measure the muscular activity of acetylcholinesterase and lactate dehydrogenase in the five target species and the levels of lipid peroxidation in the muscle of *Aleppocephalus rostratus* in order to assess fish health.

8) To characterize of histological alterations in different organs of all five target species in order to evaluate fish health.

9) To assess the impact of parasite populations on fish health at histological and biochemical levels and with different health indexes.

Chapter III

Parasite communities of the deep-sea fish *Alepocephalus rostratus* Risso, 1820 in the Balearic Sea (NW Mediterranean) along the slope and relationships with enzymatic biomarkers and health indicators

Deep-Sea Research I 99 (2015) 65–74

Contents lists available at ScienceDirect

Deep-Sea Research I

journal homepage: www.elsevier.com/locate/dsri

Parasite communities of the deep-sea fish *Alepocephalus rostratus* Risso, 1820 in the Balearic Sea (NW Mediterranean) along the slope and relationships with enzymatic biomarkers and health indicators

D. Pérez-i-García [a], M. Constenla [a], F. Padrós [a], A. Soler-Membrives [a], M. Solé [b], M. Carrassón [a,*]

[a] Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, Bellaterra, 08193 Barcelona, Spain
[b] Institut de Ciencies del Mar (ICM-CSIC), Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain

ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form
12 January 2015
Accepted 19 January 2015
Available online 7 February 2015

Keywords:
Alepocephalus rostratus
Deep-sea fish
Parasites
NW Mediterranean
Geographical indicators
Biomarkers

ABSTRACT

This study examines the parasite communities of *Alepocephalus rostratus* and its influence on some fish biochemical markers and histological alterations. *A. rostratus* constitutes the second most important fish species, in terms of biomass, inhabiting the deep slope of the Catalan Sea (Balearic Sea, NW Mediterranean). The study revealed eight different parasite species in this host: one coccidian, one digenean, one monogenean, one cestode and four nematodes. The parasite fauna of *A. rostratus* was partially dominated by larval forms (four of the seven metazoan taxa found), which combined with low species richness corresponds to a parasite fauna pattern more typical of bathypelagic fish species rather than demersal ones. The larval tetraphyllideans and cucullanid nematodes were the predominant species. In relation to depth, differences in abundance of the nematodes Cucullaninae gen. sp. and *Hysterothylacium aduncum* were found, probably due to the dietary shift in the fish host at greater depth. Thus, Cucullaninae gen. sp. and *H. aduncum* could be regarded as indicators for discriminating populations of *A. rostratus* in relation to depth in NW Mediterranean waters. Of the biochemical markers examined, acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) activities and lipid peroxidation (LP) levels, only LP showed significant differences between depths. A positive relationship was found between AChE activity and Tetraphyllidea fam. gen. sp., *Anisakis physeteris* and *H. aduncum* abundance and a negative one with the abundance of Cucullaninae gen. sp. LDH showed a positive relationship with the abundance of the parasites *Paracyclocotyla cherbonnieri* and Tetraphyllidea fam. gen. sp. At cyto-histological level, coccidians were detected in the pyloric caeca with a prevalence of 90% in Barcelona, but in the rest of organs almost no alterations were detected. The restricted macroplanktonic diet of *A. rostratus*, that maintains it distant from the sea-floor for longer periods than other demersal species, probably makes this species less susceptible to sediment-associated impacts including parasitism.

1. Introduction

Deep-sea ecosystems are known to be a large repository of biodiversity and most of them, due to their extreme conditions and remoteness, remain unexplored (Danovaro et al., 2010; Gage and Tyler, 1991). Among their inhabitants, Alepocephalidae is one of the most important families of deep-sea fishes in terms of diversity (Markle and Quéro, 1984) and they are present in the Indian, Pacific and Atlantic Oceans as well as in the Mediterranean Sea (Merrett et al., 1991; Nelson, 1994). *Alepocephalus rostratus* Risso, 1820 is the only species of Alepocephalidae described in the Mediterranean Sea and is exclusively found in the Western basin (Markle and Quéro, 1984) at depths between 500 and 2250 m with maximum abundance between 1000 and 1300 m (Morales-Nin et al., 1996; Stefanescu et al., 1992). Some biological data about this species are available, mainly about its reproduction and fecundity (Follesa et al., 2007; Golovan and Pakhorukov, 1980; Merrett, 1994) and dietary preferences (Carrassón and Matallanas, 1990, 1998; Macpherson, 1983; Marshall and Merrett, 1977). As far as we are aware no studies on parasites of *A. rostratus* have been carried out

* Corresponding author. Tel.: +34 935814637.
E-mail addresses: David.Perez.Garcia@uab.cat (D. Pérez-i-García), Maria.Constenla@uab.cat (M. Constenla), Francesc.Padros@uab.cat (F. Padrós), Anna.Soler@uab.cat (A. Soler-Membrives), msole@icm.csic.es (M. Solé), Maite.Carrasson@uab.cat (M. Carrassón).

http://dx.doi.org/10.1016/j.dsr.2015.01.009
0967-0637/

yet, and the scarce knowledge of the parasites of this fish species is based on few records of individual parasites species (Bruce et al., 1994; Bray et al., 1999; Dollfus, 1970; Dollfus and Euzet, 1973; Gaevskaya, 1989; Gaevskaya and Aleshkina, 1983; Hartwich and Kilias, 1992).

Parasites can provide valuable information about the community structure and the biology of their hosts (Marcogliese, 2004), otherwise difficult to obtain in these extreme and remote ecosystems. Parasites, which often have complex life-cycles that rely in many cases on trophic interactions, can provide information about the trophic status of hosts since they reflect the presence of intermediate host species and inform about prey–predator links within an ecosystem (Marcogliese, 2004). Due to the strict environmental requirements of parasites, in addition to their host specificity, they have been used as biological tags in fish population studies (MacKenzie, 2002; MacKenzie and Abaunza, 1998).

Deep-sea ecosystems are extremely vulnerable to changes in their conditions. Environmental changes, presence of xenobiotics and harmful biological organisms amongst others may affect the physiological and health conditions of the organisms inhabiting these ecosystems and consequently affecting their performance. Different methodologies have been used to evaluate these conditions as an indirect method to assess the presence of these changes in the environment.

Histopathology is one of the methodologies frequently used to evaluate fish health status (Au, 2004; Feist and Longshaw, 2008) allowing to recognise specific lesional patterns associated to different pathogens (including parasites), pollutants or physicochemical changes in the water. Biochemical parameters (henceforward referred as biomarkers) which are traditionally used in environment pollution monitoring, can also respond to biological and ecological variables (Van der Oost et al., 2003). For instance, some fish studies have highlighted the relationship of biomarkers to diet, sex and depth range variables (Solé et al., 2008, 2010). Parasites can also modify biomarkers responses since they are natural stressors that have detrimental effects on their hosts by definition (Combes, 1996). Moreover, the conjunction of parasitism and an environmental stress might further modify these responses. Nevertheless, the knowledge about the interactions between parasites and environmental stress on general physiological biomarkers is limited (Dallarés et al., 2014; Marcogliese and Pietrock, 2011).

The aim of this study was to comprehensively characterise the parasite communities of *A. rostratus* inhabiting the NW Mediterranean Sea. Parasite component communities were analysed at different depths and locations and in relation to environmental variables in order to evaluate their potential use as indicators for geographical differentiation within this area. The influence of parasitism on the biomarkers: acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) activities, lipid peroxidation (LP) levels and the identification of potential pathological changes at histological level is also included and discussed.

2. Materials and methods

2.1. Sample collection

In July 2010, 82 specimens of *A. rostratus* were collected on board the R/V *García del Cid* in the Balearic Sea (NW Mediterranean) (Fig. 1).

A semi-balloon otter-trawl (OTSB-14) was used for demersal sampling (Merrett and Marshall, 1981). Environmental parameters (temperature in °C, salinity in psu, oxygen concentration in mL/L and turbidity in voltage units) were recorded by casts with a SBE25 CTD profiler at 5 m above the bottom. Samples were obtained from the continental slope of two different localities (Barcelona and Balearic Islands) at depths between 1006 and 1873 m (Table 1). Morphometrical data (total length, TL and total weight, TW) was taken from individual fish immediately on board.

From a subsample of 72 individuals, the right gills, right gonad, a portion of liver (about 5 g) and spleen were fixed in buffered 10% formalin for histological analysis, a portion of the axial muscle (about 5 g) was kept at −20 °C for biochemical analysis and the rest of the fish was frozen at −20 °C for parasitological studies. The rest of captured individuals (10), exclusively devoted to histopathological analyses, were fixed entire in buffered 10% formalin.

2.2. Parasitological examination

In the laboratory, individuals were dissected and liver and gonads weight was recorded to the nearest 0.1 mg. Specimens were examined for metazoan ectoparasites and all organs, including muscles, were examined and carefully checked for endoparasites under the stereomicroscope. Parasites were preserved in ethanol 70%. For identification purposes, platyhelminths were stained in iron acetocarmine, dehydrated through a graded ethanol series, cleared in dimethyl phthalate and mounted in Canada balsam. Nematodes were cleared in glycerine before identification. For those parasites that morphological identification was not enough to reach the genus level, some samples were preserved in ethanol 100% for further molecular identification.

2.2.1. Molecular identification

A pool of 129 larval nematodes of the Family Cucullanidae was used for molecular analysis due to the small size of these parasites and the lack of visible structures. Four nematode individuals of *Anisakis physeteris* and two cestode larvae of the order Tetraphyllidea were also subjected to molecular analysis. DNA from all samples was extracted with Qiagen TM (Valencia, California) DNeasy® Blood & Tissue Kit. For the pool of cucullanid nematodes, the ribosomal 18S region was amplified by PCR using 0.5 μL of the primers ERIB1 (forward: 5′-ACCTGGTTGATCCTGCCAG-3′) and ERIB10 (reverse: 5′-CTTCCGCAGGTTCACCTACGG-3′) (Barta et al., 1997) at 25 μM in 50 μL PCR reaction volume. PCR was performed in an Applied Biosystems Veriti 96 Well thermal cycler under the following conditions: initial denaturation of 95 °C for 3 min, followed by 35 amplification cycles of 94 °C for 50 s (denaturation), 56 °C for 50 s (annealing) and 72 °C for 80 s (extension), ended by and a final incubation of 72 °C for 4 min. For *Anisakis* Type II, the ITS region was amplified by PCR using 0.5 μL of the primers A (forward: 5′-GTCGAATTCGTAGGTGAACCTGCGGAAGGATCA-3′) and B (reverse: 5′-GCCGGATCCGAATCCTGGTTAGTTTCTTTTCCT-3′) (Li et al., 2014) at 100 μM in 50 μL PCR reaction volume. PCR was performed under the following conditions: initial denaturation of 94 °C, 4 min, followed by 30 amplification cycles of 94 °C, 30 s (denaturation), 55 °C, 30 s (annealing), 72 °C, 70 s (extension) and a final incubation of 72 °C for 7 min. For cestodes, the ribosomal 28S region was amplified using 0.5 μL of the primers LSU5 (forward: 5′-TAGGTCGACCCGCTGAAYTTAAGCA-3′) and 1500R (5′-GCTATCCTGAGGGAAACTTCG-3′) (Fyler et al., 2009) at 25 μM in 50 μL PCR reaction volume. PCR was performed under the following conditions: initial denaturation of 94 °C for 1 min, followed by 40 amplification cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min, and a final incubation of 72 °C for 5 min. PCR products were checked on GelRed-stained 1% agarose gels. To identify the parasites, PCR products were purified by the Qiagen TM (Valencia, California) MinElute® PCR Purification Kit and sequenced by Macrogen (Amsterdam, Netherlands).

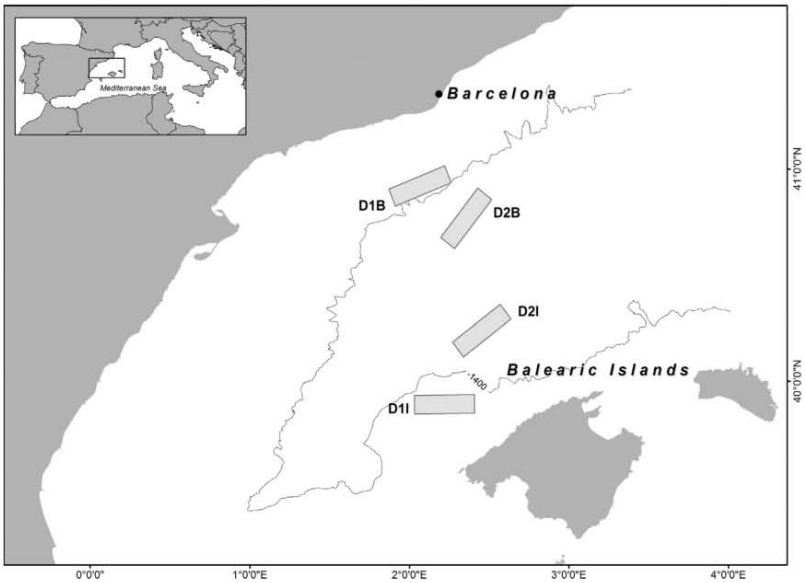


Fig. 1. Study area with sampling sites and depths within the Barcelona and Balearic slopes. Abbreviations for depth/locality categories: D1B=Barcelona at 1000–1400 m, D1I=Mallorca at 1000–1400 m, D2B=Barcelona at 1400–2000 m, D2I=Mallorca at 1400–2000 m.

Table 1
Sampling data for *Alepocephalus rostratus*.

Trawl	Date	Depth (m)	Coordinates		DepthLoc	*n*	*T* (°C)	*S* (psu)	*O* (mL/L)	Turb (V)
			Latitude	Longitude						
A103	08/07/2010	1048	40°58.06N	2°05.30 E	D1B	16	13.07	38.48	4.38	0.08
A104	08/07/2010	1024	40°58.69N	2°01.13 E	D1B	3	13.07	38.48	4.38	0.08
A105	10/07/2010	1268	40°54.35N	2°06.05 E	D1B	7 (6)	13.08	38.48	4.40	0.07
A106	10/07/2010	1308	40°53.84N	2°03.99 E	D1B	4 (3)	13.11	38.48	4.38	0.07
A109	12/07/2010	1743	40°38.39N	2°04.11 E	D2B	8	13.17	38.48	4.36	0.06
A110	12/07/2010	1787	40°30.80N	2°03.68 E	D2B	3	13.17	38.48	4.36	0.06
A111	13/07/2010	1630	40°56.40N	2°30.02 E	D2B	2	13.15	38.48	4.36	0.07
A112	13/07/2010	1422	41°03.92N	2°33.25 E	D2B	8	13.11	38.48	4.36	0.04
A117	19/07/2010	1006	39°52.39N	2°20.26 E	D1I	3	13.06	38.48	4.32	0.07
A118	19/07/2010	1059	39°53.36N	2°18.65 E	D1I	12	13.06	38.48	4.32	0.07
A119	19/07/2010	1231	39°55.16N	2°08.25 E	D1I	7 (1)	13.08	38.48	4.38	0.07
A120	20/07/2010	1604	40°08.53N	2°12.20 E	D2I	4	13.15	38.48	4.33	0.08
A121	20/07/2010	1477	40°05.37N	2°11.32 E	D2I	3	13.13	38.48	4.28	0.06
A122	21/07/2010	1873	40°23.30N	2°40.65 E	D2I	2	13.17	38.48	4.37	0.04

DepthLoc=Spatial groups: D1B=Barcelona at 1000–1400 m, D2B=Barcelona at 1400–2000 m, D1I=Balearic Islands at 1000–1400 m, D2I=Balearic Islands at 1400–2000 m; *n*=number of individuals captured in each trawl, number of individuals entirely devoted to histopathological analyses in brackets. Environmental variables: *T*=temperature, *S*=salinity, *O*=oxygen concentration, Turb=turbidity.

Sequencing primers were the same as for the PCR for 18S region and ITS region but LSU5 and 1200R (reverse: 5′-GCATAGTTCACCATCTTTCGG-3′) were used for sequencing the 28S region. The length of the Cucullaninae gen. sp. 18S rDNA PCR product was 1604 bp. The length of the *A. physeteris* PCR product was 792 bp. The lengths of the 28S rDNA PCR products for Tetraphyllidea fam. gen. sp. were ~1217 bp long. Sequences were aligned using BioEdit 7.0.1 (Hall, 1999), variable sites were checked visually for accuracy and sequences were submitted to GenBank under the following accession numbers: Cucullaninae gen. sp. (KP742487), *A. physeteris* (KP313727) and Tetraphyllidea fam. gen. sp. (KP249697 and KP249698). Uncorrected pairwise distances among sample sequences and GenBank sequences (Supplementary Table 1) were calculated in MEGA v.6 (Tamura et al., 2013).

2.3. Biomarker analysis

Muscle from all fish was used for analysis on acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) activities, while a subsample of 36 fish (9 for each combination of depth and locality) was selected for lipid peroxidation (LP) level contrasts. A portion of muscle of about 0.3 g, was homogenised in ice-cold 50 mM phosphate buffer (pH 7.4) in a 1:5 (w/v) ratio using a Polytron® blender. The homogenate was centrifuged at 10,000 × *g* for 30 min at 4 °C, and the obtained supernatant (S10) stored at −80 °C was used for biochemical analyses. All assays were carried out in triplicate at 25 °C in a 96-well plate using a Tecan Infinite M200 microplate reader. A range of six concentrations of acetylthiocholine iodide (ACT) from 0.05 to 10 mM were used to determine

Vmax and Km of AChE. For AChE determination, the concentration of the substrate (ATC) selected was 1 mM, as described in Solé et al. (2010). AChE activities were assayed according to the principle of Ellman et al. (1961) at 405 nm. For the LDH determination, 150 μL of NADH solution in phosphate buffer was mixed with 25 μL of 1:40 diluted sample and 50 μL of pyruvate solution in each microplate well. LDH activity was given by the amount of pyruvate consumed due to NADH oxidation at 340 nm (Vassault, 1983). Activity of AChE and LDH was expressed in nmol/min/mg prot. Lipid peroxidation (LPO) was determined in muscle using 200 μL of the same supernatant homogenate (S10) and mixed with 650 μL of 1-methyl-2-phenylindole in acetonitrile:methanol (3:1) and 150 μL of 37% HCl. This mixture was incubated at 45 °C for 40 min, the reaction was stopped in ice and further centrifuged at 13,000 rpm × 10 min at 4 °C to precipitate proteins. Absorbance was read at 586 nm versus a standard solution of 1,1,3,3-tetramethoxypropane treated similarly. LP content was expressed as nmol MDA (malondialdehide)/g wet weight.

Total protein content in the S10 fraction was determined by Bradford (1976) method using bovine serum albumin as standard (BSA 0.1–1 mg/mL).

2.4. Histological analysis

Samples from fixed organs and tissues (gills, gonads, liver and spleen) of 72 individuals were processed in paraffin for routine histology. The rest 10 individuals fixed entire in formalin were dissected and the mentioned organs, in addition to the digestive tract, were processed in the same way. Sections (4 μm) were stained with haematoxylin–eosin. Identification of the lesions and parasites present were performed according to their morphology in at least 3 sections of each organ. For coccidian measurements, two sizes were established due to their apparent visual differences on oocysts sizes. Twenty oocysts of each size group were randomly selected and measured under 400 × magnification using a Micro-Comp Integrated Image Analysis System.

2.5. Data analysis

Two fish size categories were assigned: size 1 (S1: $24 \leq TL \leq 34$ cm) and size 2 (S2: $34 < TL \leq 44$ cm). Data were also grouped according to depth (1000–1400 m and 1400–2000 m) and locality (Barcelona and Balearic Islands).

Parasitological terms: prevalence (*P*) and mean abundance (MA) were calculated according to Bush et al. (1997). Species with prevalence > 10% at least in one depth and locality combination (DepthLoc) are henceforth considered common. The species richness (Margalef index) and diversity (Brillouin index) of the metazoan parasites were calculated with PRIMER v6 (Anderson et al., 2008). Fish condition was assessed by condition factor ($K = TW \times 100/TL^3$), hepatosomatic index (HSI = liver weight × 100/TW) and gonadosomatic index in females (GSI = gonad weight × 100/TW).

Four spatial categories (DepthLoc) were established: 1000–1400 m off Barcelona (D1B), 1400–2000 m off Barcelona (D2B), 1000–1400 m off Balearic Islands (D1I) and 1400–2000 m off Balearic Islands (D2I). To comply for normality and homoscedasticity requirements, mean species richness (MSR) data were ln $(x+1)$-transformed and data for total mean abundance (TMA) and fish HSI were ln-transformed prior to General Linear Model analysis (GLM). Possible effects of the factor DepthLoc and host size were tested for infracommunity parasite descriptors (MSR, TMA and mean diversity) and fish condition indices (K, HSI and GSI) using GLM, with *post-hoc* pairwise comparisons.

A Factorial Correspondence Analysis (FCA) was first applied to visualize patterns in parasite abundance in relation to spatial variation (factor "DepthLoc"). A data matrix comprising component population abundance for the six common parasites and the four spatial groups was used in FCA. Based on the parasite coordinates on the two first axis obtained in the FCA, a cluster of dissimilarity was simultaneously performed to define host groups clearly. To gain insight into the suggested differences shown by the FCA, using individual fish as replicate samples, differences in abundance and prevalence among the parasite populations were tested using Generalized Linear Model (GZM) for the factors DepthLoc and fish size (applying log-binomial model for abundance and logistic model for prevalence). When a significant interaction was found between the spatial factor and fish size, both size groups were analysed separately to test spatial differences.

Moreover, a Permutational Multivariate Analysis of Similarity (PERMANOVA) was carried out with PERMANOVA+ for PRIMER v6 (Anderson et al., 2008) to test the null hypothesis of no differences in parasite community structure due to spatial factor using parasite infracommunitiy data. Bray–Curtis similarity matrices derived from square root transformed abundance data was used on the test. Permutation *P*-values were obtained under unrestricted permutation of raw data on 9999 permutations. A similarity percentages analysis (SIMPER) was also carried out with PRIMER v6 (Anderson et al., 2008) to determine the contribution of each parasite species to the similarity/dissimilarity between the four Depth–Locality groups.

Relationships between the abundance of six common parasite species vs the environmental variables were analysed by multivariate Canonical Correspondence Analysis (CCA) (Ter Braak, 1986). CCA related the abundance of each parasite with each environmental variable. Arrows in CCA plots represent explanatory variables and they are proportional in length to their importance on the explained variable. The arrow points in the direction of maximum change in the value of the associated variable.

Biochemical parameters (AChE, LDH and LP) were ln-transformed prior to GLM analyses. GLMs were carried out to test relationships between biomarkers and the infracommunity descriptors (species richness, parasite diversity and total mean abundance) and the abundance of each parasite species, with size host as covariate. Differences in biomarkers responses due to the spatial factor, fish size and fish sex were tested also by GLM. When a significant interaction was found between the spatial factor and fish size, the two size groups were analysed separately to test spatial differences in biomarker responses.

3. Results

3.1. Parasite data

All analysed fish were infected by at least one parasite species. A total of 1925 parasites belonging to seven different taxa was found (Table 2): the digenean *Paraccacladium* sp., the monogenean *Paracyclocotyla cherbonnieri* Dollfus, 1970, the plerocercoid of tetraphyllidean cestodes known collectively as *Scolex pleuronectis* (Müller, 1788); and four nematodes, *Capillostrongyloides morae* (González-Solís et al., 2014), Cucullaninae gen. sp., *A. physeteris* (Baylis, 1923) and *Hysterothylacium aduncum* (Rudolphi, 1802). Voucher material for the new host records (only parasites identified to species) is deposited in the Helminthological Collection of the Universitat Autònoma de Barcelona (UABhc) with the catalogue numbers: *C. morae*: N-8 A1, *A. physeteris*: N108 A1, *H. aduncum*: N201 A1.

The developmental stage and location of the parasites are shown in Table 2. Cucullaninae gen. sp. was the most abundant parasite (MA = 15.00) and the second most prevalent (*P* = 65%) (Fig. 2). These nematodes were small in size (746–881 μm) and they had no visible structures. Nucleotide sequences of the 18S

Table 2
Prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites of *Alepocephalus rostratus* off Barcelona and Balearic Islands along depths. Different letters showed significant differences in spatial assessment.

Depth	1000–1400 m				1400–2000 m					
	Barcelona (D1B) (n=21)				Balearic Islands (D1I) (n=21)		Barcelona (D2B) (n=21)		Balearic Islands (D2I) (n=9)	
	Location	Stage	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD
Digenea										
Paraccacladium sp.	I, Ce	J	24	0.48 ± 1.33^{ab}	14	0.19 ± 0.51^{a}	43	0.95 ± 1.53^{b}	44	0.44 ± 0.53^{ab}
Monogenea										
Paracyclocotyla cherbonnieri	G	A	62	1.29 ± 2.17^{ab}	57	2.33 ± 3.35^{a}	52	1.00 ± 1.27^{b}	44	0.56 ± 0.73^{b}
Cestoda										
Phyllobothriinae gen. sp.	I, Ce	L	100	7.95 ± 7.64^{a}	100	11.24 ± 8.86^{a}	100	7.76 ± 8.38^{a}	100	7.78 ± 5.33^{a}
Nematoda										
Capillostrongyloides morae	S	A	5	0.10 ± 0.44	–	–	–	–	–	–
-Cucullaninae gen. sp- (size 1 hosts)	I, Ce, M	L3	85	41.62 ± 57.86^{a}	80	15.80 ± 26.27^{b}	44	0.89 ± 1.27^{c}	33	0.33 ± 0.58^{c}
-Cucullaninae gen. sp. (size 2 hosts)	I, Ce, M	L3	63	5.63 ± 8.58^{a}	91	27.55 ± 40.99^{b}	33	1.00 ± 1.95^{c}	67	2.00 ± 3.46^{ac}
Anisakis physeteris	M, SW	L3	10	0.10 ± 0.30^{a}	24	0.38 ± 0.74^{a}	14	0.57 ± 1.83^{a}	33	0.33 ± 0.50^{a}
Hysterothylacium aduncum	M, I, AC	L3	5	0.05 ± 0.22^{a}	14	0.33 ± 1.11^{a}	33	1.29 ± 2.30^{b}	33	1.00 ± 2.29^{b}

Abbreviations for locations names: AC=abdominal cavity, Ce=pyloric caeca, G=gills, I=intestine, M=mesentery, S=stomach, SW=stomach wall; abbreviations for stage names: A=adult, J=juvenile, L=larvae (the number at right indicates the larval stage in nematodes).

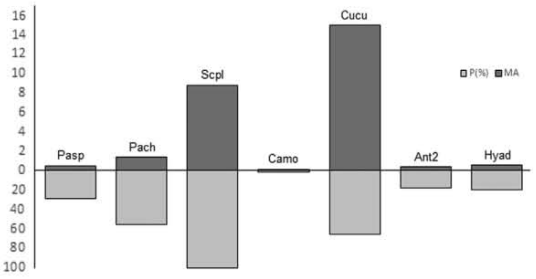


Fig. 2. Prevalence (P%) and mean abundance (MA) of the parasites of *Alepocephalus rostratus*; abbreviations for species names: Pasp=*Paraccacladium* sp., Pach=*Paracyclocotyla cherbonnieri*, Scpl=Tetraphyllidea fam. gen. sp., Camo=*Capillostrongyloides morae*, Cucu=Cucullaninae gen. sp., Ant2=*A. physeteris*, Hyad=*Hysterothilacium aduncum*.

rDNA obtained from the 129 individuals of these nematodes analysed were grouped together within other sequences from Genbank belonging to the Subfamily Cucullaninae (Supplementary Table 1) and showed a maximum p-distance of 3.7% with *Dichelyne pleuronectidis*. The tetraphyllidean cestode was the second parasite species on abundance (MA=8.83) and the most prevalent one (P=100%). The sequences of the two individuals were grouped within the Subfamily Phyllobothriinae Beauchamp, 1905 when they were compared with sequences of tetraphyllideans obtained from GenBank (Supplementary Table 1): There was a 0.1% of intraspecific variation among the Tetraphyllidea fam. gen. sp. sequences, and they were most related with *Calyptrobothrium* sp., *Phyllobothrium squali* and *Crossobothrium longicolle* (~2.1%, ~2.9% and ~3.0% p-distance, respectively). The monogenean *P. cherbonnieri*, that was found attached to the four gill arches and in the inner part of the operculum, was the third most abundant (TMA=1.42) and prevalent (P=56%) parasite species. *Anisakis* Type II showed a 100% of match among other *A. physeteris* sequences obtained from Genbank (Supplementary Table 1). When the sequences were compared to other *Anisakis* sequences, they presented maximum p-distances of 5.4%, 12.0%, 14.3%, 41.3% and 41.7% with *Anisakis brevispiculata*, *Anisakis paggiae*, *Anisakis simplex* C, *A. simplex* and *Anisakis nascettii*, respectively.

Infracommunity data, fish condition indices and the abundance of almost all parasites did not differ significantly between the two sized fish groups (GLM/GZM; $P > 0.05$ in all cases), except for Tetraphyllidea fam. gen. sp. (GZM, $X^2_3 = 10.881$, P=0.001), *H. aduncum* (GZM, $X^2_1 = 12.421$, P=0.0001) and *A. physeteris* (GZM, $X^2_1 = 7.177$, P=0.007). A higher significant parasite load of these three species has been observed in larger fish individuals. Interactions between host size and DepthLoc for parasitological descriptors (MSR, TMA and mean diversity) and condition indices (K, HSI, GSI) were not observed (GLM; $P > 0.05$ in all cases). The total mean abundance of parasites was 26.74 (Table 3) and significant differences were found between depths (GLM, $F_{3,67} = 5.631$, P=0.002), the fish captured off Barcelona and Balearic Islands at 1000–1400 m depth being more infected. Mean species richness and mean diversity showed no significant differences between spatial groups. Fish condition factor (K) was lower in fish off Barcelona at 1000–1400 m in contrast to the other groups (GLM, $F_{3,68} = 5.419$, P=0.002) (Table 3). The highest values for HSI were reached in fish off Balearic Islands at 1000–1400 m (GLM, $F_{3,65} = 13.963$, P=0.0001) while fish GSI did not show site differences.

Fig. 3 presents a plot of the first factorial plane of co-inertia analysis (98.57% of the variance explained) mostly due to the first axis (96%) of the FCA, carried out using component population data from the 6 common parasites found in *A. rostratus*. Shallower depth (1000–1400 m) and greater depth (1400–2000 m) samples had positive and negative values, respectively, along the first axis. Component populations corresponding to three parasite species exhibited the strongest correlations with the first FCA axis. These species were: Cucullaninae gen. sp., Tetraphyllidea fam. gen. sp. and *Paraccacladium* sp. ($Cosine^2$, ranging from 0.7886 to 0.9978). Component populations of *Paraccacladium* sp. ($Cosine^2$=0.6568) showed the strongest correlation with the second FCA axis. Two different groups could be identified depending on their parasite load based on FCA (Fig. 3) and cluster analysis (not shown).

The first group (A in Fig. 3; depth 1000–1400 m; samples D1B and D1I) was characterised by Cucullaninae gen. sp., *P. cherbonnieri* and Tetraphyllidea fam. gen. sp., displaying the highest abundances at these depths (Table 2). Cucullaninae gen. sp. shows interactions between size classes and DepthLoc ($GZM_{(Interaction)}$, $X^2_3 = 19.591$, P=0.0001). Fish showed site differences in Cucullaninae gen. sp. abundance being D1B the location with the highest

Table 3
Means and standard deviations of total length (TL), condition factor (*K*), hepatosomatic index (HSI), gonadosomatic index (only for females) (GSI), mean species richness, total mean abundance, mean diversity, acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) activities and lipid peroxidation (LP) levels for *Alepocephalus rostratus* along the spatial assessment.

Depth	Total	1000–1400 m		1400–2000 m	
Locality		Barcelona (D1B)	Balearic Islands (D1I)	Barcelona (D2B)	Balearic Islands (D2I)
n	72(47)	21(6)	21(17)	21(17)	9(7)
TL (cm)	33.50 ± 4.65	32.55 ± 4.21[a]	34.15 ± 3.93[a]	33.29 ± 5.45[a]	34.69 ± 5.43[a]
K	0.77 ± 0.11	0.70 ± 0.07[a]	0.81 ± 0.07[b]	0.78 ± 0.15[b]	0.80 ± 0.06[b]
HSI	1.03 ± 0.53	0.77 ± 0.30[a]	1.59 ± 0.46[b]	0.72 ± 0.33[a]	1.00 ± 0.34[a]
GSI	1.77 ± 1.27	1.31 ± 0.89[a]	1.73 ± 1.22[a]	1.89 ± 1.39[a]	1.95 ± 1.52[a]
Species richness	7	7	6	6	6
Mean species richness (Margalef)	0.74 ± 0.42	0.60 ± 0.26[a]	0.67 ± 0.39[a]	0.85 ± 0.54[a]	0.97 ± 0.36[a]
Total mean abundance	26.74 ± 34.87	37.76 ± 47.85[a]	36.43 ± 35.92[a]	12.52 ± 13.11[b]	11.56 ± 7.28[b]
Mean diversity (Brillouin Index)	0.53 ± 0.28	0.46 ± 0.22[a]	0.58 ± 0.26[a]	0.56 ± 0.37[a]	0.57 ± 0.16[a]
AChE (nmol/min/mg protein) (size 1 fish)*	25.92 ± 9.69	28.54 ± 9.83[a]	17.17 ± 6.86[b]	29.73 ± 7.06[a]	33.17 ± 6.19[a]
AChE (nmol/min/mg protein)(size 2 fish)*	14.83 ± 5.52	15.04 ± 4.97[a]	16.70 ± 6.26[a]	12.11 ± 2.79[a]	16.57 ± 7.74[a]
LDH (nmol/min/mg protein)	2496 ± 601	2412 ± 532[a]	2482 ± 668[a]	2552 ± 522[a]	2586 ± 811[a]
LP (nmol MDA/g wet weight) °°	9.38 ± 6.24	7.41 ± 5.46[a]	7.32 ± 4.35[a]	12.29 ± 7.64[b]	10.50 ± 6.57[b]

n: sample size, number of females in brackets. Different letters showed significant differences in spatial assessment.
* *n* size1 = 35, *n* size2 = 37. °*n* = 36, subgroups *n* = 9.

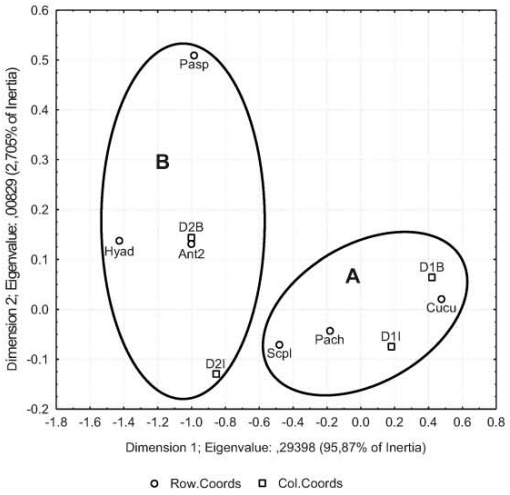


Fig. 3. Plot of the first factorial plane of co-inertia of the FCA on component population data for the 6 common parasite species in *A. rostratus*. FCA groups: *A*=Group A, *B*=Group B; abbreviations for species names are defined in Fig. 2; abbreviations for Depth/locality categories are defined in Fig. 1.

values (GZM, X^2_3=57.862, *P*=0.0001) for size 1 fish and D1I for size 2 fish (GZM, X^2_3=48.761, *P*=0.0001). Cucullaninae gen. sp. also showed site differences in prevalence (GZM, X^2_3=10.937, *P*=0.012) and *P. cherbonnieri* displayed its highest abundance at D1I (GZM, X^2_3=7.856, *P*=0.049).

The second group (B in Fig. 3; depth 1400–2000 m; samples D2B and D2I) was characterised by *H. aduncum*, *Paraccacladium* sp. and *A. physeteris*. All three parasites had their maximum abundance off Barcelona at this depth (Table 2). Fish captured at greater depths had higher abundance of *H. aduncum* (GZM, X^2_3=16.138, *P*=0.0001). Significant differences in abundance were also found for *Paraccacladium* sp. between D2B and D1I (GZM, X^2_1=6.554, *P*=0.010).

The analysis based on the replicate infracommunity samples (PERMANOVA) from all four spatial groups revealed significant effect of the factor DepthLoc on the community structure (*Pseudo-F*(2,94)=3.47; *P*(*perm*)=0.0006; 9941 unique permutations; all *post hoc* site comparisons were only significant when contrasting depths). The similarity/dissimilarity percentages analysis (SIMPER) showed that five parasite species contributed in the similarity/dissimilarities of the infracommunities within the replicate samples of the fourth DepthLoc cases (Table 4). Four of these parasites (Cucullaninae gen. sp., *P. cherbonnieri*, *Paraccacladium* sp. and *H. aduncum*) were also the ones to contribute to the differentiation of the groups *A* and *B* in FCA and GZM analyses.

The CCA relating parasites and environmental variables revealed similar spatial changes found in the FCA. The first two axes explained 98.42% of the analysis variance (Fig. 4). High near-bottom temperature was strongly linked to the abundance of *Paraccacladium* sp. (pasp) and *A. physeteris* (ant2), coinciding with the hauls performed at greater depths (right-lower part of the plot, Fig. 3). The abundance of Cucullaninae gen. sp. was associated with hauls performed at shallower depths (left-lower part of the plot, Fig. 3) and linked with turbidity.

3.2. Biomarkers

No relationship was found between the biomarkers (AChE, LDH and LP) and species richness (SR), the diversity index or total mean abundance (TMA) of parasites and fish sex (GLM; $P > 0.005$ in all cases).

AChE displayed interaction between size and DepthLocs ($GLM_{(Interaction),\ F3,63}$=6.352, *P*=0.001). The lowest significant value of AChE was observed in the small fish (Size 1) from D1I (GLM, $F_{3,29}$=4.968, *P*=0.007) (Table 3). For AChE enzyme Vmax was 32.6 nmol/min/mg prot and Km was 0.1 mM. No differences were found on LDH activity between DepthLocs. No interactions were observed between TL and parasite abundances with all three biomarkers. A significant negative relationship was found between AChE activity and abundances of Tetraphyllidea fam. gen. sp. (GLM, $F_{1,69}$=13.019, *P*=0.001), *A. physeteris* (GLM, $F_{1,69}$=7.514, *P*=0.008) and *H. aduncum* (GLM, $F_{1,69}$=5.225, *P*=0.025); whereas the relationship was positive between this activity and the abundance of Cucullaninae gen. sp. (GLM, $F_{1,69}$=5.824, *P*=0.018). A significant positive relationship was also found between LDH activity and *P. cherbonnieri* (GLM, $F_{1,69}$=4.593, *P*=0.036) and Tetraphyllidea fam. gen. sp. (GLM, $F_{1,69}$=4.656, *P*=0.034). Fish from greater depths present higher LP levels on their muscle than fish captured at shallower depths (GLM, $F_{1,34}$=4.717, *P*=0.037).

Table 4
Mean similarity/dissimilarity for parasite infracommunities sampled within the four spatial groups sampled in *Alepocephalus rostratus* and a breakdown into contributions from individual parasite species.

Species/Spatial Contrast	Similarity				Dissimilarity					
	D1B	D2I	D1I	D2B	D1I–D2B	D1B–D2B	D1I–D2I	D1B–D2I	D2B–D2I	D1B–D1I
Mean similarity/dissimilarity (%)	59.88	56.93	56.30	50.42	53.06	51.35	48.18	46.54	45.07	41.51
Cucullaninae	33.87	7.30	33.30	–	36.56	41.90	37.77	43.81	17.91	43.87
Tetraphyllidea fam. gen. sp.	56.87	76.05	55.85	75.13	21.32	19.14	19.39	17.07	24.86	20.95
Paracyclocotyla cherbonnieri	–	5.53	9.32	9.43	16.53	13.83	16.77	13.25	15.97	18.51
Paraccacladium sp.	–	6.23	–	6.65	9.41	11.53	8.40	10.14	14.58	6.98
Hysterothylacium aduncum	–	–	–	–	9.93	9.34	9.96	9.41	17.60	–
Cumulative contribution (%)	90.74	95.11	98.47	91.21	93.76	95.73	92.28	93.67	90.91	90.29

Only species contributing to more than 10% of the mean community similarity/dissimilarity within spatial groups are included. D1B: 1000–1400 m (Barcelona), D2B: 1400–2000 m (Barcelona), D1I: 1000–1400 m (Balearic Islands), D2I: 1400–2000 m (Balearic Islands).

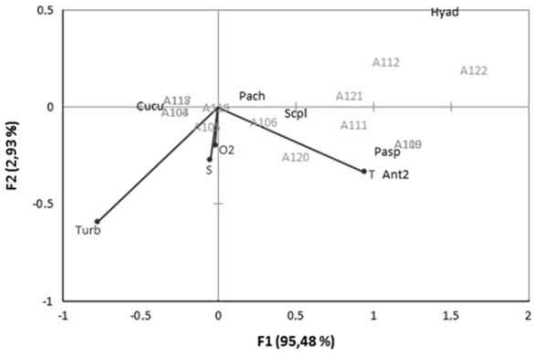


Fig. 4. Plot of the first factorial plane of co-inertia of the CCA for infracommunity data for the 6 common parasites of each trawl and environmental factors. See Fig. 2 for abbreviations of parasite species names. Hauls are depicted in grey (see Table 1). Abbreviations for environmental categories: O_2=oxygen at the benthic boundary layer, *T*=temperature, *S*=salinity, Turb=turbidity.

3.3. Histology

Of the 82 examined fish (10 entirely fixed in formalin plus 72 samples of the rest), only presence of melanomacrophage centres (MMC) were found in the spleen of 4 specimens (5% of prevalence) in low density (< 20% of the splenic parenchyma occupied), except for a single case with high density of MMC (50% of the splenic parenchyma occupied). Only a single parasite (*P. cherbonnieri*) was observed in the gill preparations, probably due to the fact that most parasites are detached during processing due to the characteristics of the haptor of this species. In addition, coccidians were found in nine of the 10 digestive tracts analysed (from the 10 fish fixed entire in formalin). They were located in the epithelium mainly of the pyloric caeca and once in the intestine. Oocysts were spherical, with a thin wall, and possessed four ellipsoidal sporocysts, each containing two sporozoites. According to this morphology they have been identified tentatively within the family Eimeriidae. However, due to the methodology used in this study, their identification at species level was not possible. Although all oocysts showed similar morphology, two different sizes were differentiated suggesting two potential different species: coccidia size 1 (small oocysts: 7.36–9.49 μm) and coccidia size 2 (large oocysts: 11.52–13.93 μm). The smaller coccidia were found in the pyloric caeca and intestine of nine out of ten fish from D1B and D1I whereas larger ones were found only in the pyloric caeca of five out of nine fish from D1B. No other relevant histological alterations were associated to these parasites in the examined organs.

4. Discussion

This is the first comprehensive parasitological study of *A. rostratus* in the Mediterranean Sea that also addresses the relationship between data on parasite communities and biomarkers, biological traits and environmental variables. The parasite fauna infecting *A. rostratus* in the Mediterranean Sea is relatively poor and partially dominated by helminthic larval forms (four of the seven metazoan parasite species found). The fact that *A. rostratus* is not among the top-predators in the deep-sea food webs (results obtained in the stable isotopes studies performed in this species; see Fanelli et al., 2013) agrees with the high abundance of larval and juvenile forms in its parasite fauna. Of the parasite species found in this study, only *P. cherbonnieri* was previously recorded in *A. rostratus* (Dollfus, 1970; Dollfus and Euzet, 1973). Some of the new parasite records were previously reported in other *Alepocephalus* spp in the Atlantic Ocean: *Paraccacladium* sp. in *Alepocephalus bairdii* (see Bray and Gibson, 1977) and *H. aduncum*, *Anisakis* sp. and *Scolex* sp. larvae in *Alepocephalus agassizii* (see Campbell et al., 1980; McDonald and Margolis, 1995). The last three species, all generalist parasites, are reported in many fish species worldwide (Gibson et al., 2005).

Parasite prevalence in benthic ichthyofaunas is more pronounced than in either mesopelagic or bathypelagic species (Campbell et al., 1980). *A. rostratus* is not an exception since all fish analysed in this study had at least one parasite individual. However, its parasite richness is low compared with some other deep-sea demersal fishes such as macrourids and morids in the Atlantic Ocean and Mediterranean Sea (Campbell et al., 1980; Dallarés et al., 2014; Klimpel et al., 2006), *A. rostratus* exhibiting values more typical for meso- and bathypelagic fish hosts (around two to seven parasite species in bathypelagic fishes versus twenty in demersal fishes) (Klimpel et al., 2006, 2010; Marcogliese, 2002). In all likelihood, a specialised diet based on a narrow range of mobile macroplanktonic (even some benthic) organisms (Carrassón and Matallanas, 1998) could be the reason for the low parasite richness observed in *A. rostratus*.

Species of some genera of cucullanids, such as *Cucullanus* spp., utilise calanoid and cyclopoid copepods as intermediate hosts (Køie, 2000). The greater abundance of Cucullaninae gen. sp. at shallower depths may be due to a higher predation of calanoid copepods by *A. rostratus* at 1000–1400 m depth as was observed by Carrassón and Matallanas (1998). Turbidity promotes an increase of the zooplanktonic invertebrate communities (as calanoid copepods) in these waters (Cartes et al., 2013) and was linked to the higher infection rates of Cucullaninae gen. sp. in the CCA. According to present results, Cucullaninae gen. sp., the most abundant parasite taxon, could be linked to calanoid copepods as possible intermediate host. *A. rostratus* probably also acts as intermediate host for Cucullaninae gen. sp. L3 before its transmission to the final host.

The order Tetraphyllidea is one of the three orders of cestodes that predominate in deep-sea fishes (Klimpel et al., 2009) and the adult individuals of this order are known to have elasmobranchs as final hosts (Euzet, 1994). The fact that all *A. rostratus* harboured the cestode larvae Phyllobothriinae gen. sp. with relatively high intensity could indicate a strong trophic link between its definitive hosts and *A. rostratus*, which may act as obligate intermediate host. The shark *Centroscymnus coelolepis* is known to include *Alepocephalus* sp. in its diet (du Buit, 1983) and Caira and Pickering (2013) found adult phyllobothriids in the shark *C. coelolepis* off Azores. Further research on *A. rostratus* predators and their parasites is needed to better understand the role of this fish in Phyllobothriinae gen. sp. transmission.

H. aduncum has been recorded in nine fish species of deep Mediterranean waters (unpublished data of the ANTROMARE project), and it is one of the most abundant parasites in the northern hemisphere (Køie, 1993). This nematode, which was frequently found in *A. rostratus* at greater depths, could arrive to the host through prey-items such as cumaceans and mysids. These crustaceans, that increase their importance in the diet of *A. rostratus* at this depth (Carrassón and Matallanas, 1998), are described as potential first intermediate hosts of this nematode (Klimpel and Rückert, 2005; Køie, 1993; Marcogliese, 1996). The trend towards the increase of the prevalence and abundance of *H. aduncum* with depth observed in this host, coincides with a similar trend in *Bathypterois mediterraneus* in the same area, that reaches maximum values of infection at the maximum depth of these waters (2000–2200 m) (Mateu et al., 2014).

The digenean life-cycle is complex, and in the case of the accacoeliid *Paraccacladium* sp. includes cnidarians and ctenophores, among other planktonic animals, as possible intermediate hosts (Bray and Gibson, 1977). Gelatinous plankton, particularly siphonophores as *Chelophyes appendiculata* and *Heteropyramis maculata*, are frequently predated by *A. rostratus* (Carrassón and Matallanas, 1998), and could act as a means of transmission of the parasite to this host. The higher abundance of *C. appendiculata* found at depths of 1500–2132 m (Cartes et al., 2013) could explain the increase of the digenean infection at greater depths. The environmental analysis performed suggests that *Paraccacladium* sp. is linked to deeper (1400–2200 m) hauls, with maxima of near-bottom temperature (as observed in CCA). This is consistent with higher temperature found at the lower slope (1500–2132 m) compared with that observed in the middle slope (1010–1282 m) in the Balearic basin (Cartes et al., 2013).

Fish coccidia are common parasites in freshwater and marine environments around the world (Molnár, 2006). Despite the low number of histological samples of digestive tract analyzed, the high prevalence (90%) of coccidians in *A. rostratus* predicts that these may be important parasites in this fish species. Within the family Eimeriidae that inhabit predominantly the digestive tract of infected fish, *Eimeria* and *Goussia* are among the most diverse genera (Lom and Dyková, 1992). Since species of these genera have already been reported in deep-sea fish species (Khan, 2009; Lom and Dyková, 1992), and taking into account the morphology observed in *A. rostratus*, *Eimeria* or *Goussia* probably parasitize this fish.

The PERMANOVA test also confirmed a clear differentiation between two habitat groups (shallower and greater depths) defined at the parasite infracommunity level with higher dissimilarities found between different depths. Probably, the parasite abundance at community level reflects the well-known dietary shift of *A. rostratus* at greater depths (Carrassón and Matallanas, 1998; Fanelli et al., 2013). All analyses revealed a clear influence of depth on parasites mainly explained by the abundance of Cucullaninae gen. sp., *H. aduncum*, but also *P. cherbonnieri* and *Paraccacladium* sp. All these parasites could be useful as depth discriminating species for *A. rostratus* in the NW Mediterranean.

Within natural stressors, the effects of parasitism on biomarkers is poorly documented in the literature, although parasites have proved to alter their hosts biomarker responses (Marcogliese and Pietrock, 2011 and references therein). In the case of general physiological markers such as AChE and LDH activities and LP levels, only a few studies have addressed the effects of parasitism on these responses; these frequently reveal controversial outcomes. For instance, while some authors have found a positive relationship between AChE and parasitism (Gupta and Agarwal, 1985), other studies revealed no relationship between them (Podolska and Napierska, 2008). Probably the nature of the parasite and its host and how they interact may be responsible of these differences. Several stressing factors inhibit AChE activity (e.g. pesticides, heavy metals, etc) but also fish size can influence this enzymatic activity (usually larger fish have lower AChE activity than smaller ones) (Koenig and Solé, 2014). In the case of parasitism, the negative relationship found between AChE activity and the parasites Tetraphyllidea fam. gen. sp., *A. physeteris* and *H. aduncum* could correspond to an expected response of this enzyme to stress. However, the positive relationship between Cucullaninae gen. sp. and AChE activity in present study is more similar to that found in Gupta and Agarwal (1985), in which hepatic AChE activity increased due to parasitism. The other enzymatic biomarker used in this study, LDH, showed higher activity when higher numbers of the parasites *P. cherbonnieri* and Tetraphyllidea fam. gen. sp. were present which it might be due to the additional energy support needed under parasite stress (Almeida et al., 2010). The effect marker, LP levels, increased in some host species due to parasite infection (Belló et al., 2000), but this relationship was not seen in *A. rostratus*. By contrast, a relationship with depth was found. In hauls from 1400 to 2000 m depth, where the values of LP are higher, *A. rostratus* eats more osteichthyes than at shallower depths (Carrassón and Matallanas, 1998). This change towards a diet on prey enriched in polyunsaturated fatty acids could explain the increase of LP levels, since these lipids make the fish more prone to suffer LP (Mourente et al., 2000).

In addition to the former biomarkers, histological alterations also respond to different kinds of stress. Surprisingly, no significant histopathological alterations/lesions in the targeted organs (mainly gills, liver and spleen) have been detected in *A. rostratus* in this study. This is an unexpected observation in contrast to the data observed in other species living at the same depth such as *Mora moro* (Dallarés et al., 2014), *Phycis blennoides* or *Lepidion lepidion* (unpublished data of the ANTROMARE project) which showed higher signs of alteration and parasite-derived effects. This lack of alterations could be associated to the particular biological and physiological characteristics of individuals of *Alepocephalus* spp., e.g. their low protein content versus their high lipid and water content compared to the other fish species (Økland et al., 2005) and their specialized diet on gelatinous plankton, mostly pyrosomids and syphonophores (Carrassón and Matallanas, 1998). This restricted macroplanktonic diet probably maintains *A. rostratus* more distant from the sea-floor for longer periods than the diet habits of other demersal species thus making this species less susceptible to sediment-associated impacts including parasitism.

Acknowledgements

We thank three anonymous reviewers for their comments and suggestions. This study was supported by Spanish Science and Innovation Ministry project ANTROMARE (CTM2009-12214-C02-02). We thank all participants in the ANTROMARE 1 cruise and the staff of the Department of Animal Biology, Vegetal Biology and Ecology of the Universitat Autònoma de Barcelona (Spain) for their assistance during

the oceanographic campaign and in the laboratory work. We are grateful to Dr. A. Kostadinova for her assistance with digenean identification, Dr. Montero and Dr. A. Pérez-del-Olmo for their assistance with some nematode identification, and X. Munill for help with preparing Fig. 1. D. Pérez-i-García benefits from a PIF PhD student grant of the Universitat Autònoma de Barcelona.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dsr.2015.01.009.

References

Almeida, J.R., Oliveira, C., Gravato, C., Guilhermino, L., 2010. Linking behavioural alterations with biomarkers responses in the European seabass *Dicentrarchus labrax* L. exposed to the organophosphate pesticide fenitrothion. Ecotoxicology 19, 1369–1381. http://dx.doi.org/10.1007/s10646-010-0523-y.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E Ltd, Plymouth.

Au, D.W.T., 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. Mar. Pollut. Bull. 48, 817–834. http://dx.doi.org/10.1016/j.marpolbul.2004.02.032.

Barta, J.R., Martin, D.S., Liberator, P.A., Dashkevicz, M., et al., 1997. Phylogenetic relationships among eight *Eimeria* species infecting domestic fowl inferred using complete small subunit ribosomal DNA sequences. J. Parasitol. 83, 262–271.

Belló, A.R.R., Fortes, E., Belló-Klein, A., Belló, A.A., Llesuy, S.F., Robaldo, R.B., 2000. Lipid peroxidation induced by *Clinostomum detruncatum* in muscle of the freshwater fish *Rhamdia quelen*. Dis. Aquat. Org. 42, 233–236. http://dx.doi.org/10.3354/dao042233.

Bradford, M.D., 1976. Rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. http://dx.doi.org/10.1006/abio.1976.9999.

Bray, R.A., Gibson, D.I., 1977. The Accacoeliidae (Digenea) of fishes from the north-east Atlantic. Bull. Br. Mus. Nat. Hist. (Zool.) 31, 51–99.

Bray, R.A., Littlewood, D.T.J., Herniou, E.A., Williams, B., Henderson, R.E., 1999. Digenean parasites of deep-sea teleosts: a review and case studies of intra-generic phylogenies. Parasitology 119, S125–S144.

Bruce, N.L., Adlard, R.D., Cannon, L.R.G., 1994. Synoptic checklist of ascaridoid parasites (Nematoda) from fish hosts. Invertebr. Taxon. 8, 583–674. http://dx.doi.org/10.1071/IT9940583.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 83 (4), 575–583. http://dx.doi.org/10.2307/3284227.

Caira, J.N., Pickering, M., 2013. Cestodes from deep-water squaliform sharks in the Azores. Deep Sea Res. II 98, 170–177. http://dx.doi.org/10.1016/j.dsr2.2013.08.008.

Campbell, R.C., Haedrich, R.L., Munroe, T.A., 1980. Parasitism and ecological relationships among deep-sea benthic fishes. Mar. Biol. 57, 301–313 http://dx.doi.org/10.1007/BF00387573.

Carrassón, M., Matallanas, J., 1990. Preliminary data about the feeding habits of some deep-sea Mediterranean fishes. J. Fish Biol. 36, 461–463. http://dx.doi.org/10.1111/j.1095-8649.1990.tb05625.x.

Carrassón, M., Matallanas, J., 1998. Feeding habits of *Alepocephalus rostratus* (Pisces: Alepocephalidae) in the western Mediterranean Sea. J. Mar. Biol. Assoc. UK 78, 1295–1306.

Cartes, J.E., Fanelli, E., López-Pérez, C., Lebrato, M., 2013. Deep-sea macroplankton distribution (at 400 to 2300 m) in the northwestern Mediterranean in relation to environmental factors. J. Mar. Syst. 113-114, 75–87. http://dx.doi.org/10.1016/j.jmarsys.2012.12.012.

Combes, C., 1996. Parasites, biodiversity and ecosystem stability. Biodivers. Conserv. 5, 953–962.

Dallarés, S., Constenla, M., Padrós, F., Cartes, J.E., Solé, M., Carrassón, M., 2014. Parasites of the deep-sea fish *Mora moro* (Risso, 1810) from the NW Mediterranean Sea and relationship with fish diet and enzymatic biomarkers. Deep Sea Res. I , http://dx.doi.org/10.1016/j.dsr.2014.07.001.

Danovaro, R., Batista-Company, J., Corinaldesi, C., et al., 2010. Deep-sea biodiversity in the Mediterranean Sea: the known, the unknown, and the unknowable. PLoS One 5, e11832. http://dx.doi.org/10.1371/journal.pone.0011832.

Dollfus, R.P., 1970. Campagne d'essais du "Jean Charcot" (3–8 décembre 1968). 4. D'un Trématode monogénétique trouvé libre en dix exemplaires parmi les matériaux récoltés par un chalutage au large de la Bretagne. Bull. Mus. Natl. Hist. Nat. 41, 1522–1530.

Dollfus, R.P., Euzet, L., 1973. Nouvelles données sur *Paracyclocotyla cherbonnieri* Dollfus, 1970 (Monogenea). Bull. Mus. Natl. Hist. Nat. (Zool.) 101, 815–819.

du Buit, M.H., 1983. Note sur l'alimentation des selaciens de profondeur a l'ouest des Iles Faeroe. Bull. Soc. Sci. Bretagne 55, 55–61.

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95. http://dx.doi.org/10.1016/0006-2952(61)90145-9.

Euzet, L., 1994. Order Tetraphyllidea Carus, 1863. In: Khalil, L.F., Jones, A., Bray, R.A. (Eds.), Keys to the Cestode Parasites of Vertebrates. CAB International, Wallingford, pp. 149–194.

Fanelli, E., Papiol, V., Cartes, J.E., Rumolo, P., López-Pérez, C., 2013. Trophic webs of Deep-sea megafauna on mainland and insular slopes of the NW Mediterranean: a comparison by stable isotope analysis. Mar. Ecol. Prog. Ser. 490, 199–221. http://dx.doi.org/10.3354/meps10430.

Feist, S., Longshaw, M., 2008. Histopathology of fish parasite infections: importance for populations. J. Fish Biol. 73, 2143–2160. http://dx.doi.org/10.1111/j.1095-8649.2008.02060.x.

Follesa, M.C., Porcu, C., Cabiddu, S., Davini, M.A., Sabatini, A., Cau, A., 2007. First observations on the reproduction of *Alepocephalus rostratus* Risso, 1820 (Osteichthyes, Alepocephalidae) from the Sardinian Channel (Central-Western Mediterranean). Mar. Ecol. 28, 75–81.

Fyler, C.A., Caira, J.N., Jensen, K., 2009. Five new species of *Acanthobothrium* (Cestoda: Tetraphyllidea) from an unusual species of *Himantura* (Rajiformes: Dasyatidae) from northern Australia. Folia Parasitol. 56, 107–128.

Gaevskaya, A.V., 1989. Revision of the genus *Lecithophyllum* (Trematoda, Lecithasteridae). Zool. Zh. 68 (9), 130–134.

Gaevskaya, A.V., Aleshkina, L.D., 1983. New data on the trematodes from fishes of the Atlantic coast of Africa. Parazitologiya 17, 12–17.

Gage, J.D., Tyler, P.A., 1991. Deep-sea Biology: A Natural History of Organisms at the Deep-sea Floor. Cambridge University Press, Cambridge.

Gibson, D.I., Bray, R.A., Harris, E.A., 2005. Host-Parasite Database of the Natural History Museum, London. WWW Page, ⟨http://www.nhm.ac.uk/research-curation/projects/host-parasites⟩.

Golovan, G.O., Pakhorukov, N.P., 1980. New data on the ecology and morphometry of *Alepocephalus rostratus* (Alepocephalidae). J. Ichthyol. 20, 77–84.

Gupta, A.K., Agarwal, S.M., 1985. Physiology of host-parasite relationships of *Pallisentis nagpurensis* and *Senga visakhapatnamensis*, parasitizing liver of *Colisa lalia* and intestine of *Channa punctatus*. Curr. Sci. 54 (17), 866–869.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hartwich, G., Kilias, I., 1992. Die Typen der Cercomeromorphae (Plathelminthes) des Zoologischen Museums in Berlin. Mitt. Zool. Mus. Berlin 68, 209–248.

Khan, R.A., 2009. Parasites causing disease in wild and cultured fish in Newfoundland. Icel. Agric. Sci. 22, 29–35.

Klimpel, S., Rückert, S., 2005. Life cycle of *Hysterothylacium aduncum* to become the most abundant anisakid fish nematode in the North Sea. Parasitol. Res. 97, 141–149.

Klimpel, S., Palm, H.W., Busch, M.W., Kellermanns, E., Rückert, S., 2006. Fish parasites in the Arctic deep-sea: poor diversity in pelagic fish species vs. heavy parasite load in a demersal fish. Deep Sea Res. I 53, 1167–1181. http://dx.doi.org/10.1016/j.dsr.2006.05.009.

Klimpel, S., Busch, M.W., Kellermanns, E., Kleinertz, S., Palm, H.W., 2009. Metazoan Deep-Sea Fish Parasites. Acta Biologica Benrodis, Supplementband I I. Heinrich-Heine University Düsseldorf.

Klimpel, S., Busch, M.W., Sutton, T., Palm, H.W., 2010. Meso- and bathy-pelagic fish parasites at the Mid-Atlantic Ridge (MAR): low host specificity and restricted parasite diversity. Deep Sea Res. I 57 (4), 596–603. http://dx.doi.org/10.1016/j.dsr.2010.01.002.

Koenig, S., Solé, M., 2014. Muscular cholinesterase and lactate dehydrogenase activities in deep-sea fish from the NW Mediterranean. Mar. Environ. Res. 94, 16–23. http://dx.doi.org/10.1016/j.marenvres.2013.11.003.

Køie, M., 1993. Aspects of the life-cycle and morphology of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). Can. J. Zool. 71, 1289–1296. http://dx.doi.org/10.1139/z93-178.

Køie, M., 2000. Life cycle and seasonal dynamics of *Cucullanus cirratus* O.F. Müller, 1777 (Nematoda, Ascaridia, Seuratoidea, Cucullanidae) in Atlantic cod, *Gadus morhua L.* Can. J. Zool. 78, 182–190. http://dx.doi.org/10.1139/cjz-78-2-182.

Li, L., Du, L.Q., Xu, Z., Guo, Y.N., Wang, S.X., Zhang, L.P., 2014. Morphological variability and molecular characterisation of Dichelyne (Cucullanellus)pleuronectidis (Yamaguti, 1935) (Ascaridida: Cucullanidae) from the flatfish Pleuronichthys cornutus (Temminck & Schlegel) (Pleuronectiformes: Pleuronectidae) in the East China Sea. Syst. Parasit 87, 87–98.

Lom, J., Dyková, I., 1992. Protozoan parasites of fishes. Developments in Aquaculture and Fisheries Science, vol. 26. Elsevier Science Publishers BV, Amsterdam.

MacKenzie, K., 2002. Parasites as biological tags in population studies of marine organisms: an update. Parasitology 124 (7), S153–S163. http://dx.doi.org/10.1017/S0031182002001518.

MacKenzie, K., Abaunza, P., 1998. Parasites as biological tags for stock discrimination of marine fish: a guide to procedures and methods. Fish. Res. 38 (1), 45–56. http://dx.doi.org/10.1016/S0165-7836(98)00116-7.

Macpherson, E., 1983. Ecología trófica de peces en las costas de Namibia I. Hábitos alimentarios. Reseñ. Exp. Cient. (Supl Inv Pesq) 11, 81–137.

Marcogliese, D.J., 1996. Larval parasitic nematodes infecting marine crustaceans in eastern Canada, 3. *Hysterothylacium aduncum*. J. Helminthol. Soc. Wash. 63, 1–18.

Marcogliese, D.J., 2002. Food webs and the transmission of parasites to marine fish. Parasitology 124 (7), S83–S99. http://dx.doi.org/10.1017/S003118200200149X.

Marcogliese, D.J., 2004. Parasites: small players with crucial roles in the ecological theater. EcoHealth 1, 151–164. http://dx.doi.org/10.1007/s10393-004-0028-3.

Marcogliese, D.J., Pietrock, M., 2011. Combined effects of parasites and contaminants on animal health: parasites do matter. Trends Parasitol. 27, 123–130.

Markle, D.F., Quéro, J.C., 1984. Family Alepocephalidae. In: Whitehead, P.J.P., Bauchot, M.L., Hureau, J.C., Nielsen, J., Tortonese, E. (Eds.), Fishes of the North-eastern Atlantic and the Mediterranean. UNESCO, Paris, pp. 228–253.
Marshall, N.B., Merrett, N.R., 1977. The existence of benthopelagic fauna in the deep-sea. Deep Sea Res. 24, 483–497.
Mateu, P., Montero, F.E., Carrassón, M., 2014. Geographical variation in metazoan parasites of the deep-sea fish *Bathypterois mediterraneus* Bauchot, 1962 (Osteichthyes: Ipnopidae) from the Western Mediterranean. Deep Sea Res. I 87, 24–29. http://dx.doi.org/10.1016/j.dsr.2014.01.008.
McDonald, T.E., Margolis, L., 1995. (I–IV). Synopsis of the Parasites of Fishes of Canada: Supplement (1978–1993), vol. 122. Canadian Special Publication of Fisheries and Aquatic Sciences, Ottawa, pp. 1–265.
Merrett, N.R., 1994. Reproduction in the North Atlantic oceanic ichthyofauna and the relationship between fecundity and species' sizes. Environ. Biol. Fishes 40, 207–245. http://dx.doi.org/10.1007/BF00023814.
Merrett, N.R., Marshall, N.B., 1981. Observations on the ecology of deep-sea bottom living fishes collected off northwest Africa (08°–27°N). Prog. Oceanogr. 9, 185–244. http://dx.doi.org/10.1016/0079–6611(80)90002-6.
Merrett, N.R., Gordon, J.D.M., Stehmann, M., Haedrich, R.L., 1991. Deep demersal fish assemblage structure in the Porcupine Seabight (eastern North Atlantic): slope sampling by three different trawls compared. J. Mar. Biol. Assoc. UK 71 (2), 329–358.
Molnár, K., 2006. Phylum Apicomplexa. In: Woo, P.T.K. (Ed.), Fish Diseases and Disorders, Volume 1: Protozoan and Metazoan Infections, second ed CAB International, Wallingford, pp. 183–204.
Morales-Nin, B., Massutí, E., Stefanescu, C., 1996. Distribution and biology of *Alepocephalus rostratus* from the Mediterranean Sea. J. Fish Biol. 48, 1097–1112. http://dx.doi.org/10.1111/j.1095-8649.1996.tb01807.x.
Mourente, G., Díaz-Salvago, E., Tocher, D.R., Bell, J.G., 2000. Effects of dietary polyunsaturated fatty acid/vitamin E (PUFA/tocopherol ratio on antioxidant defence mechanisms of juvenile gilthead sea bream (*Sparus aurata* L., Osteichthyes, Sparidae). Fish Physiol. Biochem. 23, 337–351. http://dx.doi.org/10.1023/A:1011128510062.
Nelson, J.S., 1994. Fishes of the World, third ed. John Wiley & Sons, Inc., New York, NY.
Økland, H.M.W., Stoknes, I.S., Remme, J.F., Kjerstad, M., Synnes, M., 2005. Proximate composition, fatty acid and lipid class composition of the muscle from deep-sea teleosts and elasmobranchs. Comp. Biochem. Physiol. B 140, 437–443.
Podolska, M., Napierska, D., 2008. Acetylcholinesterase activity in hosts (herring *Clupea harengus*) and parasites (*Anisakis simplex* larvae) from the southern Baltic. ICES J. Mar. Sci. 63, 161–168. http://dx.doi.org/10.1016/j.icesjms.2005.08.001.
Solé, M., Lobera, G., Aljinovic, B., Ríos, J., García de la Parra, L.M., Maynou, F., Cartes, J.E., 2008. Cholinesterases activities and lipid peroxidation levels in muscle from shelf and slope dwelling fish from the NW Mediterranean: its potential use in pollution monitoring. Sci. Total Environ. 402, 306–317. http://dx.doi.org/10.1016/j.scitotenv.2008.04.041.
Solé, M., Baena, M., Arnau, S., Carrasson, M., Maynou, F., Cartes, J.E., 2010. Muscular cholinesterase activities and lipid peroxidation levels as biomarkers in several Mediterranean marine fish species and their relationship with ecological variables. Environ. Int. 36, 202–211. http://dx.doi.org/10.1016/j.envint.2009.11.008.
Stefanescu, C., Lloris, D., Rucabado, J., 1992. Deep-living demersal fishes in the Catalan Sea (western Mediterranean) below a depth of 1000 m. J. Nat. Hist. 26, 197–213 (doi:0.1080/00222939200770081).
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729. http://dx.doi.org/10.1093/molbev/mst197.
Ter Braak, C.J.F., 1986. Canonical correspondence analysis: a new eigenvector-technique for multivariate direct gradient analysis. Ecology 67, 1167–1179.
Van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.
Vassault, A., 1983. Lactate dehydrogenase. In: Bergmeyer, M.O. (Ed.), Methods of Enzymatic Analysis, Enzymes: Oxidoreductases, Transferases. Academic Press, New York, NY, pp. 118–126.

Chapter IV

Natural variability of parasite communities of Macrouridae of the middle and lower slope of the Mediterranean Sea and their relation with fish diet and health indicators

Natural variability of parasite communities of Macrouridae of the middle and lower slope of the Mediterranean Sea and their relation with fish diet and health indicators

D. Pérez-i-García[a], M. Constenla[a], A. Soler-Membrives[a], J. E. Cartes[b], M. Solé[b], M. Carrassón[a,*]

[a] Departament de Biologia Animal, Biologia Vegetal i d'Ecologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain

[b] Institut de Ciències del Mar (ICM-CSIC), Pg. Marítim de la Barceloneta 37–49, 08003 Barcelona, Spain

*Corresponding author. Tel.: +34 935814637 E-mail address: Maite.Carrasson@uab.cat

Abstract: This study examines the parasite communities of *Coelorinchus caelorhincus*, *Coelorinchus mediterraneus*, *Coryphaenoides guentheri* and *Coryphaenoides mediterraneus* of the middle and lower slope of the Mediterranean Sea. Histopathological, enzymatic activity (acetylcholinesterase and lactate dehydrogenase), dietary and environmental (oxygen, salinity, temperature and turbidity) information were also obtained. A total of 11 parasite taxa were found among all four fish species, being the copepod *Hamaticolax resupinus* the only parasite shared by all of them. *Coelorinchus mediterraneus*, *Coryphaenoides guentheri* and *Cor. mediterraneus* exhibited rather homogeneous parasite communities, especially in the case of the latter two. *Coelorinchus mediterraneus* showed the highest richness of parasite taxa (eight species), whereas *C. guentheri* and *Cor. mediterraneus* harboured up to five and six, respectively, and *C. caelorhincus* up to three. Several of the encountered parasites occurred at very low prevalences (<10%), while only three species were exceptionally prevalent and abundant: Cucullanidae fam. gen. sp. larvae in *C. caelorhincus*; *Lepidapedon desclersae* in *Coe. mediterraneus* and *Hysterothylacium aduncum* in both *Coryphaenoides* spp. The abundance of the nematode *H. aduncum*, present in all host species except for *C. caelorhincus*, increased with water temperature and depth and became the dominant parasite below 2000 m. Salinity might be an important on the distribution of *H. resupinus*. The diet was generally homogeneous between the studied species, being *C. guentheri* more specialized on suprabenthic/benthic prey. The parasites *H. aduncum* and Tetraphylidea, and to lesser extent *Rhaphidascaris* sp., were related with the most mobile (swimming) prey consumed by macrourids (Chaetognaths, decapod larvae, and *Boreomysis arctica*). The parasites *L. desclersae, Capillostrongyloides morae* and *Otodistomum* sp. were related in *Coe. mediterraneus* with epibenthic prey (ophiuroids, isopods and tanaids). *Coryphaenoides guentheri* was

the smallest macrourid analysed, with the poorest parasite fauna with higher proportion of larval stages. Few histopathological alterations were found, being epitheliocystis the most extended and prevalent one. Few parasite effects on fish health were reflected at enzymatic and histological level, probably due to the low parasite burden in their hosts. It is possible that the role of small macrourids, especially of *C. guentheri*, is closer to act as intermediate host in deep-Mediterranean trophic webs.

Keywords: *Coryphaenoides; Coelorinchus*; Mediterranean deep-sea; parasites; fish health; diet.

1. Introduction

The Macrouridae, commonly referred as grenadiers or rattails, comprise a diverse group of deep-sea gadiform fishes extended world-wide (Cohen et al., 1990). This family is composed by approximately 300 species, 90% of them live in the continental slope between 200 and 2000 m depth (Marshall, 1965). In the Balearic deep sea, the Macrouridae is the best represented family in terms of number of species (eight species), the third in number of individuals and the fifth in terms of biomass between depths of 1000 and 2250 m (Stefanescu et al., 1992, 1993). Among Balearic Sea Macrouridae, two representatives of each both genera *Coelorinchus* and *Coryphaenoides* can be found: *Coelorinchus caelorhincus* (Risso, 1810); *Coelorinchus mediterraneus* Iwamoto & Ungaro, 2002; *Coryphaenoides guentheri* (Vaillant, 1888) and *Coryphaenoides* (*Chalinura*) *mediterraneus* (Giglioli, 1893) (previously named *Chalinura mediterranea* and actually accepted as senior synonym). *Coelorinchus caelorhincus* is found in the upper slope, mainly between 300 and 500 m (Macpherson, 1979), whereas the rest of species are found in the middle and lower slope: *Coe. mediterraneus* inhabits between 1046 and 2201 m and *C. guentheri* and *Cor. mediterraneus* dwell between 1308 and 2251 m (Stefanescu et al., 1992). At depths below 500 m, *Coe. mediterraneus* is the third fish species in number of individuals. Below 2000 m both *Coryphaenoides* species become especially relevant; being the second and third more abundant species and representing the third and fifth position in terms of biomass (D'Onghia et al., 2004; Stefanescu et al., 1993). The knowledge of their biology has increased thanks to some studies on their diets (Carrassón and Matallanas, 2002, Macpherson, 1979), on their longevity (D'Onghia et al., 2000; Massutí et al., 1995; Morales-Nin, 1990; Sion et al., 2012) and some aspects of their reproduction (D'Onghia et al., 2008; Fernandez-Arcaya et al., 2013; Massutí et al., 1995); however, information about their parasitic communities in the Mediterranean

Sea remains unknown, despite the importance of this fish group in the Mediterranean realm (Klimpel et al., 2001; Marcogliese, 2005). The first assessment on the parasite communities of macrouridae species in the Mediterranean Sea, was carried out recently by Constenla et al. (2015) on *Hymenocephalus italicus, Nezumia aqualis* and *Trachyrincus scabrus* of the upper slope. To date, no study has been devoted to the parasite communities of *C. caelorhincus*, *Coe. mediterraneus*, *C. guentheri* nor *Cor. mediterraneus*. However, parasite fauna of these fishes is not completely unknown, since some parasitological studies have been carried out in the Atlantic Ocean: 12 different species, mainly trematodes have been described in *C. caelorhincus* (Klimpel et al., 2009) and a single parasite, *Lepidapedon arlenae*, has been recorded in *Coe. mediterraneus* (Bray and Gibson, 1995). Two parasites, *Sarcotaces* sp. and *Lepidapedon sommervillae*, are recorded in *C. guentheri* (Bray and Gibson, 1995; Bullock et al., 1986) and *Cor. mediterraneus* accounts for 16 parasite species (Kellermanns et al. 2009).

The study of parasites can provide valuable biological information of their hosts (Marcogliese, 2004), otherwise difficult to obtain in deep sea. Because many parasites are trophically-transmitted and possess complex life-cycles with different hosts, they reflect trophic interactions between organisms (Campbell et al., 1980). In addition, several studies highlighted the usefulness of parasites as biological tags for fish stock discrimination and for environmental stress and pollution on aquatic ecosystems (MacKenzie, 2002; MacKenzie et al., 2013; Marcogliese, 2005; Marcogliese and Pietrock 2011; Sasal et al., 2007; Sures, 2008). Besides, parasites can cause pathologies in different organs that can affect the fitness of their hosts (Feist and Longshaw, 2008). Host responses to the presence of parasites depend on the affected organ, but histopathological changes tend to be localized in the region of attachment of the parasites (Feist and Longshaw, 2008). Therefore, histopathological techniques allow the assessment of the impact of these organisms on their hosts. In a similar way, biochemical markers, which are traditionally used as markers of environmental stress and pollution (Van der Oost et al., 2003), may be modified by parasitic burden (Dallarés et al., 2014; Espinal-Carrión et al., 2010; Pérez-i-García et al., 2015) and provide a complementary assessment to histopathology on the their impact.

Therefore, the aim of the present paper is to provide, for the first time, a description of the natural variability of the parasite communities of *Coelorinchus caelorhincus*, *Coelorinchus mediterraneus*, *Coryphaenoides guentheri* and *Coryphaenoides mediterraneus* in the Mediterranean Sea. Different environmental parameters (temperature, salinity, oxygen and turbidity) were used to test their influence on those

communities. General fish condition, diet items, muscular biochemical markers and fish potential pathologies are also explored in order to test for possible relationships with the parasite load.

2. Materials and methods

In summer (July 2010), autumn (October 2011) and spring (May 2012), fish samples were collected on board the R/V García del Cid with a semi-balloon otter-trawl (OTSB-14) (Merrett and Marshall, 1980) in the Balearic Sea (NW Mediterranean) at depths between 442 and 2223 m from the continental slope off Barcelona and Balearic Islands (Fig.1):

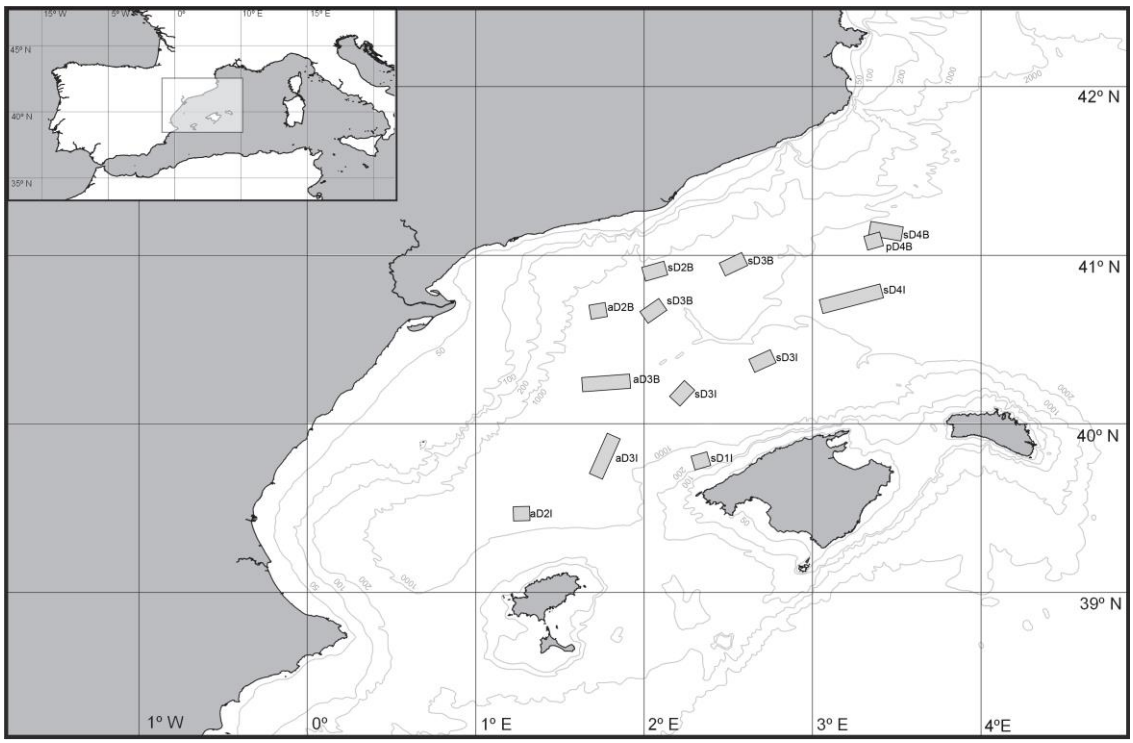


Figure 1. Study area with sampling seasons, depths and sites within the Barcelona and Balearic slopes. Abbreviations for spatiotemporal groups (season/depth/locality categories): sD1I: Balearic Islands at 600–1000 m in summer, sD2B: Barcelona at 1000–1400 m in summer, sD2I: Balearic Islands at 1000–1400 m in summer, aD2B: Barcelona at 1000–1400 m in autumn, aD2I: Balearic Islands at 1000–1400 m in autumn; sD3B: Barcelona at 1400–2000 m in summer, sD3I: Balearic Islands at 1400–2000 m in summer, aD3B: Barcelona at 1400–2000 m in autumn, aD3I: Balearic Islands at 1400–2000 m in autumn, sD4B: Barcelona at 2000–2200 m in summer, sD4I: Balearic Islands at 2000–2200 m in summer.

Coelorinchus caelorhincus (16 specimens from 457 m in summer in Balearic Islands), *Coelorinchus mediterraneus* (103 specimens from 1236 – 1751 m in summer and autumn in both slopes), *Coryphaenoides guentheri* (199 specimens from 1477 – 2197 m in spring, summer and autumn in both slopes) and *Coryphaenoides mediterraneus* (63 specimens from 1549 – 2197 m in spring, summer and autumn in both slopes) (Table 1).

Table 1. Sampling data of *Coelorinchus caelorhincus*, *Coelorinchus mediterraneus*, *Coryphaenoides guentheri* and *Coryphaenoides mediterraneus*. SeasDepthLoc: Spatiotemporal groups: sD1I: Balearic Islands at 600–1000 m in summer, sD2B: Barcelona at 1000–1400 m in summer, sD2I: Balearic Islands at 1000–1400 m in summer, aD2B: Barcelona at 1000–1400 m in autumn, aD2I: Balearic Islands at 1000–1400 m in autumn; sD3B: Barcelona at 1400–2000 m in summer, sD3I: Balearic Islands at 1400–2000 m in summer, aD3B: Barcelona at 1400–2000 m in autumn, aD3I: Balearic Islands at 1400–2000 m in autumn, sD4B: Barcelona at 2000–2200 m in summer, sD4I: Balearic Islands at 2000–2200 m in summer; n: number of individuals captured in each trawl. Environmental variables: T: temperature, S: salinity, O_2: oxygen concentration, Turb: turbidity.

Trawl	Date (dd/mm/yy)	Depth (m)	Coordinates		SeasDepthLoc	*Coelorinchus caelorinchus*	*Coelorinchus mediterraneus*	*Coryphaenoides guentheri*	*Coryphaenoides mediterraneus*	Environmental variables			
			Latitude	Longitude		n	n	n	n	T (ºC)	S (psu)	O_2 (mL/L)	Turb (V)
A105	10/07/10	1269	40º54.35N	02º06.06E	sD2B	-	15	-	-	13.08	38.48	4.41	0.06
A106	10/07/10	1308	40º53.85N	02º04.00E	sD2B	-	5	-	-	13.08	38.48	4.41	0.06
A109	12/07/10	1744	40º38.40N	02º04.12E	sD3B	-	1	29	3	13.14	38.48	4.37	0.06
A110	12/07/10	1787	40º30.81N	02º03.68E	sD3B	-	-	14	5	13.14	38.48	4.37	0.06
A111	13/07/10	1630	40º56.40N	02º30.03E	sD3B	-	1	-	-	13.15	38.35	4.36	0.07
A112	13/07/10	1422	41º03.92N	02º33.26E	sD3B	-	3	-	-	13.15	38.43	4.28	0.09
A113	16/07/10	2057	40º38.82N	03º06.70E	sD4I	-	-	4	1	13.20	38.48	4.35	0.05
A114	16/07/10	2194	40º37.59N	03º27.82E	sD4I	-	-	2	4	13.22	38.48	4.34	0.06
A116	17/07/10	457	39º46.73N	02º21.85E	sD1I	16	-	-	-	13.07	38.49	4.15	0.90
A120	20/07/10	1605	40º08.53N	02º12.21E	sD3I	-	7	3	-	13.15	38.48	4.33	0.09
A121	20/07/10	1477	40º05.37N	02º11.33E	sD3I	-	17	4	-	13.15	38.48	4.33	0.09
A122	21/07/10	1874	40º23.30N	02º40.65E	sD3I	-	-	20	9	13.17	38.48	4.36	0.04
A124	22/07/10	2197	41º04.31N	03º16.74E	sD4B	-	-	27	9	13.22	38.48	4.34	0.06
A304	15/10/11	1236	40º41.96N	01º37.46E	aD2B	-	16	-	-	13.12	38.48	4.25	0.28
A305	16/10/11	1516	40º10.24N	01º38.26E	aD3B	-	1	-	-	13.18	38.49	4.32	0.21
A306	16/10/11	1751	40º09.65N	02º00.23E	aD3B	-	4	42	7	13.18	38.49	4.32	0.21
A310	18/10/11	1272	39º25.31N	01º16.84E	aD2I	-	7	-	-	13.10	38.49	4.16	0.23
A311	19/10/11	1626	39º56.20N	01º37.91E	aD3I	-	-	33	10	13.15	38.49	4.31	0.20
A312	19/10/11	1407	39º45.53N	01º44.88E	aD3I	-	14	-	-	13.12	38.49	4.31	0.33
A313	21/10/11	1549	40º15.13N	01º39.66E	aD3B	-	12	1	2	13.12	38.49	4.31	0.22
A3b02	04/05/12	2174	41º04.64N	3º18.23E	pD4B	-	-	20	13	13.13	38.49	4.27	0.20
Total						16	103	199	63				

Individual fish morphometrical data (pre-anal length, PAL and total weight, TW) were taken immediately on board. Environmental parameters (temperature in °C, salinity in psu, oxygen concentration in mL/L and turbidity in voltage units) were recorded by casts with a SBE25 CTD profiler at 5 m above the bottom. Captured specimens were processed in different ways: (I) 16 *C. caelorhincus*, 94 *Coe. mediterraneus*, 80 *C. guentheri* and 45 *Cor. mediterraneus* were processed for both parasitological and histological studies: half liver and spleen and right gills were fixed in 10% buffered formalin for histopathological studies, and the rest of the specimen was frozen at –20 ºC for the subsequent parasitological study; (II) 7 *Coe. mediterraneus*, 50 *C. guentheri* and 18 *Cor. mediterraneus* were immediately frozen at –20 ºC after capture and were added to the parasitological study; (III) a portion of the axial muscle (about 2 g) was kept at –20 ºC from 10 *C. caelorhincus*, 60 *Coe. mediterraneus*, 66 *C. guentheri* and 16 *Cor. mediterraneus* for biomarker analyses; (IV) 2 *Coe. mediterraneus* and 69 *C. guentheri* were fixed “in toto” in 10% buffered formalin with the abdominal cavity opened to help the fixation and preservation of internal organs and were added to the histopathological study.

2.1 Parasitological examination

In the laboratory, fish individuals were thawed, dissected and the liver weight was recorded to the nearest 0.1 mg. Specimens were examined for metazoan ectoparasites and all organs, including muscles, were examined and carefully checked for endoparasites under the stereomicroscope. Parasites were preserved in ethanol 70%. For identification purposes, platyhelminths were stained in iron acetocarmine, dehydrated through a graded ethanol series, cleared in dimethyl phthalate and mounted in Canada balsam. Nematodes were cleared in glycerine before identification.

2.3 Diet analysis

Guts (stomachs and intestines) of 10 *C. caelorhincus*, 51 *Coe. mediterraneus*, 90 *C. guentheri* and 37 *Cor. mediterraneus*, of individuals that were first examined for the occurrence of parasites, were further analysed for diet. Gut contents were individually weighed to the nearest 0.001 g and prey were identified to the lowest possible taxonomic level under a stereomicroscope (X10-X40). It was not possible to analyze all specimens used for parasitology also for diet due to different limitations (e.g. specimens presenting everted stomachs, absence of identifiable prey in some cases). Diet was considered by prey number to do results directly comparable with parasitological data. In fish with everted stomachs (to 55% of fish for *C. guentheri* in some hauls) only intestine contents were analysed. As macrourids consumed mainly

prey with hard structures (crustaceans, basically, having hard specific structures like mandibles, telsons) underestimation of prey analysing intestines was not significant.

Further than the description of diet as a function of the different factors here explored for parasite communities (i.e. locality, season or depth), we pretend to relate fish parasites and prey as potential intermediate vectors.

2.4 Biomarker analysis

Muscle tissues were used for analysis on acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) activities. A portion of muscle of about 0.3 g was homogenised in ice-cold 50 mM phosphate buffer (pH 7.4) in a 1:5 (w/v) ratio using a Polytron® blender. The homogenate was centrifuged at 10,000 × g for 30 min at 4 ºC, and the obtained supernatant (S10) stored at –80 ºC was used for biochemical analyses. Both assays were carried out in triplicate at 25 ºC in a 96-well plate using a Tecan Infinite M200 microplate reader. For AChE determination, the concentration of the substrate (ATC) selected was 1 mM, as described in Solé et al. (2010). AChE activities were assayed according to the principle of Ellman et al. (1961) at 405 nm with diluted samples at 1:5 except those of *Coe. mediterraneus*, which were diluted to 1:10. For the LDH determination, 150 µL of NADH solution in phosphate buffer was mixed with 25 µL of 1:20 to 1:125 diluted sample (dilution varied as needed) and 50 µL of pyruvate solution in each microplate well. LDH activity was given by the amount of pyruvate consumed due to NADH oxidation at 340 nm (Vassault, 1983). Activity of AChE and LDH was expressed in nmol/min/mg prot. Total protein content in the S10 fraction was determined by Bradford (1976) method using bovine serum albumin as standard (BSA 0.1–1 mg/mL).

2.5 Histological analysis

Fishes and organs fixed in 10% buffered formalin were processed by routine paraffin histology techniques. Sections of 4 µm of each organ were stained with Haematoxylin and Eosin. Identification of the present alterations was performed according to their morphology. Prevalences of the different alterations found in each host were calculated.

For splenic macrophagic centres analysis, three fields of view (0.23 mm^2 / screen) were randomly selected from each section of the spleen at 200x of magnification and were microscopically examined. Area and number of macrophagic centres were calculated per square millimetre for each field using a MicroComp Integrated Image Analysis System.

2.6 Data analysis

Data were grouped according to the season (p: spring, s: summer and a: autumn), depth (D1: upper slope at 400–800 m, D2: middle slope from 1000 to 1400 m, D3: lower slope ranges 1400–2000 m and D4: lower slope deepest station between 2000-2200 m) and locality (B: Barcelona and I: Balearic Islands). Different spatiotemporal categories (SeasDepthLoc) were stablished for each of the host species (Table 1).

Parasitological terms: prevalence (P) and mean abundance (MA) were calculated according to Bush et al. (1997). The species richness and diversity (Brillouin index) of the metazoan parasites were calculated with PRIMER v6 (Anderson et al., 2008). Fish condition was assessed by condition factor ($K=TW \times 100/PAL^3$) and hepatosomatic index (HSI=liver weight × 100/TW).

To comply for normality and homoscedasticity requirements some data were ln (x)-transformed prior to General Linear Model analysis (GLM): HSI (in *Coe. mediterraneus* and *C. guentheri*), K (in *Coe. mediterraneus*, *C. guentheri* and *Cor. mediterraneus*), fish PAL (in all four species), activities of AChE (in *Coe. mediterraneus*), LDH (in *Coe. mediterraneus* and *C. guentheri*) and the number and area occupied per mm^2 of macrophagic centres (in *C. guentheri*). Possible effects of the factor SeasDepthLoc and host size were tested for infracommunity parasite descriptors (mean species richness MSR, total mean abundance TMA and mean diversity MDI) using GLM and Generalized Linear Model Analysis (GZM) (only for TMA), with post-hoc pairwise comparisons. The possible effects of the factor SeasDepthLoc were also tested for fish PAL and condition indices (K and HSI) using GLM, with post-hoc pairwise comparisons.

A Factorial Correspondence Analysis (FCA) was first applied to visualize patterns in parasite abundance in relation to spatiotemporal variation (factor “SeasDepthLoc”) with all four hosts. A data matrix comprising component population abundance of parasites and the spatiotemporal groups was used in FCA. Since *C. caelorhincus* was completely isolated from the other three species and in order to gain more resolution, another FCA was applied excluding this species. Based on the parasite coordinates of the two first axes obtained in FCA, a cluster of dissimilarity was simultaneously performed to define host groups. Differences in abundance and prevalence among the parasite populations of each host were tested using GZM in order to gain insight into the suggested differences shown by the FCA. These were tested using individual fish as replicate samples, for the spatiotemporal factors and fish size (applying negative-binomial model for abundance and logistic model for prevalence).

Relationships between parasites and environmental variables (T, S, O_2 and turbidity) and between parasites and preys were analysed by multivariate canonical

correspondence analysis (CCA) (TerBraak, 1986) for each host. Vectors in CCA plots represented explanatory variables and were proportional in length to their importance on the explained variable (TerBraak, 1986). A matrix was generated with individuals with parasitological and dietary information, being further analysed by Canonical Correspondence Analysis (CCA) (Ter Braak, 1986). We considered parasites and prey with occurrences ≥ 3 in the matrix, accumulating a total of 8 parasites and 34 prey types. In such matrix, number of individuals per haul was between 3 and 19. Diet was presented to broad taxa, while for CCA we considered prey to genus/species levels, because within a same taxon we can find species with e.g. different swimming capacity, as happens within isopods.

The relation between the enzymatic activities (AChE, LDH) and the abundance of the different parasites abundance was tested by GLM for each host species. In addition, GLM was also used to test the relation between these markers and the infracommunity descriptors (MSR, TMA and MDI) and condition indices (K, HSI), as well the differences of biochemical markers in the spatiotemporal factor and fish size.

Differences in the spatiotemporal factor and fish size were tested for all the prevalence of pathologies by GZM (applying logistic model) and by GLM (only for number and area per mm^2 occupied by macrophagic centres). The possible relation of the presence and number and area occupied per mm^2 of macrophagic centres with the different infracommunity descriptors (MSR, TMA and MDI) and the most abundant endoparasites for each host, condition indices (K, HSI) and enzymes (AChE, LDH) were tested by GZM (applying logistic model for prevalence of the alterations) and by GLM (for number and area occupied by macrophagic centres per mm^2).

3. Results

3.1 Parasitological analysis

The analysis of the 310 individuals from the four macrourid species revealed a total of 1025 parasites belonging to 11 taxa: the copepods *Hamaticolax resupinus* and *Sarcotaces* sp., the isopod *Gnathia* sp., the digeneans *Bathycreadium brayi*, *Otodistomum* sp. and *Lepidapedon desclersae*, the cestode plerocercoid Tetraphyllidea fam. gen. sp. and the nematodes *Capillostrongyloides morae*, Cucullanidae gen. sp., *Hysterothylacium aduncum* and *Raphidascaris* sp. The parasite fauna of all four macrourids was rather homogeneous; the four hosts were parasitized by *H. resupinus* and Tetraphyllidea fam. gen. sp. The nematodes *H. aduncum* and *Raphidascaris* sp. were shared among *Coe. mediterraneus*, *C. guentheri* and *Cor. mediterraneus* (Table 2).

Table 2. Parasites found in *Coelorinchus caelorhincus*, *Coelorinchus mediterraneus*, *Coryphaenoides guentheri* and *Coryphaenoides mediterraneus*; infracommunity descriptors (MSR: mean species richness, TMA: total mean abundance, MDI: mean diversity index), histological alterations and biochemical markers (AChE: acetylcholinesterase, LDH: lactate dehydrogenase). n: total number of specimens sampled for the parasitological study, number of specimens sampled for histopathological study in brackets. Developmental stage: A, adult and juveniles; L, larvae; Mt, metacercariae. Location: G, gills; M, mouth; S, stomach; I, intestine; PC, pyloric caeca; Li, liver; Go, gonads; P, peritoneum; Mu, muscle; SC, subcutaneous. Prevalence (P%) and mean abundance (MA ± standard deviation, SD). Different superscript letters indicate significant differences.

	Developmental stage	Location	*Coelorinchus caelorhincus*		*Coelorinchus mediterraneus*		*Coryphaenoides guentheri*		*Coryphaenoides mediterraneus*	
n			16 (16)		101 (94)		130 (149)		63 (45)	
Parasites			P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD
Copepoda										
Hamaticolax resupinus	A	G	19	0.19 ± 0.40	34	0.69 ± 1.26	4	0.11 ± 0.72	25	0.65 ± 1.67
Sarcotaces sp.	A	SC	-	-	2	0.05 ± 0.36	-	-	2	0.06 ± 0.50
Isopoda										
Gnathia sp.	A	M	-	-	-	-	-	-	2	0.02 ± 0.14
Digenea										
Bathycreadium brayi	A	I, PC	13	0.13 ± 0.34	-	-	-	-	-	-
Lepidapedon desclersae	A	I, PC	-	-	69	2.87 ± 4.86	-	-	-	-
Otodistomum sp.	Mt	Mu	-	-	15	0.18 ± 0.46	4	0.05 ± 0.25	-	-
Cestoda										
Tetraphyllidea fam. gen. sp.	L	S, I, PC	-	-	7	0.13 ± 0.59	1	0.01 ± 0.09	6	0.25 ± 1.25
Nematoda										
Capillostrongyloides morae	A	S	-	-	8	0.13 ± 0.52	-	-	-	-
Cucullanidae gen. sp.	L	I, PC	81	7.88 ± 7.54	-	-	-	-	-	-
Hysterothylacium aduncum	A, L	I, P, Li, Go	-	-	11	0.14 ± 0.45	65	2.08 ± 2.43	80	2.47 ± 2.63
Raphidascaris sp.	L	I, PC	-	-	3	0.02 ± 0.14	1	0.01 ± 0.09	2	0.02 ± 0.14
Infracommunity descriptors										
MSR				1.31 ± 0.48^{A}		1.48 ± 0.89^{A}		0.74 ± 0.54^{B}		1.13 ± 0.61^{AB}
TMA				8.19 ± 7.41^{A}		4.18 ± 5.19^{A}		2.27 ± 2.61^{B}		3.39 ± 3.35^{A}
MDI				0.09 ± 0.14^{A}		0.24 ± 0.24^{B}		0.03 ± 0.13^{A}		0.10 ± 0.18^{A}
Histological alterations										
Cysts of unknown etiology			25		-		-		-	
Epitheliocystis					45		32		22	
Hepatocellular inclusions			25		-		-		-	
Macrophagic centers			-		78		42		38	
-Number/mm^2				-		28.44 ± 18.47		6.49 ± 11.20		26.17 ± 38.03
-Area/mm^2				-		124793 ± 98394		2629 ± 5716		31784 ± 55617
Inflammatory reactions			-		6		2		2	
Biochemical markers										
AChE*				17.31 ± 6.86^{A}		68.09 ± 36.52^{B}		33.77 ± 11.06^{C}		35.55 ± 9.06^{C}
LDH*				1383 ± 467^{A}		650 ± 290^{B}		507 ± 228^{B}		120 ± 28^{C}

*n: *C. caelorhincus* = 10; *Coelorinchus mediterraneus* = 60; *C. guentheri* = 66; *Coryphaenoides mediterraneus* = 16

There were significant differences between the four hosts with respect to parasite infracommunity descriptors (MSR, MDI, TMA) (GLM: $F_{3,293}$=22.821, p=0.000; GLM: $F_{3,231}$=24.843, p=0.000; and GZM: X^2_3=32.399, p=0.000; respectively). *Coryphaenoides guentheri* harboured the lowest values of all infracommunity descriptors, whereas *Coe. mediterraneus* harboured the highest MSR and MDI values and *C. caelorhincus* the highest TMA (Table 2).

The cluster analysis of the spatiotemporal categories (SeasDepthLoc) evidenced a clear differentiation on parasite communities of *C. caelorhincus* from the other three hosts (not shown). The FCA applied, excluding *C. caelorhincus,* presented a plot of the first factorial plane of co-inertia analysis (70.69% of the variance explained, mostly due to the first axis (57.23%)). Four different groups could be identified depending on their parasite load (Fig. 2) and cluster analysis (not shown): A) *Coe. mediterraneus* in autumn in the middle slope, B) *Coe. mediterraneus* in summer and in autumn in the lower slope, C) *C. guentheri* and *Cor. mediterraneus* in spring and summer, D) *C. guentheri* and *Cor. mediterraneus* in autumn.

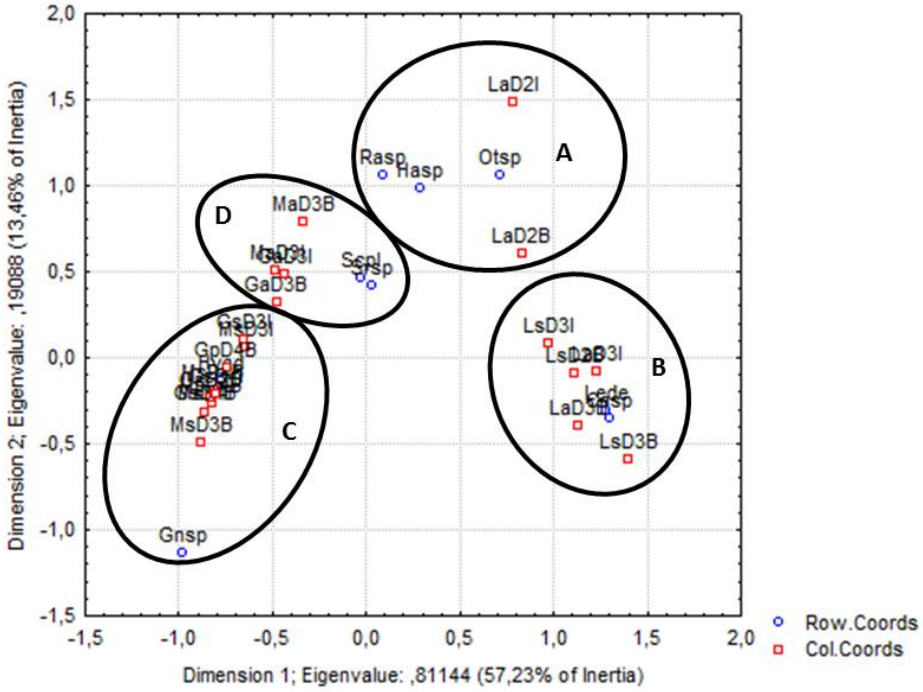


Figure 2. Plot of the first factorial plane of co-inertia analysis of the factorial correspondence analysis (FCA) on component population data for the parasite species in *Coelorinchus mediterraneus* (abbreviated as L), *Coryphaenoides guentheri* (abbreviated as G) and *Coryphaenoides mediterraneus* (abbreviated as M). A, B, C and D indicate the groups established in the parasite fauna description. Abbreviations for spatiotemporal groups of each host: are defined in Figure 1. Abbreviations for parasite names: basp, *Bathycreadium brayi*; casp, *Capillostrongyloides morae*; cucu, Cucullanidae gen. sp.; gnsp, *Gnathia* sp.; hasp, *Hamaticolax resupinus*; hyad, *Hysterothylacium aduncum*; lede, *Lepidapedon desclersae*; otsp, *Otodistomum* sp.; rasp, *Raphidascaris* sp.; scpl, Tetraphyllidea fam. gen. sp.; srsp, *Sarcotaces* sp.

3.1.1 Coelorinchus caelorhincus

This macrourid showed the lowest parasite richness of all the Macrouridae in the area, with only three parasite species: *H. resupinus*, *Bathycreadium brayi* and Cucullanidae sp. (Table 2). The most abundant and prevalent one was Cucullanidae gen. sp. larvae, which was found in the intestine. Few individuals were parasitized with *B. brayi* and *H. resupinus* in intestine and gills, respectively. The copepod is the only parasite also found in the other three hosts.

Preanal length of fish ranged from 4.3 to 6.7 cm. Fish size was associated neither with any of the infracommunity parameters nor with the abundance of the three parasite species identified (GLM/GZM, $p>0.005$ in all cases). Condition parameters were not affected by parasitism (GZM, $p>0.005$ in all cases).

3.1.2 Coelorinchus mediterraneus

Eight parasite species were isolated from *Coe. mediterraneus* (Table 2). The most abundant and prevalent parasite was *Lepidapedon desclersae*. *Hamaticolax resupinus* was the second most abundant and prevalent parasite, followed by *Otodistomum* sp.

The *Coe. mediterraneus* group of autumn in the middle slope at 1000–1399 m identified by the FCA (Fig. 2, A) was characterized by *Hamaticolax resupinus*, *Otodistomum* sp. and *Raphidascaris* sp., displaying the highest abundance in this spatiotemporal group. *Hamaticolax resupinus* abundance was significantly higher at individuals of Barcelona slope (GZM, $X^2_5=12.895$, $p=0.024$) (Table 3). No differences were found for parasite prevalences.

Table 3. Prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites, infracommunity descriptors (MSR: mean species richness, TMA: total mean abundance, MDI: mean diversity index) and histological alterations of *Coelorinchus mediterraneus*. Different superscript letters and numbers indicate significant differences in abundance and prevalence, respectively.

	1000 – 1399 m						1400 – 1999 m							
	Summer		Autumn				Summer				Autumn			
	Barcelona		Barcelona		Balearic Islands		Barcelona		Balearic Islands		Barcelona		Balearic Islands	
n	20 (20)		15 (15)		8 (5)		5 (5)		22 (22)		17 (15)		14 (12)	
Parasites	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD
Copepoda														
Hamaticolax resupinus	55^{1}	1.15 ± 1.53^{A}	47^{1}	1.40 ± 1.92^{A}	38^{1}	0.50 ± 0.76^{AB}	-	-	27^{1}	0.59 ± 1.14^{AC}	12^{1}	0.18 ± 0.53^{BC}	36^{1}	0.43 ± 0.65^{AC}
Sarcotaces sp.	-	-	13	0.33 ± 0.90	-	-	-	-	-	-	-	-	-	-
Digenea														
Lepidapedon desclersae	75^{1}	5.40 ± 9.11^{A}	53^{1}	1.73 ± 2.87^{BC}	38^{1}	0.50 ± 0.76^{B}	100^{1}	5.00 ± 4.80^{ABC}	73^{1}	2.27 ± 2.90^{C}	71^{1}	2.47 ± 2.90^{C}	79^{1}	2.50 ± 1.56^{AC}
Otodistomum sp.	5^{1}	0.05 ± 0.22^{A}	7^{1}	0.07 ± 0.26^{A}	63^{1}	0.63 ± 0.52^{A}	20^{1}	0.20 ± 0.45^{A}	18^{1}	0.27 ± 0.63^{A}	6^{1}	0.06 ± 0.24^{A}	14^{1}	0.21 ± 58^{A}
Cestoda														
Tetraphyllidea fam. gen. sp.	25^{1}	0.55 ± 1.23^{A}	7^{1}	0.07 ± 0.26^{B}	-	-	-	-	-	-	6^{1}	0.06 ± 0.24^{B}	-	-
Nematoda														
Capillostrongyloides morae	20^{1}	0.40 ± 0.10^{A}	13^{1}	0.20 ± 0.56^{A}	-	-	40^{1}	0.40 ± 0.55^{A}	-	-	-	-	-	-
Hysterothylacium aduncum	5^{1}	0.10 ± 0.45^{A}	7^{1}	0.07 ± 0.26^{A}	-	-	-	-	23^{1}	0.32 ± 0.72^{A}	24^{1}	0.24 ± 0.44^{A}	-	-
Raphidascaris sp.	5^{1}	0.05 ± 0.22^{A}	-	-	13^{1}	0.13 ± 0.35^{A}	-	-	-	-	-	-	-	-
Infracommunity descriptors														
MSR		1.90 ± 0.97^{A}		1.47 ± 0.92^{A}		1.50 ± 0.76^{A}		1.60 ± 0.55^{A}		1.41 ± 0.96^{A}		1.18 ± 0.88^{A}		1.29 ± 0.73^{A}
TMA		7.70 ± 9.39^{A}		3.87 ± 3.64^{AB}		1.75 ± 0.89^{B}		5.60 ± 4.28^{AB}		3.45 ± 3.20^{B}		3.00 ± 3.10^{B}		3.14 ± 1.79^{B}
MDI		0.35 ± 0.26^{A}		0.27 ± 0.28^{A}		0.16 ± 0.24^{A}		0.22 ± 0.20^{A}		0.25 ± 0.26^{A}		0.17 ± 0.22^{A}		0.19 ± 0.20^{A}
Histological alterations														
Epitheliocystis	80^{1}		73^{1}		60^{12}		40^{12}		23^{2}		20^{2}		25^{2}	
Macrophagic centers	95^{A}		87^{1}		80^{1}		80^{1}		68^{1}		87^{1}		42^{1}	
-Number/mm²		27.21 ± 15.49^{AB}		26.94 ± 13.53^{AB}		9.93 ± 17.13^{A}		30.49 ± 7.02^{AB}		35.21 ± 22.50^{AB}		43.01 ± 18.83^{B}		
-Area/mm²		135032 ± 87545^{AB}		216583 ± 132354^{B}		51049 ± 69840^{A}		87532 ± 56243^{AB}		114470 ± 73373^{AB}		149516 ± 166614^{AB}		
Inflammatory reactions	10^{1}		-		-		-		18^{1}		-		-	

Coelorinchus mediterraneus of summer in the middle slope (1000–1399 m) and summer and autumn in the lower slope, 1400–1999 m (Fig. 2, B) were characterized by *L. desclersae* and *C. morae*. Significant differences in the spatiotemporal factor were detected for *L. desclersae* (GZM, X^2_6=19.734, p=0.003), being more abundant in summer in the middle slope and in the lower slope (Table 3).

Fish PAL ranged from 2.6 to 8.5 cm, being significantly smaller in Balearic Islands (GLM, $F_{6,96}$=9.543, p=0.000). Concerning fish condition, HSI was significantly higher in summer than in any of the other seasons (GLM, $F_{6,85}$=14.720, p=0.000) and K was also significantly higher in summer in the lower slope of the Balearic Islands (GLM, $F_{6,94}$=10.847, p=0.000) (Table 4). Parasite MSR and MDI were positively related to PAL (GLM: $F_{1,99}$=7.373, p=0.008; and GLM: $F_{1,85}$=5.371, p=0.023; respectively). However, neither the TMA nor the abundance of each parasite species were associated to it (GZM, p>0.05 in all cases). The prevalence of *C. morae* was positively related with PAL (GZM, X^2_1=5.455, p=0.020). There was no relation between any of the condition parameters and parasitism (GLM, p>0.005 in all cases).

Table 4. Means and standard deviations of pre-anal length (PAL), hepatosomatic index (HSI), condition factor (K) and acetylcholinesterase (AChE) and lactate dehydrogenase (LDH) activities for *Coelorinchus caelorhincus*, *Coelorinchus mediterraneus*, *Coryphaenoides guentheri* and *Coryphaenoides mediterraneus*. Abbreviations for spatiotemporal groups: are defined in Table 1. n: total number of samples, number of samples devoted to biochemical analyses in brackets.

Host species	Spatiotemporal groups	n	PAL	HSI	K	AChE	LDH
Coelorinchus caelorhincus	sD1I	16 (10)	4.98 ± 0.68	3.28 ± 0.58	13.31 ± 1.31	17.31 ± 6.86	1383.46 ± 467.47
Coelorinchus mediterraneus	sD2B	20 (10)	7.48 ± 0.76^{A}	3.35 ± 1.62^{A}	5.87 ± 0.82^{A}	56.04 ± 13.45^{AB}	834.66 ± 247.44^{A}
	aD2B	15 (10)	7.62 ± 0.69^{A}	1.56 ± 0.33^{B}	5.21 ± 0.73^{A}	53.32 ± 19.45^{AB}	704.97 ± 268.95^{AB}
	aD2I	8 (5)	5.88 ± 1.87^{B}	1.83 ± 1.56^{A}	5.56 ± 0.70^{A}	49.81 ± 20.50^{A}	737.05 ± 237.88^{AB}
	sD3B	5 (5)	7.06 ± 0.70^{A}	3.13 ± 0.92^{A}	5.46 ± 0.63^{A}	93.46 ± 26.00^{B}	921.34 ± 463.66^{A}
	sD3I	24 (10)	5.41 ± 0.90^{B}	3.04 ± 1.26^{A}	8.20 ± 3.28^{B}	95.31 ± 69.57^{AB}	653.18 ± 294.76^{AB}
	aD3B	17 (10)	6.53 ± 1.61^{AB}	1.72 ± 0.84^{B}	5.08 ± 0.55^{A}	55.37 ± 22.63^{AB}	486.03 ± 126.87^{BC}
	aD3I	14 (10)	5.21 ± 0.99^{B}	1.71 ± 0.96^{B}	5.65 ± 0.52^{A}	76.84 ± 17.56^{AB}	392.22 ± 79.44^{C}
Coryphaenoides guentheri	sD3B	43 (10)	5.17 ± 0.63^{A}	3.52 ± 1.18^{AB}	10.21 ± 2.00^{A}	26.43 ± 5.07^{A}	500.04 ± 253.43^{AB}
	sD3I	27 (10)	5.13 ± 0.85^{A}	4.86 ± 1.78^{A}	10.51 ± 2.40^{AB}	25.65 ± 13.22^{A}	548.22 ± 219.50^{AB}
	aD3B	43 (10)	4.43 ± 0.68^{BC}	2.32 ± 1.02^{B}	10.25 ± 2.95^{A}	47.86 ± 11.44^{B}	601.77 ± 368.62^{AB}
	aD3I	33 (10)	4.20 ± 0.46^{B}	2.50 ± 0.89^{B}	9.70 ± 2.02^{A}	34.54 ± 5.94^{AC}	394.68 ± 92.22^{B}
	pD4B	20 (9)	4.71 ± 0.42^{C}	1.08 ± 0.44^{C}	8.45 ± 1.61^{B}	39.24 ± 8.52^{C}	620.43 ± 172.67^{A}
	sD4B	27 (10)	5.00 ± 0.61^{A}	3.10 ± 0.93^{AB}	9.39 ± 1.24^{AB}	30.88 ± 4.66^{C}	373.02 ± 60.67^{B}
	sD4I	6 (6)	5.00 ± 0.89^{A}	2.88 ± 0.27^{AB}	10.36 ± 0.97^{A}	31.38 ± 5.93^{AC}	539.62 ± 214.15^{AB}
Coryphaenoides mediterraneus	sD3B	8 (0)	5.19 ± 0.92^{A}	2.21 ± 1.37^{A}	15.23 ± 2.48^{A}	*	*
	sD3I	9 (0)	4.89 ± 0.83^{A}	2.62 ± 0.78^{A}	15.61 ± 2.49^{A}	*	*
	aD3B	9 (7)	4.33 ± 1.45^{A}	1.38 ± 0.54^{A}	13.70 ± 0.89^{A}	33.73 ± 10.67^{A}	133.16 ± 27.64^{A}
	aD3I	10 (9)	4.89 ± 1.24^{A}	1.28 ± 0.37^{A}	1.38 ± 1.60^{A}	36.96 ± 7.95^{A}	110.69 ± 26.67^{A}
	pD4B	13 (0)	3.85 ± 1.08^{A}	0.76 ± 0.55^{A}	14.35 ± 1.75^{A}	*	*
	sD4B	9 (0)	5.28 ± 2.17^{A}	2.53 ± 1.36^{A}	13.65 ± 1.27^{A}	*	*
	sD4I	5 (0)	4.78 ± 0.86^{A}	1.63 ± 0.80^{A}	15.67 ± 1.50^{A}	*	*

* Data not available

The CCA relating parasites and environmental variables (85.8% of accumulated variance explained by the first to axes) linked temperature with the abundance of *H. aduncum* (upper part of the plot), *H. resupinus* with salinity and *Otodistomum* sp. was associated to turbidity (right-mid part of the plot) (Fig. 3, A).

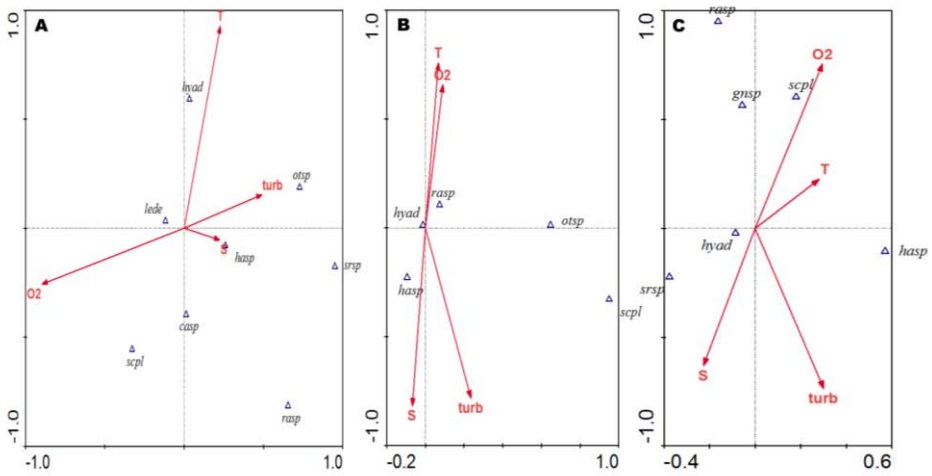


Figure 3. Canonical correspondence analysis showing relationships between the parasites in *Coelorinchus mediterraneus* (A), *Coryphaenoides guentheri* (B) and *Coryphaenoides mediterraneus* (C) and environmental data. Abbreviations for parasite names: are defined in Figure 2. Abbreviations for environmental variables: O, oxygen levels at the benthic boundary layer; S, salinity; T, temperature; turb, turbidity.

3.1.3 Coryphaenoides guentheri

Five different species of parasites were found, being *Hysterothylacium aduncum* the most abundant and prevalent one (Table 2). The rest of parasite species were found at low abundances and prevalences.

The *C. guentheri* group of spring and summer identified by the FCA (Fig. 2, C) was characterized by *H. aduncum*, displaying its highest abundance in this group. This species was significantly more abundant in spring and summer at the deepest station of the lower slope (GZM, X^2_6=53.737, p=0.000) (Table 5). It was also significantly more prevalent in spring and summer at the deepest station of the lower slope, attaining its lowest values in autumn at the middle slope (GZM, X^2_6=20.667, p=0.002).

Coryphaenoides guentheri of autumn (Fig. 2, D) was characterized by Tetraphyllidea fam. gen. sp. This parasite was only found in this season (Table 5).

Table 5. Prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites, infracommunity descriptors (MSR: mean species richness, TMA: total mean abundance, MDI: mean diversity index) and histological alterations of *Coryphaenoides guentheri*. Different superscript letters and numbers indicate significant differences in in abundance and prevalence, respectively.

	1400 – 1999 m								2000 – 2200 m					
	Summer				Autumn				Spring		Summer			
	Barcelona		Balearic Islands		Barcelona		Balearic Islands		Barcelona		Barcelona		Balearic Islands	
n	21 (33)		20 (23)		21 (35)		22 (28)		20		20 (24)		6 (6)	
Parasites	P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD
Copepoda														
Hamaticolax resupinus	-	-	5^{1}	0.20 ± 0.89^{A}	-	-	5^{1}	0.05 ± 0.21^{A}	15^{1}	0.45 ± 1.57^{A}	-	-	-	-
Digenea														
Otodistomum sp.	-	-	5^{1}	0.05 ± 0.22^{A}	10^{1}	0.14 ± 0.48^{A}	5^{1}	0.05 ± 0.21^{A}	-	-	-	-	17^{1}	0.17 ± 0.41^{A}
Cestoda														
Tetraphyllidea fam. gen. sp.	-	-	-	-	5	0.05 ± 0.22	-	-	-	-	-	-	-	-
Nematoda														
Hysterothylacium aduncum	67^{1}	1.57 ± 1.75^{A}	65^{1}	1.30 ± 1.34^{AB}	38^{12}	0.52 ± 0.75^{AB}	18^{2}	0.23 ± 5.53^{B}	100^{3}	4.15 ± 2.58^{C}	95^{3}	4.35 ± 2.62^{C}	100^{3}	4.33 ± 2.42^{AC}
Raphidascaris sp.	5	0.05 ± 0.22	-	-	-	-	-	-	-	-	-	-	-	-
Infracommunity descriptors														
MSR		0.71 ± 0.46^{AB}		0.75 ± 0.55^{AB}		0.52 ± 0.60^{BC}		0.27 ± 0.46^{C}		1.15 ± 0.37^{D}		0.95 ± 0.22^{AD}		1.17 ± 0.41^{D}
TMA		1.70 ± 1.72^{A}		1.55 ± 1.67^{A}		0.71 ± 1.01^{AB}		0.32 ± 0.57^{B}		4.60 ± 3.22^{C}		4.35 ± 2.62^{C}		4.50 ± 2.43^{AC}
MDI		0.00 ± 0.00^{A}		0.03 ± 0.14^{A}		0.03 ± 0.15^{A}		0.00 ± 0.00^{A}		0.09 ± 0.23^{A}		0.00 ± 0.00^{A}		0.08 ± 0.20^{A}
Histological alterations														
Epitheliocystis	45^{1}		43^{1}		29^{1}		14^{1}				25^{1}		50^{1}	
Macrophagic centers	52^{1}		48^{1}		34^{1}		14^{1}				67^{1}		50^{1}	
-Number/mm^2		2.79 ± 4.81^{A}		5.06 ± 6.91^{A}		9.42 ± 15.21^{B}		5.06 ± 11.58^{B}				9.49 ± 13.57^{B}		7.33 ± 8.04^{AB}
-Area/mm^2		1412 ± 3653^{A}		1901 ± 2713^{AB}		2857 ± 5763^{C}		769 ± 1579^{BC}				5791 ± 9791^{C}		2511 ± 3534^{AC}
Inflammatory reactions	-		4^{1}		3^{1}		-				4^{1}		-	

Fish PAL ranged from 3.2 to 7.0 cm, being significantly larger in summer than in the other seasons (GLM, $F_{6,192}$=11.499, p=0.000). HSI was significantly higher in summer (GLM; $F_{6,178}$=23.601, p=0.000) and K significantly lower in spring (GLM; $F_{6,192}$=2.748, p=0.014) (Table 4). Parasite TMA and MSR were positively related to PAL (GZM, X^2_6=49.331, p=0.022 and GLM, $F_{1,122}$=8.314, p=0.010, respectively). Parasite MDI and the abundance of each parasite species were not associated to PAL (GLM/GZM, p>0.005 in all cases) except for *H. aduncum*, which was significantly more abundant in individuals with higher PAL (GZM, X^2_1=6.705, p=0.010). The prevalence of *H. aduncum* was also positively related with PAL (GZM, X^2_1=19.712, p=0.000). TMA and MSR showed differences in the spatiotemporal factor; being significantly higher in the lowest slope deepest station (GZM, X^2_6=52.254, p=0.000) and (GLM, $F_{6,122}$=7.419, p=0.000), respectively (Table 5). HSI was negatively related to the abundance of *H. aduncum* (GLM; $F_{1,114}$=9.749, p=0.002), and K was positively related to *H. aduncum* (GLM; $F_{1,128}$=26.133, p=0.000) and *H. resupinus* (GLM; $F_{1,128}$=4.380, p=0.038).

The CCA relating parasites and environmental variables (88.2% of accumulated variance explained by the first to axes) slightly linked temperature and oxygen concentration with the abundance of *Raphidascaris* sp. (left-mid part of the plot). *Hamaticolax resupinus* was slightly associated to salinity (left-mid part of the plot) (Fig. 3, B).

3.1.4 Coryphaenoides mediterraneus

Six different species of parasites were isolated, being the most abundant and prevalent parasite *H. aduncum*, followed by *H. resupinus* (Table 2).

Based on the FCA output, *Cor. mediterraneus* at spring and summer (Fig. 2, C) was characterized by *Gnathia* sp., and *H. aduncum*. Spring samples of Barcelona from the deepest station of the lower slope displayed significant higher abundance of *H. aduncum* (GZM, X^2_6=20.001, p=0.003). *Gnathia* sp. was only present in this group (Table 6).

Coryphaenoides mediterraneus at autumn (Fig. 2, D) was characterized by *H. resupinus* and Tetraphyllidea fam. gen. sp. The abundance of *H. resupinus* displayed interaction between the spatiotemporal factor and fish PAL ($GZM_{(Interaction)}$, X^2_1=4.248, p=0.039). Therefore, the differences among spatiotemporal groups for the abundance of *H. resupinus* in this species depended on fish size, autumn being the season with the significantly higher values for this parasite (GZM, X^2_1=3.980, p=0.046) (Table 6).

Table 6. Prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites, infracommunity descriptors (MSR: mean species richness, TMA: total mean abundance, MDI: mean diversity index) and histological alterations of *Coryphaenoides mediterraneus*. Different superscript letters and numbers indicate significant differences in abundance and prevalence, respectively.

	1400 – 1999 m								2000 – 2200 m					
	Summer				Autumn				Spring		Summer			
	Barcelona		Balearic Islands		Barcelona		Balearic Islands		Barcelona		Barcelona		Balearic Islands	
n	8 (8)		9 (9)		9 (7)		10 (7)		13		9 (9)		5 (5)	
Parasites	P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD	P(%)	MA ± SD
Copepoda														
Hamaticolax resupinus	-	-	11^{1}	0.11 ± 0.33^{A}	71^{1}	1.67 ± 2.92^{B}	60^{1}	1.70 ± 2.06^{B}	-	-	-	-	-	-
Sarcotaces sp.	-	-	-	-	-	-	-	-	8	0.31 ± 1.11	-	-	-	-
Isopoda														
Gnathia sp.	13	0.13 ± 0.35	-	-	-	-	-	-	-	-	-	-	-	-
Cestoda														
Tetraphyllidea fam. gen. sp.	-	-	11^{1}	0.89 ± 2.67^{A}	11^{1}	0.44 ± 1.33^{A}	10^{1}	0.10 ± 0.32^{A}	-	-	-	-	-	-
Nematoda														
Hysterothylacium aduncum	75^{1}	1.50 ± 1.31^{A}	89^{1}	3.00 ± 2.00^{A}	67^{1}	2.33 ± 2.69^{A}	70^{1}	3.70 ± 4.40^{A}	100^{1}	7.62 ± 4.43^{B}	100^{1}	2.22 ± 1.64^{A}	80^{1}	1.80 ± 1.92^{A}
Raphidascaris sp.	-	-	-	-	-	-	-	-	-	-	11	0.11 ± 0.33	-	-
Infracommunity descriptors														
MSR		0.88 ± 0.64^{AB}		1.22 ± 0.67^{AB}		1.33 ± 1.12^{A}		1.40 ± 0.52^{B}		1.08 ± 0.28^{B}		1.00 ± 0.00^{B}		0.80 ± 0.45^{AB}
TMA		1.63 ± 1.41^{A}		4.00 ± 3.35^{ABC}		4.44 ± 4.22^{ABC}		5.50 ± 4.20^{BC}		7.92 ± 4.73^{B}		2.22 ± 1.64^{AC}		1.80 ± 1.92^{C}
MDI		0.60 ± 0.15^{A}		0.13 ± 0.19^{A}		0.33 ± 0.24^{A}		0.14 ± 0.19^{A}		0.04 ± 0.14^{A}		0.00 ± 0.00^{A}		0.00 ± 0.00^{A}
Histological alterations														
Epitheliocystis	25^{1}		33^{1}		14^{1}		14^{1}				33^{1}		-	
Macrophagic centers	25^{1}		22^{1}		71^{1}		57^{1}				33^{1}		20^{1}	
-Number/mm^2		23.04 ± 38.06^{A}		22.12 ± 47.12^{A}		29.78 ± 32.98^{A}						49.10 ± 49.36^{A}		12.19 ± 27.27^{A}
-Area/mm^2		44351 ± 86967^{A}		34214 ± 74491^{A}		12149 ± 14671^{A}						51130 ± 48496^{A}		15605 ± 34893^{A}
Inflammatory reactions	13		-		-		-				-		-	

Fish PAL for this species ranged from 1.7 to 9.3 cm and did not show significant differences in the spatiotemporal factor (GLM, $p>0.05$). Parasite MSR and MDI displayed interaction between the spatiotemporal factor and fish PAL ($GLM_{(Interaction)}$, $F_{6,49}=4.143$, $p=0.002$) and ($GLM_{(Interaction)}$, $F_{6,42}=2.451$, $p=0.040$), respectively (Table 6). Therefore, the differences among spatiotemporal groups for MSR in this species depended on fish size, autumn being the season with the significantly higher values for MSR (GLM, $F_{6,49}=3.004$, $p=0.014$). Parasite TMA significantly increased with fish PAL (GZM, $X^2_1=5.976$, $p=0.016$) and was significantly higher in spring (GZM, $X^2_6=19.028$, $p=0.004$) (Table 6). The only parasite whose abundance increased with fish PAL was *H. aduncum* (GZM, $X^2_1=7.426$, $p=0.006$). The prevalence of *H. aduncum* was positively related with PAL (GZM, $X^2_1=5.124$, $p=0.024$). No spatiotemporal factor effect was observed in the fish condition parameters (Table 4). HSI was negatively related to the abundance of *H. resupinus* (GLM, $F_{1,46}=5.081$ $p=0.029$). No other factors were related to any parasite abundance.

The CCA relating parasites and environmental variables (89.8% of accumulated variance explained by the first to axes) linked oxygen concentration with the abundance of Tetraphyllidea fam. gen. sp. (upper part of the plot) (Fig. 3, C).

3.2 Diet analysis

Diets of the four macrourids examined were based, in term of occurrence, on the same prey, though the proportion of prey groups varied depending of the species. In brief (Fig. 4, broad taxa), *C. caelorhincus* based its diet in polychates (*Harmothoe* spp.) and gammaridean amphipods.

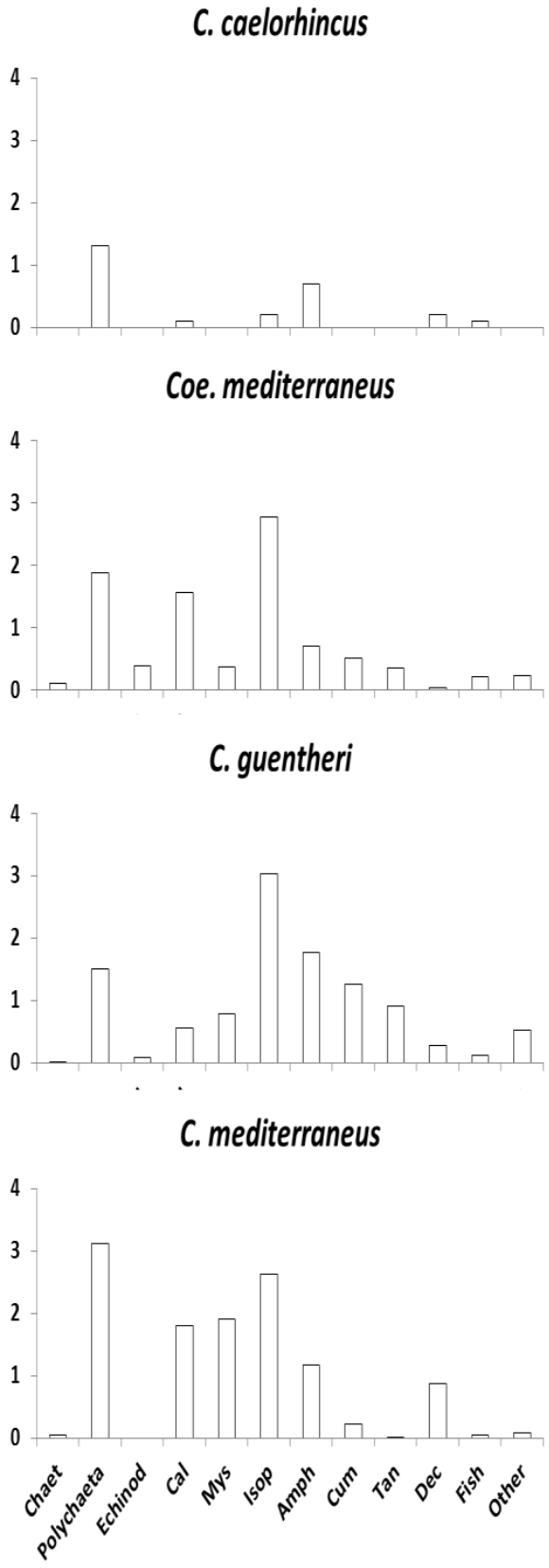


Figure 4. Diet (in number of prey x 10 individuals) of the four species of macrourids analysed in this study: *Coelorinchus caelorhinchus*, *Coelorinchus mediterraneus*, *Coryphaenoides guentheri* and *Coryphaenoides mediterraneus*. Prey were grouped in broad taxa: Chaet: chaetognaths; Echinod: Ophiuroids and asteroids; Cal: calanoid copepods; Mys: mysids; Isop: isopods; Amph: amphipods; Cum: cumaceans; Tan: tanaids; Dec: decapods (see also Figure 5 heading).

Polychaetes (*Harmothoe* spp.) were also important in *Coe. mediterraneus* diet, but this was more diversfied with isopods being the main prey-group (30.1% of prey) and calanoid copepods the third one (17.3% of prey). The two deepest species (*C. guentheri* and *Cor. mediterraneus*) preyed on the same type of prey, but, with *C. guentheri* preying on groups more linked to the substrate. Hence isopods (Asellota), amphipods, polychates and cumaceans represented 70.0% of *C. guentheri* diet. In *Cor. mediterraneus* amphipods decreased (9.8% of prey) while mysids and calanoid copepods increased as dominant prey reaching 15-16% of the total prey.

The CCA accumulated 49.6% of constrained variance (Fig. 5). CCA evidenced certain degree of segreggation among *Coe. mediterraneus* respect the other two species considered (the unique sample of *C. caelorhincus* was excluded from the analysis) associated to most abundant parasites and prey in their diets.

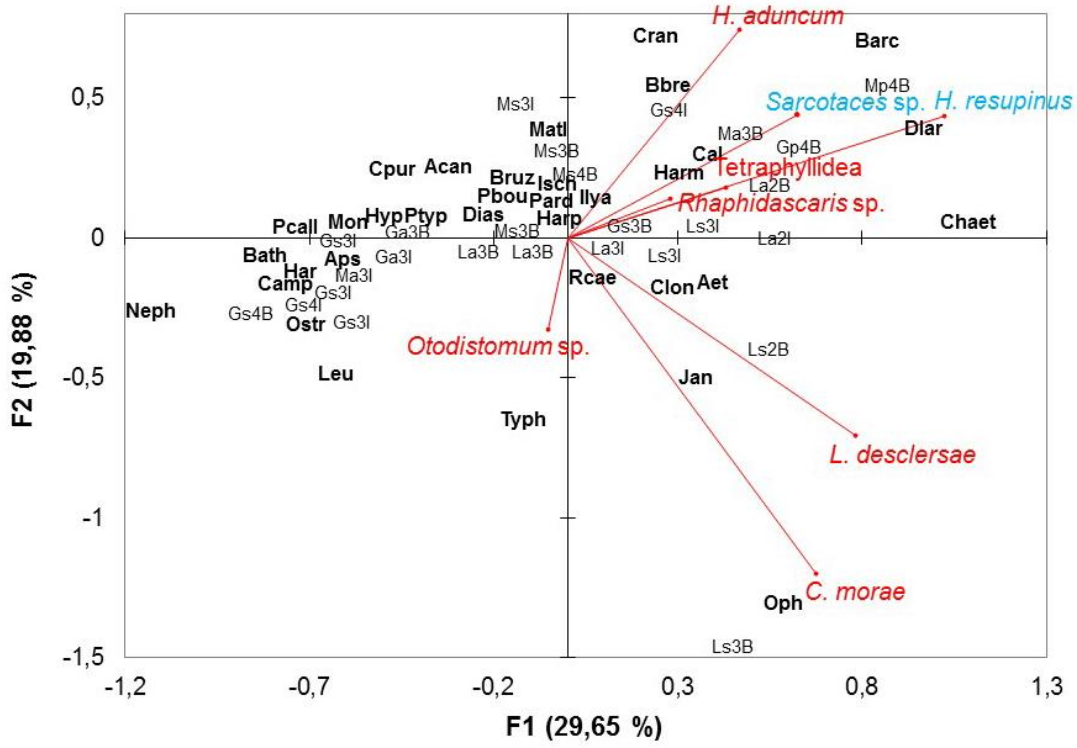


Figure 5. Canonical correspondence analysis showing relationships between the prey items of *Coelorinchus mediterraneus* (labelled as L), *Coryphaenoides guentheri* (labelled as G) and *Coryphaenoides mediterraneus* (labelled as M) and fish parasites. Abbreviations for spatiotemporal groups of each host: are defined in Figure 1. Abbreviations for prey groups: Amphipods (Bath: *Bathymedon* sp.; Har: *Harpinia* spp.; Mon: *Monoculodes* sp.; Bruz: *Bruzelia typica*; Pbou: *Pseudotiron bouvieri*; Rcae: *Rhachotropis caeca*; Pard: pardaliscids; Hyp: hyperiids); Chaet: chaetognaths; Copepods (Cal: calanoids; Aet: aetidids; Harp: harpacticoids); Cumaceans (Bbre: *Bathycuma brevirostre*; Camp: *Campylaspis* sp.; Ptyp: *Platysympus typicus*; Dias: Diastylidae; Clon: *Cyclaspis longicaudata*; Leu: leuconids); Decapods (Dlar: larvae; Acan: *Acanthephyra eximia*; Cran: crangonids); Isopods (Matl: *Munnopsurus atlanticus*; Isch: *Ischnomesus bispinosus*; Ilya: *Ilyarachna* sp.; Jan: *Janirella* sp.); Mysids (Barc: *Boreomysis arctica*; Cpur: *Calyptomma puritani*; Pcall: *Pseudomma calloplura*); Oph: Ophiuroids; Ostr: ostracods; Polychaetes (Harm: *Harmothoe* spp.; Neph: nephthydae); Tanaids (Aps: apseudids, Typh: *Typhlotanais* sp.).

The two ectoparasites included in CCA (two copepods: *Hamaticolax resupinus* and *Sarcotaces* sp.) were related (right-upper in the plot) with the most mobile (swimming) prey consumed by macrourids (Chaetognaths, decapod larvae, part of zooplankton; *Boreomysis arctica*, a bentho-bathypelagic mysid), in turn related with the species *Cor. mediterraneus* and *C. guentheri* of autumn and spring and to some *Coe. Mediterraneus* of autumn, *Cor mediterraneus* and *Coe. mediterraneus* consuming more mobile prey (including some prey of zooplankton) than *C. guentheri*. However, such relationships should not be attributable to any role by prey as intermediate vector because *H. resupinus* and *Sarcotaces* sp are ectoparasites.

Among endoparasites, the most clear prey-parasite relationship was that *L. desclersae* and *C. morae* and to lesser extent *Otodistomum* sp., were, in general, linked to *Coe. mediterraneus* and *H. aduncum* to *Cor. mediterraneus*, and *C. guentheri*. A number of parasites (upper-left part of the plot), mainly *H. aduncum* and Tetraphylidea and to lesser extent *Rhaphidascaris* sp., were related with the same more mobile prey cited above for the ectoparasites, but other parasites (*L. desclersae, C. morae* and *Otodistomum* sp.) were related in *Coe. mediterraneus* with epibenthic prey (ophiuroids, the isopod *Janirella* sp., the tanaid *Thyphlotanais* sp.). Most prey belonging to benthos (the polychaetes *Nephthydae* sp.) or less mobile suprabenthos (e.g. most cumaceans, tanaids and amphipods; even mysids like *Calyptomma puritani* and *Pseudomma calloplura* with lower natatory capacity than *B. arctica*) were (consistently with diet results) related with *C. guentheri* at the left part of the plot, where we did not found hardly parasites. So, most parasites are transmitted among deep-sea macrourids at > 1000 m, via zooplankton and swimming suprabenthos, and the rest via benthic invertebrates.

3.3 Biochemical analysis

The enzymatic analysis of AChE and LDH activities on all four fish species revealed significant differences between them (GLM, $F_{3,138}$=52.100, p=0.000) and (GLM, $F_{3.138}$=101.892, p=0.000), respectively. *Coelorinchus caelorhincus* had the lowest AChE and the highest LDH activities, while the lowest LDH activity corresponded to *Cor. mediterraneus* (Table 2).

3.3.1 Coelorinchus caelorhincus

Due to the sample limitations, a spatiotemporal assessment for muscle enzymatic activities was not possible for this species. AChE levels were negatively related with the abundance of larvae of Cucullanidae gen. sp. (GLM, $F_{1,8}$=8.351, p=0.020). No other

relationships were found between enzymatic levels and parasite load (GLM, P>0.05 in all cases).

3.3.2 Coelorinchus mediterraneus

LDH activity displayed interaction between the spatiotemporal factor and PAL ($GLM_{(Interaction)}$, $F_{6,46}$=2.512, P=0.035), therefore the differences among spatiotemporal groups for the levels of this enzyme in this species depended on fish size, being Barcelona in summer the spatiotemporal group with the significantly higher values (GLM, $F_{6,46}$=3.161, p=0.011). Differences in AChE activities were found for the spatiotemporal factor (GLM, $F_{6,53}$=3.333, P=0.007), being these values higher in summer and in the lower slope (Table 6). No relationship was seen between enzyme activities and parasitism in any case (GZM, p>0.05).

3.3.3 Coryphaenoides guentheri

The levels of AChE in this species showed an interaction between the spatiotemporal factor and fish PAL ($GLM_{(Interaction)}$, $F_{6,42}$=2.347, p=0.045). Therefore, the differences among spatiotemporal groups for AChE in this species depended on fish size, autumn in Barcelona lower slope being the group with the significantly higher levels of AChE (GLM, $F_{6,51}$=3.069, p=0.012) (Table 6). LDH levels significantly increased with PAL (GLM, $F_{1,57}$=12.944, p=0.001) and were significantly higher in spring samples of 2000–2200 m (GLM, $F_{6,57}$=3.113, p=0.010) (Table 6). No relationships were found between enzymatic activities and parasitism (GZM, p>0.005 in all cases).

3.3.4 Coryphaenoides mediterraneus

Enzymatic activities showed no variation between Barcelona and Balearic slopes, neither any relationship with parasite load (GZM, p>0.05 in all cases) (Table 6).

3.4 Histological analysis

A total of five different histological alterations were found among all four fish species: cysts of unknown etiology (CUEs) and epitheliocystis in gills; hepatocyte fusiform inclusions in liver; and macrophagic centres and inflammatory reactions in liver and spleen. CUEs were spherical in shape, with a homogeneous core of acellular, amorphous and eosinophilic material, surrounded by an acellular basophilic material. Connective tissue and capillaries were observed around the cyst. They were located on the primary gill filaments, and always one in a section of a gill arch. Epitheliocystis was characterized by the presence of hypertrophied gill epithelial cells with a large basophilic inclusion in all three hosts. The hepatocyte fusiform inclusions consisted in

discrete aggregations of slightly enlarged hepatocytes with several unknown fusiform refractive structures. Those structures were not stained by Haematoxylin and Eosin and they were located inside the lipid vacuoles. In liver and spleen, macrophagic centres consisted usually in rounded yellow-brown colored aggregations of macrophages. The number and size of these aggregations varied between all three species (see below). The inflammatory reactions from liver and spleen were rounded and consisted in foci of macrophages and neutrophiles. Some of these inflammatory reactions were granulomatous. Those granulomas were rounded in shape and displayed a calcified core surrounded by a layer of inflammatory cells and fibroblasts.

Epitheliocystis and macrophagic centres were common to *Coe.mediterraneus*, *C. guentheri* and *Cor. mediterraneus* (Table 2).

3.4.1 Coelorinchus caelorhincus

Two different histological alterations were detected in this species: CUEs on gills and hepatic fusiform inclusions, both with a prevalence of 25% (Table 2). Neither of both alterations were related with fish PAL or with parasite load (GZM, $p>0.05$ in all cases).

3.4.2 Coelorinchus mediterraneus

Epitheliocystis was detected in the gills with a prevalence of 46% being significantly higher in the middle slope (GZM= $X^2_6=22.088$, $p=0.001$). Macrophagic centers were found in liver and spleen (prevalence of 78%) and they were more prevalent in fishes with higher PAL (GZM, $X^2_1=17.935$, $p=0.000$). Moreover, the number and area of macrophagic centers per mm^2 had significant differences between spatiotemporal samples (GLM, $F_{5,20}=3.161$, $p=0.029$; GLM, $F_{5,20}=3.242$, $p=0.026$, respectively) (Table 3). In both cases, the lowest values were found in Balearic Islands in autumn at 1000–1399 m (Table 3). The area of macrophagic centers per mm^2 was negatively related to the abundance of *Otodistomum* sp. (GLM, $F_{1,24}=4.890$, $p=0.037$). Few individuals (n = 6) presented inflammatory reactions in liver.

3.4.3 Coryphaenoides guentheri

Epitheliocystis in gills had a prevalence of 32% which was significantly more present in individuals with higher PAL (GZM, $X^2_1=15.228$, $p=0.000$). Spleen and liver presented macrophagic centers (prevalence 42%) being more present in individuals with higher PAL (GZM, $X^2_1=41.277$, $p=0.000$) (Table 2). The number of centers and the area they occupied per mm^2 was significantly higher in bigger (PAL) individuals (GLM, $F_{1,119}=114.696$, $p=0.000$; GLM, $F_{1,119}=102.878$, $p=0.000$, respectively). Significant variations in spatiotemporal samples were found on the number of centers per mm^2

(GLM, $F_{5,119}$=6.987, p=0.000) and in area occupied by centers per mm^2 (GLM, $F_{5,119}$=4.812, p=0.000). The highest number and area occupied by the macrophagic centers were determined in the summer sampling in Barcelona at depth between 2000–2200 m. The lowest number of centers per mm^2 were recorded also in the summer sampling in Barcelona but at depths between 1400–1999 m and the lowest area occupied by them corresponded to the autumn sampling at the Balearic Islands (Table 5). The prevalence of macrophagic centres was negatively related to parasite TMA and MSR and to the abundance of *H. aduncum* (GZM, X^2_1=11.143, p=0.001; GZM, X^2_1=12.997, p=0.000; GZM, X^2_1=11.893, p=0.010, respectively). The number of macrophagic centres per mm^2 was also negatively related to parasite MSR (GLM, $F_{1,63}$=6.245, p=0.015). Only two individuals presented granulomatous inflamatory reactions in liver and spleen.

3.4.4 Coryphaenoides mediterraneus

Gills presented epitheliocystis with a prevalence of 22% (Table 2). Macrophagic centers were found in liver and spleen and they significantly increased both in number and area occupied per mm^2 with PAL (GLM, $F_{1,19}$=29.470, p=0.000; GLM, $F_{1,19}$=14.568, p=0.001, respectively). The prevalence of macrophagic centres was negatively related to parasite TMA, MSR and MDI (GZM, X^2_1=7.519, p=0.006; GZM, X^2_1=9.817, p=0.002; GZM, X^2_1=7.399, p=0.007, respectively). The number and area occupied per mm^2 by the macrophagic centres was positively related to the abundance of Tetraphyllidea fam. gen. sp. (GLM, $F_{1,16}$=5.785, p=0.027; GLM, $F_{1,16}$=8.629, p=0.008, respectively).

4. Discussion

This is the first study of the parasite communities of Mediterranean macrourids living at > 1000 m, which include a number of the deepest living fish in Mediterranean Sea (Stefanescu et al., 1993; Gates et al., 2012). Likewise, it is the first study that addresses the relationship between data on those parasites communities and biological traits, environmental variables and fish health (biochemical markers and histopathology). The results of this study together with the one analising the parasite communities of the macrourids *Hymenocephalus italicus*, *Nezumia aequalis* and *Trachyrincus scabrus* in the upper slope (Constenla et al., 2015), characterise the parasite fauna of this fish family in the deep-sea Mediterranea. All parasite species found in this study in *C. caelorhincus*, and *C. guentheri* represent new records for their respective hosts. All parasites except *H. resupinus* are new records for *Coe. mediterraneus. Gnathia* sp. and *Raphidascaris* sp. are new records for *Cor. mediterraneus* and *Sarcotaces* sp., Tetraphyllidea fam. gen. sp. are first time cited in

this macrourid in the Mediterranean Sea. *Coryphaenoides guentheri* was characterized by having the less rich and diverse parasite community of all four species. This is spite *C. guentheri* is the most benthofagous species analysed. Stronger benthic habitats promote high diversity and density of invertebrates that promote parasite transmission (Campbell et al. 1980; Marcogliese, 2002). In the NW Mediterranean this tendency has been observed in relation with dietary habits of deep-sea fish, with a greater richness and diversity of parasites in benthofagous (*Mora moro, Phycis blennoides,* macrourids) than in planktophagous (*Lampanyctus crocodilus*, *Alepocephalus rostratus*) species (Constenla et al., 2015; Dallarés et al., 2014, 2016; Pérez-i-García et al., 2015). Deep Mediterranean macrourids living at > 1000 m did not follow this trend, they had in general low parasite richness and diversity, which was especially obvious in the two deepest host, especially in the most benthofagous species, *C. guentheri*. This could be related with low organic matter on the sediment, with a parallel decrease of benthic prey in the Balearic basin, especially at > 1500 m depth (Cartes et al., 2009). In fact the diet of *C. guentheri* was based on suprabenthic crustaceans (swimming benthos) while benthic taxa more strictly related with mud like bivalves, gastropods or polychates were secondary as prey.

The parasite fauna of the studied fishes was poor in prevalence and richness compared with these same species in the Atlantic Ocean. For instance, *Cor. mediterraneus* accounts up to 16 parasites in the Mid-Atlantic Ridge (Kellermanns et al. 2009) and only six species in the Mediterranean Sea. From shallower depths, 11 parasite species are reported from *C. caelorhincus* in the Atlantic Ocean (Blend, 1996; Blend et al., 2000; Bray, 1973; Bray and Gibson, 1997; Carpart, 1959; Dronen et al., 1994; Gibson, 1995; Manter, 1934) versus three in the present study. Most Mediterranean fishes tend to harbour a low number of parasite species (Sasal et al., 1997), as happens also among fishes inhabiting the lower slope, e.g. *Alepocephalus rostratus* and *Bathypterois mediterraneus*, having a low parasite richness (with 7 and 5, species, respectively) and prevalence (Mateu et al., 2014; Pérez-i-García et al., 2015). The macrourids of middle and lower slope also had limited parasite communities despite they have high dietary diversities. Their parasite communities tended to be dominated by a single endoparasite species, namely *L. desclersae* in *Coe. mediterraneus* and *H. aduncum* in both *Coryphaenoides* species. A main factor for this disparity on parasite richness is likely due to the smaller size of Mediterranean fish species compared with the same species in the Atlantic, which may limit available habitat for their parasites (Poulin, 1997). This tendency was also observed within the four macrourids analysed, i.e. the smallest species, *C. guentheri*, had the poorest

parasite fauna. This is attributable to two peculiarities of the deep Mediterranean: i) the high temperature of Mediterranean deep waters, which enhances organic matter degradation, is though increase rest metabolism with a consequent low growth among deep-Mediterranean fish (as discussed in Cartes and Sardà, 1992; Stefanescu et al., 1992), and ii) the low prey availability (i.e., low density of intermediate hosts) and likely food consumption of Mediterranean deep sea fishes (Carrassón and Matallanas, 2002). Either of both factors could, directly or indirectly, restrict parasite transmission at high depths between Mediterranean organisms. It is also worthy that high proportion of macrurid parasites were in larval or juvenile stages (as metacercaria in *Otodistomum* sp.), which was again especially true in the smallest species analysed, *C. guentheri*.

Highly successful parasites such as *H. aduncum* (Klimpel and Rückert, 2005) might tend to monopolise those host parasite communities at extreme (>1500 m) depths in the Mediterranean Sea. Therefore, fish inhabitings these depths, *Coryphaenoides guentheri* and *Cor. mediterraneus*, have the same centre of gravity (around 1900 m, Stefanescu, 1992), with little geographical distance between the mainland and the insular slope in the Balearic Basin. In addition to the same habitat, *Coryphaenoides* spp. were phylogenetically very close. All these factors would explain that both species have similar parasite communities, with subtle differences due to differences in the proportion of prey consumed. A common feature of *Coryphaenoides* spp. parasite communities was also the high proportion of larvae stages found. This tendency was also observed in the other two abundant teleosts of the deep Mediterranean, *Alepocephalus rostratus* and *Bathypterois mediterraneus* (Mateu et al., 2014; Pérez-i-García et al., 2015).

Mediterranean deep waters are often considered a stable environment with some narrow seasonal variability in temperature, salinity and organic carbon fluxes (Fanelli et al., 2011; Miquel et al., 1994; Papiol et al., 2012). However, these small differences are already responsible for the distribution of megafauna in deep Mediterranean Sea (Cartes et al., 2013; Papiol et al., 2012). Near-bottom oxygen and turbidity varied seasonally (Rumolo et al., 2015) while both temperature and salinity slightly decreased with depth, especially at > 1200 m (Cartes et al., 2013). The ectoparasite *Hamaticolax resupinus* was linked to salinity in all CCA. Since most parasitic copepods have free-living naupliar stages (Boxshall, 2005), salinity could have some influence on its distribution. In addition, it is worth to note that CCA relating fish diet and parasites, *H. resupinus* was associated with other zooplanktonic prey (chaetognaths, decapod larvae), reinforcing the influence of variables defining water masses on the distribution of this parasite during its free living stages.

Hamaticolax resupinus was present in all macrourid species of the present study. This copepod was only recently reported for the first time in *Coe. mediterraneus* and *Cor. mediterraneus* in the western Mediterranean Sea (Pérez-i-García et al., 2017). Therefore, the current work represents the first record also in *C. caelorhincus* and *C. guentheri*, where it is found in less abundance than in the other hosts. This copepod represents the second Bomolochidae from macrourids worldwide, the other one being *Hamaticolax maleus* Oldewage, 1994 from *Malacocephalus laevis* (Oldewage, 1994). Despite the overlapping distribution of *C. caelorhincus* and *Coe. mediterraneus* with other Mediterranean macrourids off the upper slope, no crustacean parasites were reported in *H. italicus*, *N. aequalis* and *T. scabrus* in Constenla et al. (2015). As it was recently proposed by Pérez-i-García et al. (2017), the higher densities of *Coe. mediterraneus* and *Cor. mediterraneus* populations between 1500 and 2000 m depth in the Mediterranean Sea may facilitate the direct transmission of this ectoparasite.

Hysterothylacium aduncum is one of the most common marine parasites of the northern hemisphere and it has a high number of intermediate and final hosts (Klimpel and Rückert, 2005). This parasite is not unknown among deep sea fishes in the studied area: it has been found in *A. rostratus*, *B. mediterraneus* and *M. moro* up to 2223 m depth (Dallarés et al., 2014; Mateu et al., 2014; Pérez-i-García et al., 2015). Three macrourids of the upper slope, *H. italicus*, *N. aequalis* and *T. scabrus*, also presented this parasite (Constenla et al., 2015). From previous works and the present data, *H. aduncum* seems to be an important component of the parasite communities below a depth of 2000 m, achieving high abundances and prevalences on deep-dwellers such as *B. mediterraneus* (Mateu et al., 2014), *C. guentheri* and *Cor. mediterraneus.* The low abundance and prevalence values observed in *Coe. mediterraneus* could be related to its shallowest depth distribution regarding the two species of *Coryphaenoides* (Stefanescu et al., 1992). In addition, the nematode *H. aduncum* was associated with increasing temperatures only in *Coe. mediterraneus* as seen in the CCA. It is known that under experimental conditions temperature is an important factor affecting the survival and development rate of *H. aduncum* larvae (Køie, 1993; Möller, 1978). The slightly colder temperature found in the middle slope might make this area a little bit less suitable for the nematode. Køie (1993) highlighted the diversity of intermediate hosts of *H. aduncum* like crustaceans, polychaetes, ophiuroids and fishes. Crustaceans are first obligatory intermediate host of *H. aduncum* and they were the main prey in the diet of deep macrourids in our study. Further, *H. aduncum was linked* with crustaceans as prey (e.g. the mysid *Boreomysis arctica* or crangonid decapods) in the CCA, which suggests that these groups could be main transmissors of this

nematode for macrourids. The polychate *Harmothoe* spp. was a common prey for all macrourids analysed in our study, though it seems more related to *H. aduncum.* Under experimental conditions, these polychaetes are known to act as intermediate hosts for this nematode (Køie, 1993).

Coelorinchus mediterraneus is closely related to *Coelorinchus labiatus* (Köhler, 1896) and *Coelorinchus occa* (Goode & Bean, 1885) (Iwamoto and Ungaro, 2002). The copepod *Sarcotaces* sp. has been found in low prevalence both in the Atlantic *C. labiatus* (Bullock et al., 1986) and in *Coelorinchus mediterraneus* (present study). Parasites belonging to the genus *Lepidapedon* have been found in *Coe. mediterraneus* (*Lepidapedon arlenae*) (Bray and Gibson, 1995) and in *C. labiatus* (*L. arlenae* and *Lepidapedon rachion*) (Bray and Kuchta, 2006), in both cases from the Atlantic Ocean. In the present study, *L. desclersae* was the most abundant and prevalent parasite of *Coe. mediterraneus* and, in conjunction with *Lepidapedon guevarai*, it is one of the two *Lepidapedon* spp. that have been recorded from western Mediterranean macrourids (Constenla et al. 2015). It also presented a general higher abundance in the mainland slope in the Balearic basin. The higher complexity of trophic webs of the mainland slope respect the insular slope (Cartes et al. 2009; Fanelli et al., 2013) might promote the completition of complex life-cycles of digeneans like *L. desclersae.* Among those organisms that could act as intermediate hosts of *L. desclersae*, the diet of *Coe. mediterraneus* pointed out that it might use ophiuroids and isopods as *Janirella* sp., i.e. both epibenthic organisms .Ophiuroids, are known to potentially act as intermediate host of *Lepidapedon elongatum* (Lebour, 1908) under experimental conditions. The exposing of cercariae of *L. elongatum* to the brittle star *Ophiura albida* Forbes leaded to the recovery of metacercariae of this digenean after 24 to 48 h (Køie, 1985).

A new species of nematode, *Raphidascaris macrouri* Pérez-i-García et al. 2015, first described from *N. aequalis* and *T. scabrus* of the Mediterranean upper slope and later encountered in *H. italicus* (Constenla et al., 2015) is one of the most abundant and prevalent parasites in its hosts, being found as stage four larvae and adults. Three macrourid species of the present work harboured stage three larvae of an unidentified species of *Raphidascaris.* It is currently not possible to assign these larvae to *Raphidascaris macrouri* based only on morphological evidences, since only the adults were described. *Capillostrongyloides morae* is a nematode originally described from the gadiform fishes *Mora moro* (Risso) and *Lepidion lepidion* (Risso) (González-Solís et al., 2014). Since then, it has been reported from *A. rostratus* and the macrourids *H. italicus* and *N. aequalis* at relatively low abundances (Constenla et al., 2015; Pérez-i-García et al., 2015). The present work adds *Coe. mediterraneus* to the list of hosts for

this nematode. The pathway of this parasite to the final host is still unclear. The studies on the parasite communities of *M. moro, P. blennoides* and macrourids of middle slope carried out in the same area related the abundance of *C. morae* to different pelagic and suprabenthic organisms (Dallarés et al., 2014; 2016; Constenla et al., 2015). CCA related *C. morae* of macrourids of the lower slope with benthic organisms (ophiuroids).

Cucullanidae gen. sp. was the main parasite in prevalence and abundance of *C. caelorhincus* of the present work. In the same area of study, Pérez-i-García et al. (2015) reported high abundance of cucullanidae larvae in *A. rostratus* between 1000 and 1400 m depth. Due to the small size and lack of structures of the larvae found in both fishes, it is uncertain if they belong to the same cucullanid species. Also from the same area, *P. blennoides* harbours adults of two *Cucullanus* spp. (Dallarés et al., 2016). To date, *P. blennoides* is the only known fish species that could potentially act as final host of these cucullanid larvae due to its bathymetric distribution and fish size (Stefanescu et al., 1992). The high abundance of *Cucullanus* sp. in *C. caelorhincus* might be related to one of the main preys of this fish, *Harmothoe* spp. (polychaeta). Few cucullanid life cycles are known, but at least two species, *Cucullanus heterochrous* Rudolphi, 1802 and *Dichelyne* (*Cucullanellus*) *minutus* (Rudolphi, 1819), also use polychaetes as intermediate hosts (Køie, 2000; 2001).

In the last decade, some authors assessed the natural variability of different enzymes commonly used as biomarkers of environmental pollution in the NW Mediterranean Sea (Koenig and Solé, 2012; Koenig and Solé, 2014; Solé et al. 2010). However, of all four targeted species in the present work, only *Coe. mediterraneus* enzymatic activity of muscular AChE and LDH has been described before and the values reported are within those encountered in here. In the reported study of Koenig and Solé (2014), they also found a direct relationship between AChE and fish length, which was not confirmed in the present paper, probably due to the generally narrower fish size range in this present work. LDH activity has been described to increase with fish size in order to maintain burst-swimming capacities in bigger fish (Somero and Childress, 1980). Considering that *C. guentheri* most common preys are linked to the benthos and presumably poor mobility, it is astonishing to find relatively high levels of LDH activity, although it may be beneficial to escape from possible predators. Moreover, *Cor. mediterraneus* displayed lower LDH activity although it mainly preys on organisms with higher swimming capabilities from the suprabenthos.

The evaluation of the possible effects of parasitism on fish health by means of alterations on enzymatic activities is yet to be fully understood. While there are studies

that found a relationship between parasitism and alterations on their hosts (Marcogliese and Pietrock, 2011 and references therein), others failed to find any relation between them (Podolska and Napierska, 2006). AChE has proven to decrease its activity when the fish is under stressful conditions caused by chemical exposures and for this reason is a common biomarker for environmental monitoring (Greco et al, 2007; Koenig and Solé, 2014; Vieira et al., 2009). Despite *C. caelorhincus* AChE activity levels responded to *Cucullanus* sp. as expected since the parasite acts as a stressor for its host, a similar study carried out in *A. rostratus* from the same area found the opposite response with larvae of a cucullanid species (Pérez-i-García et al., 2015). Probably, the specific interactions between a particular parasite species and its host may be responsible of discrepancies in this field. On the other three macrurid species none of the parasites could be associated to the activities of AChE or LDH. Maybe the parasite load on all three species from highest depth did not reach a threshold on fish that could compromise their health to be reflected on the enzyme activities.

All four macrourids of this study present few histological alterations and most of them in low prevalence, which difficults to relate them to parasitism and confirms the low stressing impact by parasites in these species. For instance, the presence of most inflammatory reactions in liver may probably be associated to the occasional presence of the nematode *H. aduncum* in this organ since the alterations were more or less circular in shape. Others, such as the hepatocyte fusiform inclusions, had an unknown origin. These inclusions were similar to the hepatocellular fibrillar inclusions described by Feist et al. (2004), although these fibrillar inclusions were found inside the cytoplasm and could be stained.

The three macrourids from depths below 1000 m generally presented low prevalence of epitheliocystis, although *Coe. mediterraneus* reached high prevalence values at the mainland slope. Usually, epitheliocystis is found in lower prevalence in wild fishes than in cultured ones (Nowak and LaPatra, 2006). This pathology was first described in the freshwater fish *Cyprinus carpio* L. by Plehn, 1920, but it was not correctly identified as a bacterial disease until Hoffman et al. (1969). Several species belonging to the Chlamydiales are suspected to cause this pathology and it has been found in up to 50 wild and cultured fish species (Nowak and LaPatra, 2006). It was also found in *T. scabrus* (Constenla et al., 2015). Several factors have been suggested to affect epitheliocystis, such as temperature (Crespo et al., 1990; Nowak and Clark, 1999; Turnbull, 1993) and nutrients concentration (Nowak, 1996). Epitheliocystis was exceptionally prevalent on *Coe. mediterraneus* from the middle slope. At higher depths, where temperature was warmer, epitheliocystis was observed only on around one third

of the samples, regardless of the targeted species. Considering the high abundance of potential hosts for the Chlamydiales at the lower slope, where all three macrourids cohabit, temperature might act as a restricting factor for the bacteria.

The number of macrophagic centres is known to increase with fish age (Brown and George, 1985). So is the case in the macrouridae of the present study (excluding *C. caelorhincus*), since higher prevalence of this alteration and higher number and area occupied by them per mm^2 were described in larger specimens. Macrophagic centres also usually increase in size and frequency due to the action of different stressors on fish (Agius and Roberts, 2003). Therefore, the negative reationship between macrophagic centres and parasite community descriptors, *Otodistomum* sp. in *Coe. mediterraneus* and *H. aduncum* in *C. guentheri* in the present study would indicate that the level of stress caused by these parasites could be limited.

In summary, *C. caelorhincus*, *Coe. mediterraneus*, *C. guentheri* and *Cor. mediterraneus* showed a rather homogeneous parasite fauna represented by few taxa. Both species of *Coryphaenoides*, phylogenetically very close, had similar parasite communities, dominated by juvenile or larval stages mainly at the deepest samples. All parasites represent new records for their respective hosts, excluding *H. resupinus* for *Cor. guentheri* and *Sarcotaces* sp. and Tetraphyllidea fam. gen. sp. for *Cor. mediterraneus*. *Hysterothylacium aduncum* seems to be a key component of the parasite communities of the Mediterranean deep sea, being the most abundant parasite not only in the deepest macrourids, but also in other recent studies carried out in the same area. *Coelorinchus mediterraneus*, and *Cor. mediterraneus* exploit more mobile prey than *C. guentheri* that exploits prey more related with sediments (suprabenthos). In this way *C. guentheri* was the smallest macrourid analysed, with the poorest parasite fauna with higher proportion of larval stages. It is possible that the role of such small species, e.g. *C. guentheri*, is closer to act as intermediate host in deep-Mediterranean trophic webs. The effects of parasitism on fish enzymatic activities and histological lessions were limited which confirms no negative impact by the parasite community but also highlight the still poor knowledge about the relation between these variables. At histological level, epitheliocystis and macrophagic centres were worth mentioning as they were widely distributed in most macrourid individuals.

Acknowledgements

We are indebted with all participants in the cruises of the project ANTROMARE and the staff of the Department of Animal Biology, Vegetal Biology and Ecology of the Universitat Autònoma de Barcelona (Spain) for their assistance during the

oceanographic campaigns and in the laboratory. We would like to thank Mònica Monje and Ester Carreras for their assistance during the collection of parasites.

Funding

This study was supported by the Spanish Ministry of Science and Innovation (MICYT) project ANTROMARE (CTM2009–12214–C02–01, CTM2009–12214–C02–02). D.P. benefits of a PhD student fellowship from the Universitat Autònoma de Barcelona (UAB, Spain).

Bibliography

Agius, C., Roberts, R., 2003. Melano-macrophage centres and their role in fish pathology. J. Fish Dis. 26, 499–509. http://dx.doi.org//10.1046/j.1365-2761.2003.00485.x

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E Ltd, Plymouth.

Blend, C.K., 1996. The digenetic trematodes parasitizing macrourid fishes from the Gulf of Mexico and Caribbean Sea: An examination of their systematics, zoogeography and host-parasite ecology. M. Sc. thesis, Texas A&M University, 1–305.

Blend, C.K., Dronen, N.O., Armstrong, H.W., 2000. Six new species of *Lepidapedon* Stafford, 1904 (Digenea: Lepocreadiidae) from deep-sea macrourid fishes from the Gulf of Mexico and Caribbean Sea, with revised keys to the species of the genus. Syst. Parasitol. 45, 29–51. http://dx.doi.org//10.1023/A:1006281324802

Boxshall, G., 2005. Crustacean parasites. Copepoda (copepods), in: Rohde, K. (Ed.), Marine Parasitology. CSIRO PUBLISHING, Collingwood, pp. 123–138.

Bradford, M.D., 1976. Rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. http://dx.doi.org//10.1006/abio.1976.9999

Bray, R.A., 1973. Some digenetic trematodes in fishes from the Bay of Biscay and nearby waters. Bull. Br. Mus. (Nat. Hist.) Zool. 26, 149–183.

Bray, R.A., Gibson, D.I., 1995. The Lepocreadiidae (Digenea) of fishes from the north-east Atlantic: a review of the genus Lepidapedon Stafford, 1904. Syst. Parasitol. 31(2), 81–132.

Bray, R.A., Gibson, D.I., 1997. The Lepocreadiidae Odhner, 1905 (Digenea) of fishes from the north-east Atlantic: summary paper, with keys and checklists. Syst. Parasitol. 36, 223–228.

Bray, R.A., Kuchta, R. 2006. Digeneans from deep-sea marine teleosts off the Outer Hebrides, Scotland, including the description of *Brachyenteron helicoleni* sp. nov. (Zoogonidae). Acta Parasitol. 51(3), 169–175. http://dx.doi.org//10.2478/s11686-006-0027-3

Brown, C., George, C., 1985. Age-dependent accumulation of macrophage aggregates in the yellow perch, *Perca flavescens* (Mitchill). J. Fish Dis. 8, 135–138.

Bullock, A.M., Phillips, S.E., Gordon, J.D.M., Roberts, R.J., 1986. *Sarcotaces* sp., a parasitic copepod infection in two deep-sea fishes, *Lepidion eques* and *Coelorhynchus occa*. J. Mar. Biol. Assoc. U.K. 66, 835–843

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 83 (4), 575–583. http://dx.doi.org//10.2307/3284227

Campbell, R.A., Haedrich, R.L., Munroe, T.A., 1980. Parasitism and ecological relationships among deep-sea benthic fishes. Mar. Biol. 57, 301–313. http://dx.doi.org//10.1007/BF00387573

Carpart, A., 1959. Copépodes parasites. Expéd. Océanogr. Belg. Eaux Côtières Afr. Atl. Sud (1948-1949) III(5), 56-126.

Carrassón, M., Matallanas, J., 2002. Diets of deep-sea macrourid fishes in the western Mediterranean. Mar. Ecol. Prog. Ser. 234, 215–228.

Cartes, J., Sardà, F., 1992. Abundance and diversity of decapod crustaceans in the deep-Catalan Sea (Western Mediterranean). J. Nat. Hist. 26, 1305–1323.

Cartes, J., Fanelli, E., López-Pérez, C., Lebrato, M., 2013. Deep-sea macroplankton distribution (at 400 to 2300 m) in the northwestern Mediterranean in relation to environmental factors. J. Mar. Syst. 113–114, 75–87. http://dx.doi.org//10.1016/j.jmarsys.2012.12.012

Cartes, J., Maynou, F., Fanelli, E., Romano, C., Marmouridis, V., Papiol, V., 2009. The distribution of megabenthic, invertebrate epifauna in the Balearic Basin (Western Mediterranean) between 400 and 2300 m: Environmental gradients influencing assemblages composition and biomass trends. J. Sea Res. 61, 244–257. http://dx.doi.org/10.1016/j.seares.2009.01.005

Cohen, D.M., Inada, T., Iwamoto, T., Scialabba, N., 1990. Gadiform fishes oftheworld (order Gadiformes). An annotated and illustrated catalogue of cods, hakes, grenadiers and other gadiform fishes knowntodate. FAO Fish. Synop. 10, 1–442.

Constenla, M., Montero, F.E., Padrós, F., Cartes, J.E., Papiol, V., Carrassón, M., 2015. Annual variation of parasite communities of deep-sea macrourid fishes from the western Mediterranean Sea and their relationship with fish diet and histopathological alterations. Deep-Sea Res. I 104, 106–121. http://dx.doi.org//10.1016/j.dsr.2015.07.002

Crespo, S., Grau, A., Padrós, F., 1990. Epitheliocystis disease in the cultured amberjack, *Seriola dumerili* Risso (Carangidae). Aquaculture 90, 197–207. http://dx.doi.org//10.1016/0044-8486(90)90245-I

Dallarés, S., Constenla, M., Padrós, F., Cartes, J.E., Solé, M., Carrassón, M., 2014. Parasites of the deep-sea fish *Mora moro* (Risso, 1810) from the NW Mediterranean Sea and relationship with fish diet and enzymatic biomarkers. Deep Sea Res. I 92, 115–126. http://dx.doi.org//10.1016/j.dsr.2014.07.001

Dallarés, S., Moyà-Alcover, C.M., Padrós, F., Cartes, J.E., Solé, M., Castañeda, C., Carrassón, M., 2016. The parasite community of *Phycis blennoides* (Brünnich, 1768) from the Balearic Sea in relation to diet, biochemical markers, histopathology and environmental variables. Deep Sea Res. I 118, 84–100. http://dx.doi.org/10.1016/j.dsr.2016.11.001

D'Onghia, G., Basanisi, M., Tursi, A., 2000. Population structure, age and growth of macrourid fish from the upper slope of the Eastern-Central Mediterranean. J. Fish Biol. 56(5), 1217–1238.

D'Onghia, G., Maiorano, P., Sion, L., 2008. A review on the reproduction of grenadiers in the Mediterranean with new data on the gonad maturity and fecundity. Am. Fish Soc. Symp. 63, 169–184.

D'Onghia, G., Lloris, D., Politou, C., Sion, L., Dokos, J., 2004. New records of deep-water teleost fish in the Balearic Sea and Ionian Sea (Mediterranean Sea). Sci. Mar. 68(S3), 171–183.

Dronen, N.O., Blend, C.K., McEachran, J.D., 1994. *Echinobreviceca coelorynchae* n. gen., n. sp. (Echinobrevicecinae n. subf.), a fellodistomid from *Coelorhynchus coelorhynchus* (Macrouridae) from the Gulf of Mexico. J. Parasitol. 80(2), 309–311.

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 8895. http://dx.doi.org//10.1016/0006–2952(61)90145-9

Espinal-Carrión, T., López-López, E., 2010. Helminths and lipid peroxidation in *Astyanax aeneus* (Pisces: Characidae) from a river in the humid subtropics of southeastern Mexico. Dis. Aquat. Org. 88(3), 215–224. http://dx.doi.org//10.3354/dao02144

Fanelli, E., Cartes, J.E., Papiol, V., López-Pérez, 2013. Environmental drivers of megafaunal assemblage composition and biomass distribution over mainland and insular slopes of the Balearic Basin (Western Mediterranean). Deep-Sea Res. I 78, 79–94. http://dx.doi.org//10.1016/j.dsr.2013.04.009.

Fanelli, E., Papiol, V., Cartes, J., Rumolo, P., Brunet, C., Sprovieri, M., 2011. Food web structure of the epibenthic and infaunal invertebrates on the Catalan slope (NW Mediterranean): Evidence from δ13C and δ15N analysis. Deep-Sea Res. I 58(1), 98–109. http://dx.doi.org// 10.1016/j.dsr.2010.12.005

Feist, S.W., Longshaw, M., 2008. Histopathology of fish parasite infections-importance for populations. J. Fish Biol. 73, 2143–2160. http://dx.doi.org//10.1111/j.1095-8649.2008.02060.x

Feist, S.W., Lang, T., Stentiford, G.D., Köhler, A., 2004. Biological effects of contaminants: Use of liver pathology of the European flatfish dab (*Limanda limanda* L.) and flounder (*Platichthys flesus* L.) for monitoring. ICES TIMES, 38, p. 50.

Fernandez-Arcaya, U., Ramirez-Llodra, E., Rotllant, G., Recasens, L., Murua, H., Quaggio-Crassiotto, I. Company, J.B., 2013. Reproductive biology of two macrourid fish, *Nezumia aequalis* and *Coelorinchus mediterraneus*, inhabiting the NW Mediterranean continental margin (400–2000 m). Deep-Sea Res. II 92, 63–72.

Gates, A., Jones, D.O.B., Cartes, J.E., 2012. Video observations of deep-water benthic megafauna from the eastern Mediterranean Sea, offshore Egypt. Afr. J. Mar. Sci. 32, 215–222.

Gibson, D.I., 1995. *Allopodocotyle margolisi* n. sp. (Digenea: Opecoelidae) from the deep-sea fish *Coryphaenoides* (*Chalinura*) *mediterraneus* in the northeastern Atlantic. Can. J. Fish. Aquat. Sci. 52(S1), 90–94.

González-Solís, D., Carrassón, M., Pérez-del-Olmo, A., 2014. *Capillostrongyloides morae* sp. n. (Nematoda: Capillaridae) from deep-sea fish (Teleostei, Moridae) in the western Mediterranean Sea. Folia Parasitol. 61(1), 63–68.

Greco, L., Capri, E., Rustad, T., 2007. Biochemical responses in *Salmo salar* muscle following exposure to ethynylestradiol and tributyltin. Chemosphere 68(3), 564–571. http://dx.doi.org//10.1016/j.chemosphere.2006.12.033

Hoffman, G.I., Dunbar, C.E., Wolf, K., Zwillenberg, L.O., 1969. Epitheliocystis, a new infectious disease of bluegill (*Lepomis macrochirus*). Antonie van Leevenhoek J. Microbiol. Serol. 35, 146–158.

Iwamoto, T., Ungaro, N., 2002. A new grenadier (Gadiformes, Macrouridae) from the Mediterranean). Cybium 26(1), 27–32.

Kellermanns, E., Klimpel, S., Palm, H., 2009. Parasite fauna of the mediterranean grenadier *Coryphaenoides mediterraneus* (Giglioli, 1893) from the Mid-Atlantic Ridge (MAR). Acta Parasitol. 54(2), 158–164. http://dx.doi.org//10.2478/s11686-009-0017-3

Klimpel, S., Rückert, S., 2005. Life cycle strategy of *Hysterothylacium aduncum* to become the most abundant anisakid fish nematode in the North Sea. Parasitol. Res. 97(2), 141–149. http://dx.doi.org//10.1007/s00436-005-1407-6

Klimpel, S., Seehagen, A., Palm, H.W., Rosenthal, H., 2001. Deep-water Metazoan Fish Parasites of the World. Logos, Berlin.

Klimpel, S., Busch, M.W., Kellermanns, E., Kleinertz, S., Palm, H.W., 2009. Metazoan Deep-Sea Fish Parasites. Acta Biologica Benrodis, Supplementband II. Heinrich-Heine University Düsseldorf.

Koenig, S., Solé, M., 2012. Natural variability of hepatic biomarkers in mediterranean deep-sea organisms. Mar. Environ. Res. 79, 122–131. http://dx.doi.org//10.1016/j.marenvres.2012.06.005

Koenig, S., Solé, M., 2014. Muscular cholinesterase and lactate dehydrogenase activities in deep-sea fish from the NW Mediterranean. Mar. Environ. Res. 94, 16–23. http://dx.doi.org// 10.1016/j.marenvres.2013.11.003

Køie, M., 1985. On the morphology and life-history of *Lepidapedon elongatum* (Lebour, 1908) Nicoll, 1910 (Trematoda, Lepocreadiidae), Ophelia, 24(3), 135–153.

Køie, M., 1993. Aspects of the life cycle and morphology of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). Can. J. Zool. 71(7), 1289–1296. http://dx.doi.org//10.1139/z93-178

Køie, M., 2000. The life-cycle of the flatfish nematode *Cucullanus heterochrous*. J. Helminthol. 74, 323–328. http://dx.doi.org//10.1017/S0022149X00000470

Køie, M., 2001. The life cycle of *Dichelyne* (*Cucullanellus*) *minutus* (Nematoda: Cucullanidae). Folia Parasitol. 48(4), 304–310.

MacKenzie, K., 2002. Parasites as biological tags in population studies of marine organisms: an update. Parasitol. 124(7), S153–S163. http://dx.doi.org//10.1017/S0031182002001518

MacKenzie, K., Brickle, W., Hemmingsen, W., George-Nascimiento, M., 2013. Parasites of hoki, *Macruronus magellanicus*, in the Southwest Atlantic and Southeast Pacific Oceans, with an assessment of their potential value as biological tags. Fish. Res. 145, 1–5. http://dx.doi.org//10.1016/j.fishres.2013.03.008

Macpherson, E., 1979. Ecological overlap between Macrourids in the western Mediterranean Sea. Mar. Biol. 53, 149–159.

Manter, H.W., 1934. Some digenetic trematodes from deep-water fish of Tortugas, Florida. Publ. Carnegie Ints. Wash. 435, 257–345.

Marcogliese, D., 2002. Food webs and the transmission of parasites to marine fish. Parasitol. 124, S83–S99. http://dx.doi.org// 10.1017/S003118200200149X

Marcogliese, D.J., 2004. Parasites: Small Players with Crucial Roles in the Ecological Theater. EcoHealth 1, 151–164. http://dx.doi.org//10.1007/s10393-004-0028-3

Marcogliese, D.J., 2005. Parasites of the superorganism: Are they indicators of ecosystem health? Int. J. Parasitol. 35, 705–716.

Marcogliese, D., Pietrock, M., 2011. Combined effects of parasites and contaminants on animal health: parasites do matter. Trends Parasitol. 27(3), 123–130. http://dx.doi.org//10.1016/j.pt.2010.11.002

Marshall, N.B., 1965. Systematical and biological studies of the Macrourid fishes (anacanthini- Teleostii). Deep-Sea Res. 12, 299–322.

Massutí, E., Morales-Nin, B., Stefanescu, C., 1995. Distribution and biology of five grenadier fish (Pisces: Macrouridae) from the upper and middle slope of the northwestern Mediterranean. Deep-Sea Res. I 42(3), 307–330.

Mateu, P., Montero, F.E., Carrassón, M., 2014. Geographical variation in metazoan parasites of the deep-sea fish *Bathypterois mediterraneus* Bauchot, 1962 (*Osteichthyes*: *Ipnopidae*) from the Western Mediterranean. Deep-Sea Res. I 87, 24–29. http://dx.doi.org//10.1016/j.dsr.2014.01.008

Merrett, N.R., Marshall, N.B., 1981. Observations on the ecology of deep-sea bottom living fishes collected off northwest Africa (081–271N). Prog. Oceanogr. 9, 185–244. http://dx.doi.org//10.1016/0079–6611(80)90002-6

Miquel, J.C, Fowler, S.W., La Rosa, J., Buat-Menard, P., 1994. Dynamics of the downward flux of particles and carbon in the open northwestern Mediterranean Sea. Deep-Sea Res. I 41, 243–261.

Möller, H. (1978). The effects of salinity and temperature on the development and survival of fish parasites. J. Fish Biol. 12, 311–323. http://dx.doi.org//10.1111/j.1095-8649.1978.tb04176.x

Morales-Nin, B., 1990. A first attempt at determining growth patterns of some Mediterranean deep-sea fishes. Sci. Mar., 54(3), 241–248.

Nowak, B.F., 1996. Health of red morwong, *Cheilodactylus fuscus*, and rock cale, *Crinodus lophodon*, from Sydney cliff-face sewage outfalls. Mar. Pollut. Bull. 33, 281–292.

Nowak, B., Clark, A., 1999. Prevalence of epitheliocystis in Atlantic salmon, *Salmo salar* L., farmed in Tasmania, Australia. J. Fish. Dis. 22, 73–78.

Nowak, B., LaPatra, S., 2006. Epitheliocystis in fish. J. Fish Dis. 29, 573–588. http://dx.doi.org//10.1111/j.1365-2761.2006.00747.x

Oldewage, W.H., 1994. A New Species of *Holobomolochus* Vervoort, 1969 (Copepoda, Poecilostomatoida) from the West Coast of South Africa. Crustaceana 67(3), 324–328.

Papiol, V., Cartes, J., Fanelli, E., Maynou, F., (2012). Influence of environmental variables on the spatio-temporal dynamics of bentho-pelagic assemblages in the middle slope of the Balearic Basin (NW Mediterranean). Deep-Sea Res. I 61, 84–99. http://dx.doi.org//10.1016/j.dsr.2011.11.008

Pérez-i-García, D., Carrassón, M., Boxshall, G.A., 2017. A new species of *Hamaticolax* Ho & Lin, 2006 (Copepoda: Bomolochidae) from deep water macrourids in the Mediterranean. Syst. Parasitol. 94, 243–254. http://dx.doi.org/10.1007/s11230-016-9693-1

Pérez-i-García, D., Constenla, M., Padrós, F., Soler-Membrives, A., Solé, M., Carrassón, M., 2015. Parasite communities of the deep-sea fish *Alepocephalus rostratus* Risso, 1820 in the Balearic Sea (NW Mediterranean) along the slope and relationships with enzymatic biomarkers and health indicators. Deep-Sea Res. I 99, 65–74. http://dx.doi.org// 10.1016/j.dsr.2015.01.009

Plehn, M., 1920. Praktikum der fischkrankheiten. In: Demoll, R. and Maier, H. (Eds.), Handbuch der Binnenfischeri Mitteleuropas. E. Schweyerbart, Stuttgart, pp. 301–309.

Podolska, M., Napierska, D., 2006. Acetylcholinesterase activity in hosts (herring *Clupea harengus*) and parasites (*Anisakis simplex* larvae) from the southern Baltic. ICES J. Mar. Sci. 63(1), 161–168. http://dx.doi.org//10.1016/j.icesjms.2005.08.001

Poulin, R., 1997. Species Richness of Parasite Assemblages: Evolution and Patterns. Annu. Rev. Ecol. Syst. 28, 341–358.

Rumolo P., Cartes, J.E., Fanelli, E., Papiol, V., Sprovieri, M., Mirto, S., Gherardi, S., Bonanno, A., 2015. Seasonal variations in the source of sea bottom organic matter off Catalonia coasts (western Mediterranean): links with hydrography and biological response. J. Oceanogr. 71,325–343.

Sasal, P., Morand, S., Guégan, J.-F., 1997, Determinants of parasite species richness in Mediterranean marine fishes. Mar. Ecol. Prog. Ser. 149, 61–71. http://dx.doi.org//10.3354/meps149061

Sasal, P., Mouillot, D., Fichez, R., Chifflet, S., Kulbicki, M., 2007. The use of fish parasites as biological indicators of anthropogenic influences in coral-reef lagoons: a case study of Apogonidae parasites in New-Caledonia. Mar. Pollut. Bull. 54(11), 1697–1706. http://dx.doi.org//10.1016/j.marpolbul.2007.06.014

Sion, L., Maiorano, P., Carlucci, R., Capezzuto, F., Indennidate, A., Tursi, A., D'Onghia, G., 2012. Review of the literature on age and growth of grenadiers in the Mediterranean and new data on age of *Trachyrincus scabrus* (Macrouridae) in the Ionian Sea. J. Ichthyol. 52(10), 740–749.

Solé, M., Baena, M., Arnau, S., Carrasson, M., Maynou, F., Cartes, J.E., 2010. Muscular cholinesterase activities and lipid peroxidation levels as biomarkers in several Mediterranean marine fish species and their relationship with ecological variables. Environ. Int. 36, 202–211. http://dx.doi.org//10.1016/j.envint.2009.11.008

Somero, G.N., Childress, J.J., 1980. A violation of the metabolism-size scaling paradigm: activities of glycolytic enzymes in muscle increase in larger-size fish. Physiol. Zool. 53(3), 322–337.

Stefanescu, C., Lloris, D., Rucabado, J., 1992. Deep-living demersal fishes in the Catalan Sea (western Mediterranean) below a depth of 1000 m. J. Nat. Hist .26, 197–213.

Stefanescu, C., Lloris, D., Rucabado, J.C., 1993. Deep-sea fish assemblages in western Mediterranean. Deep-Sea Res. I 40, 695–707.

Sures, B., 2008. Environmental Parasitology. Interactions between parasites and pollutants in the aquatic environment. Parasite 15(3), 434–438. http://dx.doi.org//10.1051/parasite/2008153434

Ter Braak, C.J.F., 1986. Canonical correspondence analysis: a new eigenvector-technique for multivariate direct gradient analysis. Ecology 67, 1167–1179.

Turnbull, J.F., 1993. Epitheliocystis and salmonid rickettsial septicaemia. In: Inglis, V., Roberts, R.J., Bromage, N.R. (Eds.), Bacterial Diseases of Fish. Blackwell Scientific Publications, Boston, pp. 237–254.

Van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.

Vassault, A., 1983. Lactate dehydrogenase. In: Bergmeyer, M.O. (Ed.), Methods of Enzymatic Analysis, Enzymes: Oxidoreductases, Transferases. Academic Press, New York, NY, pp. 118–126.

Vieira, L., Gravato, C., Soares, A., Morgado, F., Guilhermino, L., (2009). Acute effects of copper and mercury on the estuarine fish *Pomatoschistus microps*: linking biomarkers to behaviour. Chemosphere 76(10), 1416–1427. http://dx.doi.org// 10.1016/j.chemosphere.2009.06.005