Tesi doctoral

Parasites of three fish species of commercial interest from the north-western Mediterranean Sea: *Mullus barbatus*, *Spicara maena* and *Trachinus draco* (Osteichthyes, Perciformes). Use as tags of environmental conditions

Marta Carreras i Aubets

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Directors:
M. Carrassón López de Letona
F. E. Montero Royo

UAB
Universitat Autònoma de Barcelona

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

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Memòria de tesi doctoral presentada per Marta Carreras i Aubets per a optar al grau de Doctor en Zoologia sota la direcció de la Dra. Maite Carrassón López de Letona i el Dr. Francisco Esteban Montero Royo.

Aquesta tesi s'ha inscrit dins del programa de doctorat d'Aqüicultura, amb menció de qualitat, de la Universitat Autònoma de Barcelona.

Els directors
Maite Carrassón López de Letona
Francisco Esteban Montero Royo

La doctoranda
Marta Carreras i Aubets

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"Hi ha una força motriu més poderosa que el vapor, l'electricitat i l'energia atòmica: LA VOLUNTAT"

Atribuïda a Albert Einstein

"El treball sempre ajuda, ja que treballar no és fer el que hom imaginava, sinó descobrir el que hom té a dins"

Boris L. Pasternak

"... i ella de dia teixia aquell gran ordit, i aleshores, cada nit, tan bon punt posava les torxes a prop, la desfeia..."

"L'Odissea", Cant II

Homer, s. IX a. C.

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1. ABSTRACT / RESUM

Changes induced in marine ecosystems as a consequence of human activity and its influence on organisms can have important effects on the abundance and quality of natural resources and therefore on the economic development.

The parasites of fish can reflect the host life habits, including their interactions with the benthic, planktonic and fish communities. In aquatic ecosystems, parasites are frequently used as biological tags since parasites can provide information on fish stock separation, fish recruitment migrations, fish diet and feeding behavior, and host phylogenetic and systematics. A typical parasite life cycle may include the fish definitive host and several intermediate invertebrate hosts, and for the parasite to survive, all hosts must co-occur in a stable community structure. Changes in environmental conditions that affect any of the hosts, directly or indirectly, will have a significant effect on the prevalence and intensity of the infection, and on the diversity of parasites which infect the fish. In this sense, parasite communities of fish have been used as comprehensive tags of ecosystem health, mainly focusing on the structure of parasite communities and their relationship to pollution.

The general target of the present thesis is twofold: on the one hand we aim to provide a better understanding of the composition and structure of parasite communities in the perciform teleosts *Mullus barbatus* L., *Spicara maena* (L.) and *Trachinus draco* L. from the north-western Mediterranean (specifically, the Catalonian coasts); on the other hand we aimed to test whether variations in parasite community structure can be related to pollution loads and/or with natural variability (geographical and temporal).

To achieve these aims, we shall develop the following concrete three objectives:

1. Exhaustive revision of the parasite fauna communities of the three fish hosts along four seasons and two localities sampled and taxonomical description of some parasites interesting for science.

2. Assessment of the usefulness of the fish parasite populations to reflect small-scale differences in the pollution loads of PCBs in a recognized sentinel species, *Mullus barbatus*, collected in two close areas of the Catalan Sea.

3. Assessment of the geographical and seasonal variations in parasite community structure and discussion about their use as ecological tags, in the bentho-pelagic fish *Spicara maena* and the benthic fish *Trachinus draco*.

Sampling took place in 2007 in the north-western Mediterranean Sea, in front of the coast of Barcelona (north-eastern Spain) on the continental shelf at depth of 50-68m, at the mouth of the Besòs River and off the coast of Vilanova i la Geltrú, within the framework of the Spanish Science and Technology Ministry project BIOMARE (CTM2006-13508-CO2-02/MAR). Fish samples for the Besòs locality were obtained at the four seasons along the year: winter (February-March), spring (April-May), summer (June-July) and autumn (October-November) in 2007. Fish collected off the coast of Vilanova i la Geltrú city, situated further south, was collected in summer (July 2007).

A total of 117 specimens of *Mullus barbatus*, 81 of *Spicara maena* and 74 of *Trachinus draco* was collected. Once on board, individuals were measured (total length) and weighted (total weight). They were immediately frozen at -20ºC in an individual plastic bag for posterior procedures in the laboratory. Thawed specimens were processed and examined for ectoparasites and endoparasites under the stereomicroscope. All parasites collected were counted and processed following parasitological procedures.

In the first part of the fifth chapter of the present thesis, the status of the trematode *Aponurus laguncula* Looss, 1907 in the western Mediterranean was re-assessed by means of a comparative morphological study and rDNA

sequences based on newly fresh collected material. *A. laguncula* (*sensu stricto*) was redescribed from *Trachinus draco* and a new cryptic species of the '*A. laguncula* complex', *Aponurus mulli* n. sp., was described on the basis of abundant material from *Mullus barbatus* (type-host) and *M. surmuletus* off the Spanish Mediterranean coasts. The new species was differentiated from *A. laguncula* (*sensu stricto*) by its: significantly larger elongate body with maximum width at the level of ventral sucker; shorter forebody; distinctly larger sinus-sac, seminal receptacle and seminal vesicle, with the latter also being more elongate; vesicular pars prostatica; more anteriorly located vitellarium, which consists of eight globular follicles; and distinctly smaller eggs, which are also smaller in relation to body size and have both opercular and anopercular poles rounded. The variability and the allometric growth of the morphological characters in the new species were studied in detail, resulting in additional distinguishing features. Sequences of the large subunit rRNA (28S) gene (domains D1–D3) and ITS2 rRNA gene region for the new species have been submitted to GenBank.

In the second part of the fifth chapter of the present thesis, two frequently reported but poorly known Hemiuridae digeneans, *Lecithochirium musculus* (Looss, 1907) (Lecithochiriinae), from the stomach of *Trachinus draco* and *Citharus linguatula*, and *Ectenurus lepidus* Looss, 1907 (Dinurinae), from the stomach of *Spicara maena*, were redescribed based on material from off the Barcelona coast of the western Mediterranean. The two species were commented upon, and *Lecithochirium israelense* Fischthal, 1980 was considered a synonym of *L. musculus*. Records of the two species in the Mediterranean Basin and North East Atlantic region were summarised.

In the sixth chapter of the present thesis, parasite communities of the Mediterranean sentinel fish species, *Mullus barbatus*, sampled at a small-scale PCB gradient at the shelf sediments, were examined. A recurrent feature at both the population and community level was the differentiation of the samples along the increasing PCB levels simultaneously registered in the sediments. Both directly transmitted ectoparasites and endoparasites with complex life-

cycles transmitted via food chains exhibited a decrease in abundance with the increase in PCB levels. Parasite numerical responses translated into significant differences in infracommunity structure with decreasing predictability associated with increasing PCB levels. The abundance of two species, the specialist digenean *Opecoeloides furcatus* and the generalist nematode *Hysterothylacium fabri*, contributed substantially to the observed dissimilarity between infracommunity samples along the gradient. The observed parasite responses to moderate levels of pollution were simultaneously validated by both chemical monitoring and biochemical biomarkers effects.

In the seventh chapter of the present thesis, we described the parasite communities of *Spicara maena* (L.) off the north-western Mediterranean, captured during a seasonal survey at Besòs River mouth and during a summer cruise near the city of Vilanova i la Geltrú, with a view of using parasite species as environmental tags. Over the seasonal survey, a total of 33 different taxa of parasites were identified. The raphidascarid nematodes *H. fabri* and *H. aduncum* were the most prevalent and abundant parasites. Nine common species with prevalence higher than 10% were identified and considered as potential tag species: the myxozoan *Unicapsula pflugfelderi*, the tetraphyllidean metacestode (traditionally reported under the collective name *Scolex pleuronectis*), the digeneans *Cardiocephaloides longicollis*, *Aphanurus stossichii* and *E. lepidus*, the nematodes *H. fabri*, *H. aduncum*, *Contracaecum* sp., and the isopod *Ceratothoa oestroides*. A recurrent pattern at both the infracommunity and component community levels was the differentiation of the samples along the spatial/temporal groups. The abundances of *H. fabri* and *H. aduncum*, two generalist parasites, contributed substantially to the observed dissimilarity between infracommunities along the seasons. The high abundance of *C. oestroides* characterised the Vilanova summer group whereas that of the metacercaria of *C. longicollis* characterised the Besòs autumn, both of them due to their life cycles. This study underlined the potentiality of the study of the parasites of *S. maena* from the Catalan Sea for ecosystem monitoring, suggesting, in this particular case, the usefulness of these four parasite species as biological tags for environmental studies.

In the eighth chapter of the present thesis, spatial and temporal variation of the parasite communities of the perciform *Trachinus draco* L. from two points of the north-western Mediterranean were studied at infracommunity and component community levels. In total, 87 fish were collected between October 2006 and August 2007. A total of 2.177 parasites corresponding to 22 categories of parasite taxa was identified. Nine species were common (prevalence higher than 10%): the myxozoan *Kudoa* sp., the digenean *Helicometra fasciata*, a tetraphyllidean metacestode (traditionally reported under the collective name *S. pleuronectis*), the nematodes *H. fabri*, *H. aduncum*, *Phyllometra globiceps*, *Contracaecum* sp. and *Ascarophis* sp., and the isopod *Gnathia* sp. A recurrent pattern at both the infracommunity and the component community levels was observed since some parasite species presented temporal changes in their infection levels, as well as differences in infection parameters between Besòs and Vilanova. Some parasite populations of *T. draco* from the north-western Mediterranean are suggested as ecological tags to discriminate its host populations and the seasonal influence in them. Specially, the nematodes *H. fabri*, *P. globiceps* and *Ascarophis* sp. contributed most to the similarity and dissimilarity in community analyses, so they are purposed for future studies in ecological biomonitoring for *Trachinus draco* from the Catalan Sea.

Els canvis que ocorren en els ecosistemes marins a conseqüència de l'activitat humana, i la seva influència en els organismes, poden tenir efectes importants en l'abundància i la qualitat dels recursos naturals, i en el desenvolupament econòmic.

Els paràsits dels peixos poden reflectir els hàbits de vida del seu hoste, incloent-hi les seves interaccions amb les comunitats bentòniques, planctòniques i les altres comunitats íctiques. En els ecosistemes aquàtics, els paràsits són usats freqüentment com a marcadors biològics, ja que els paràsits poden proporcionar informació sobre la separació dels estocs íctics, el reclutament migratori, la dieta i el comportament alimentari, així com també la filogènia i la sistemàtica de l'hoste. El típic cicle de vida d'un paràsit pot incloure un hoste íctic definitiu i diversos hostes intermediaris invertebrats, i tots ells han de coexistir en l'estructura estable de la comunitat per a la supervivència del paràsit. Els canvis en la condició ambiental que afectin qualsevol dels hostes, directament o indirecta, tindran un efecte significatiu en la prevalença i la intensitat de la infecció, i en la diversitat dels paràsits que infecten el peix. En aquest sentit, les comunitats de paràsits de peixos han estat usades com a eines integrals per a la salut de l'ecosistema, focalitzades principalment en l'estructura de la comunitat parasítica i la seva relació amb la pol·lució.

L'objectiu general de la present tesi és doble: d'una banda es preten proporcionar una millor comprensió de la composició i l'estructura de les comunitats parasítiques dels teleostis perciformes *Mullus barbatus* L., *Spicara maena* (L.) i *Trachinus draco* L. del nord-oest Mediterrani (especialment, la costa de Catalunya) i d'altra banda testar si les variacions en l'estructura de la comunitat parasítica poden estar relacionades amb les càrregues de pol·lució i/o amb la varietat natural (geogràfica i temporal).

Per conseguir aquests objectius, varem desenvolupar els següents tres objectius concrets:

1. Revisió exhaustiva de les comunitats parasítiques al llarg de les quatre estacions i les dues localitats mostrejades en tres hostes íctics i descripció taxonòmica d'alguns paràsits interessants per a la ciència.

2. Valoració de la utilitat de les poblacions de paràsits de peixos per a reflectir diferències a petita escala en les càrregues de pol·lució de bifenils policlorats (PCBs), en una reconeguda espècie bioindicadora, *Mullus barbatus*, mostrejada en dues àrees properes del mar Català.

3. Valoració de les variacions geogràfiques i estacionals en l'estructura de les comunitats de paràsits i discussió sobre el seu ús com a marcadors ecològics en *Spicara maena*, peix bentopelàgic, i en *Trachinus draco*, peix bentònic.

El mostreig va tenir lloc el 2007 al nord-oest del Mar Mediterrani, davant de la costa de Barcelona (Catalunya, nord-est d'Espanya) a la plataforma continental, a profunditats d'entre 50-68 metres, a la desembocadura del riu Besòs i la costa de Vilanova i la Geltrú, dins del marc del projecte espanyol concedit pel Ministeri de Ciència i Tecnologia, BIOMARE (CTM2006-13508-CO2-02/MAR). Els peixos de la localitat de Besòs foren obtinguts en les quatre estacions de l'any: hivern (Febrer-Març), primavera (Abril-Maig), estiu (Juny-Juliol) i tardor (Octubre-Novembre) del 2007. Els peixos mostrejats a prop de la costa de Vilanova i la Geltrú, situada més al sud, foren recollits a l'estiu (Juliol de 2007).

Es va recollir un total de 117 espècimens de *Mullus barbatus*, 123 de *Spicara maena* i 87 de *Trachinus draco*. Una vegada al vaixell, els individus foren mesurats (longitud total) i pesats (pes total). Els espècimens foren immediatament congelats a -20ºC en bosses de plàstic individuals per a posteriors procediments al laboratori. Els individus descongelats foren processats i examinats per buscar ectoparàsits i endoparàsits sota l'estereomicroscopi. Tots els paràsits recollits foren comptats i processats seguint les tècniques parasitològiques.

A la primera part del cinquè capítol d'aquesta tesi, es reavaluà l'estatus del trematode *Aponurus laguncula* Looss, 1907 a l'oest Mediterrani, per mitjà d'un estudi morfològic comparatiu i de seqüències de rDNA basades en nou material fresc recollit. A. *laguncula* (*sensu stricto*) fou redescrit en *Trachinus draco* i una nova espècie críptica pertanyent al "complexe *A. laguncula", Aponurus mulli* n. sp., fou descrita en base a l'abundant material trobat en *Mullus barbatus* (hoste tipus) i en *M. surmuletus* capturats prop de les costes Mediterrànies espanyoles. La nova espècie es diferenciava de *A. laguncula* (*sensu stricto*) pel seu: cos significativament més llarg amb la màxima amplada a nivell de la ventosa ventral; "forebody" o part anterior del cos més curta; sac del sinus distintivament més llarg; receptacle seminal i vesícula seminal amb la part posterior més allargada; *pars prostàtica* vesicular; *vitellarium* localitzat més anteriorment, i consistent en vuit fol·licles globulars; i ous distintivament més petits, els quals també eren més petits en relació a la mida corporal i tenien ambdós pols, operculat i anoperculat, arrodonits. La variabilitat i el creixement al·lomètric dels caràcters morfològics en la nova espècie foren estudiats en detall, obtenint caràcters distintius addicionals. Les seqüències de la subunitat gran de rRNA (28S, dominis D1–D3) i de ITS2 de la nova espècie foren dipositades al GenBank.

En la segona part del cinquè capítol d'aquesta tesi, es varen redescriure dos digenis Hemiuridae que són freqüentment citats però molt poc coneguts, en base al material capturat a la costa de Barcelona de l'oest Mediterrani, *Lecithochirium musculus* (Looss, 1907) (Lecithochiriinae), trobat a l'estómac de *Trachinus draco* i *Citharus linguatula*, i *Ectenurus lepidus* Looss, 1907 (Dinurinae), trobat a l'estómac de *Spicara maena*. Es varen descriure les dues espècies i es va considerar que *Lecithochirium israelense* Fischthal, 1980 era sinònim de *L. musculus*. Es presentaren també totes les cites existents de les dues espècies de les regions del Mediterrani i del Nord- est Atlàntic.

En el sisè capítol d'aquesta tesi, s'examinaren les comunitats parasítiques de *Mullus barbatus* L., una espècie mediterrània bioindicadora, mostrejada en un gradient de variacions a petita escala de PCBs en els sediments de la plataforma continental. La diferenciació de les mostres al llarg

dels nivells creixents de PCBs en sediments fou un caràcter recurrent en ambdós nivells estudiats, poblacional i d'infracomunitat. Tant els ectoparàsits transmesos directament com els endoparàsits amb complexes cicles de vida transmesos per mitjà de les cadenes alimentàries presentaren un descens en l'abundància amb l'increment dels nivells de PCBs. Les respostes numèriques dels paràsits es correspongueren amb diferències significatives en l'estructura de la infracomunitat, amb una predictibilitat menor associada als nivells creixents de PCBs. L'abundància de dues espècies, el digeni especialista *Opecoeloides furcatus* i el nematode generalista *Hysterothylacium fabri*, varen contribuir substancialment a la dissimilaritat observada entre les mostres de la infracomunitat al llarg del gradient. Les respostes dels paràsits als nivells moderats de pol·lució observades foren validades simultàniament amb el monitoratge químic i amb l'efecte dels biomarcadors bioquímics.

En el setè capítol d'aquesta tesi, vàrem descriure les comunitats de paràsits de *Spicara maena* (L.), del nord-oest Mediterrani, capturades durant un mostreig estacional fet a la desembocadura del riu Besòs i durant una campanya d'estiu a prop de la ciutat de Vilanova i la Geltrú, amb una visió en l'ús dels paràsits com a marcadors ambientals. Durant el mostreig estacional, es van identificar un total de 33 tàxons d'espècies paràsites. Els raphidascaridae *H. fabri* i *H. aduncum* foren els paràsits més prevalents i abundants. S'identificaren nou espècies com a comunes, amb una prevalença superior al 10%, i es consideraren espècies potencialment marcadores: el mixozou *Unicapsula pflugfelderi*, un metacestode Tetraphyllidea (citat tradicionalment sota el nom col·lectiu de *S. pleuronectis*), els digenis *Cardiocephaloides longicollis*, *Aphanurus stossichii* i *E. lepidus*, els nematodes *H. fabri*, *H. aduncum*, *Contracaecum* sp., i l'isòpode *Ceratothoa oestroides*. El patró recurrent tant a nivell de comunitat component com de infrapoblació fou la diferenciació de les mostres al llarg dels grups espacial/ temporals. Les abundàncies d' *H. fabri* i *H. aduncum*, dos paràsits generalistes, va contribuir substancialment a la dissimilaritat observada entre infracomunitats al llarg de les estacions. L'alta abundància de *C. oestroides* va caracteritzar el grup de Vilanova estiu mentre que la de la metacercària de *C. longicollis* va caracteritzar el grup Besòs tardor, cadascuna d'elles a causa del seu cicle de

vida, respectivament. Aquest estudi subratllà la potencialitat de l'estudi dels paràsits de *S. maena* del mar Català en el monitoratge de l'ecosistema, suggerint, en aquest cas particular, la utilitat d'aquestes quatre espècies paràsites com a marcadors biològics per estudis ambientals.

En el vuitè capítol d'aquesta tesi, s'estudiaren les variacions espacials i temporals de les comunitats de paràsits del peix perciforme *Trachinus draco* provinent de dos punts del nord-oest Mediterrani, als nivells de comunitat component i d'infracomunitat. Es recolliren en total 87 espècimens hoste entre octubre de 2006 i agost de 2007. S'identificaren un total de 2.177 paràsits corresponents a 22 categories taxonòmiques, i es designaren nou espècies comunes (prevalença superior al 10%): el mixozou *Kudoa* sp., el digeni *Helicometra fasciata*, un metacestode Tetraphyllidea (citat tradicionalment sota el nom col·lectiu de *S. pleuronectis*), els nematodes *H. fabri*, *H. aduncum*, *Phyllometra globiceps*, *Contracaecum* sp., *Ascarophis* sp., i l'isòpode *Gnathia* sp. Es va observar un patró recurrent a tots dos nivells, de comunitat component i d'infracommunitat, ja que alguns paràsits presentaven canvis temporals en els nivells d'infecció, així com també s'observaren diferències en els paràmetres d'infecció entre Besòs i Vilanova. Algunes poblacions paràsites de *T. draco* del nord-oest Mediterrani es suggeriren com a marcadors ecològics per discriminar les seves poblacions d'hostes i la influència estacional. Especialment, els nematodes *H. fabri*, *P. globiceps* and *Ascarophis* sp. foren els que varen contribuir més en les anàlisis de similaritat i dissimilaritat, per tant foren proposats per a futurs estudis de monitoratge ecològic en *T. draco* del mar Català.

1. INTRODUCTION

2.1. The continental Shelf of the north-western Mediterranean Sea

The Mediterranean is a semi-enclosed sea with a mean depth of about 1.500m, representing 0.7% of the surface of the world's oceans, and 0.3% of their volume (Bethoux et al., 1999). Because it is relatively isolated from oceanic advection, the Mediterranean Sea offers an effective opportunity to monitor climate and environmental changes (Bethoux et al., 1999). The Catalan Sea is enclosed within the Balearic Sea (NW Mediterranean) and extends from the Balearic Islands to the province of Girona in the north of Catalonia and the Cape Nao in the south. The continental shelf, to the north of Ebro River in Tarragona, is narrow to about 15-25 km, and appears crossed by numerous submarine canyons (Serra, 1981).

2.2. Anthropogenic impacts

The functional integrity of marine ecosystems and the sustainable management of their natural resources are frequently threatened, especially in heavily polluted areas, such as the Mediterranean Sea, subject to historical anthropogenic impacts (deforestation, river damming, etc.). Diminishing the effect of pollution on organisms and ecosystems constitute nowadays one of the key topics to improve quality of life in developed countries. Changes induced in marine ecosystems as a consequence of human activity and its influence on organisms can have important effects on the abundance and quality of natural resources and therefore on the economic development, since they can alter both biodiversity and the functioning of ecosystems and their carrying capacity.

Biodiversity in the Mediterranean has been undergoing significant changes as a result of anthropogenic and climatic influences (Bethoux et al., 1999). Many changes concern additions of species mainly through Lesseptian migrating from the Red Sea (more than 300 new species have already migrated and become established in the eastern basin), although exotic species have also arrived by means of accidental introductions into the western basin. The shallow soft-bottom sediments on the coasts of Catalonia are continuously stressed by man-made disturbances such as trawling fisheries, dredging and

recreation activities and offshore construction, so the associated communities are permanently affected.

Eutrophication in polluted coastal areas tends to reduce species richness, while climatic warming is favoring the development of a more tropical fauna (Galil, 1993). These biological changes concern both fish and phytoplankton that produce nuisance and toxic blooms with concomitant socio-economic consequences for fishing industry and tourism. Growing concern about human influence on marine ecosystems conflicts with our inability to separate manmade impacts from natural change (Duarte et al., 1992). Environment managers need, as a consequence, new tools or criteria to properly monitor and manage the health status of marine ecosystems, in order to find measures to minimize risks and impacts.

Mediterranean coastal waters suffer the deepest ecological problems since they concentrate the major part of human activities, such as maritime traffic, mineral and marine sources extraction, recreational activities, chemical effluents, and urbanization of the coast. Fishing activities are often an important impact on marine communities. Soft bottoms of continental shelves are highly disturbed by trawl fishing (Gray et al., 2006). Many of these habitats might be essential fish habitats that are important for refuge, feeding, growth and recruitment of commercial species, or sensitive habitats that support highly vulnerable organisms which are often of biological interest (de Juan et al., 2011). Although the fishing activities can determine the main impacts on sea communities, the increase of human population and human activities exert a strong pressure on marine communities (pollution, large scale impacts determined by climate change, changes in water masses and wrong management of hydrological resources) with evident effects not only on coastal ecosystems but also on deep sea.

Knowledge of species composition changes at different time scales is crucial to understanding the dynamics of marine communities. Due to the scarceness of a previous historic data baseline on ecosystems functioning, trials to evaluate human impact on marine systems are often extremely difficult

to be successfully applied. Thus, studies focused in anthropogenic impact on the marine ecosystem should take into account the basic knowledge of ecosystems natural dynamics.

The rivers that discharge near the cities are highly polluted, and most of the waste discharged by them accumulates in the nearshore area. This situation is aggravated by the sewers discharging along the city littoral. Domestic wastewater treatment plants do not contribute to improving the quality of the littoral waters. Many of the contaminants are associated with sludge and river particles, so the transport and fate of the contaminants are also associated with the transport and deposition of the suspended particulate matter (SPM) discharged into the sea (Palanques and Diaz, 1994). After sedimentation, contaminants and waste can be resuspended either by bioturbation or by physical erosion. The final situation is the effect of a dynamic interaction between anthropogenic input and natural processes.

Thus, continental shelves influenced by densely populated coastal areas and located off rivers mouths experience complex interactions between continental influences, marine processes and direct and indirect anthropogenic impacts. Studies around the world have demonstrated that changes in the fluvial discharge and human activities modify the oceanographic conditions of the shelf waters and the accumulated seafloor sediments (Dounas et al., 2007; Hartwell, 2008). The Barcelona continental shelf, off the Barcelona city, is a good area to study these complex relationships, since it is under the influence of two typical Mediterranean rivers, Llobregat and Besòs, the second being the sampling area of this thesis. About five million people live in the Barcelona metropolitan area (World Gazetteer, 2012). The major sources of metals on this continental shelf are the Besòs River, the littorals sewers and the pipeline of the Barcelona-Besòs wastewater treatment plant (Palanques, 1994).

The persistent organic pollutants (POPs) are ubiquitous marine pollutants reaching the marine environment through rivers and continental runoff in coastal zones and in the open sea by atmospheric deposition. They have four main basic features: their toxicity, their persistence, their capacity for long-range

transport and their capacity to bioaccumulate in throphic chains. Once in the marine environment, these hydrophobic substances are absorbed by organisms or adsorved in suspended particles that will deposit in the bottom sediments and will accumulate in benthic organisms, entering again in the food chain (Nhan et al., 1997). Halogenated aromatic hydrocarbons (HAHs), such as polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), enter the environment from a number of potential sources. These hydrophobic chemicals are highly persistent in the environment and have a strong affinity with sediments and a high potential for accumulating in biological tissues (Eljarrat et al., 2001).

The contamination of sediments may also pose a severe risk to aquatic organisms, which tend to bioaccumulate PCDDs, PCDFs, PCBs and polycyclic aromatic hydrocarbons (PAHs), and to wildlife and humans through the ingestion of contaminated fish and sellfish. Following a sampling study done in the north-western Mediterranean, PCBs were detected in sediments at concentrations ranging from 1.1 to 311 ng/g dry weight, whereas PAHs concentrations ranged between 13.4 ng/g d.w. and 17ng/g d.w., being the Besòs area the most contaminated (Eljarrat et al., 2001). Furthermore, although the use and production of some organohalogen compounds have been restricted (chlorinated paraffins (CPs)) or banned (polychlorinated napthalenes (PCNs)), Castells et al. (2008) reported levels of PCNs and CPs in all the marine sediments analyzed in the Barcelona coast, especially near the submarine emissary, showing their ubiquity in the north-western Mediterranean coastal area, and suggesting a diffuse but uniform inputs of these pollutants to the marine environment.

Nevertheless, there is still poor knowledge on dynamic processes (e.g. in terms of persistence or bioaccumulation) or ways followed by different pollutants of anthropogenic origin until they accumulate in tissues of fish and other marine predators. Likewise, detailed knowledge about trophic fluxes in marine ecosystem is still scarce, and not only in deep water, but also in shallow (continental shelf) ecosystems (Grémare et al., 1997).

2.3. The marine fish parasites as pollution or ecological tags

The parasites of fish can reflect the life habits of the fish, including their interactions with the benthic, planktonic and fish communities (Landsberg et al., 1998). In aquatic ecosystems, parasites are frequently used as biological tags for various aspects of host biology. Parasites can provide information on fish stock separation, fish recruitment migrations, fish diet and feeding behavior, and host phylogenetic and systematics (Williams et al., 1992; Barber et al., 2000). There is an increasing interest in using parasite species as biological or ecological tags of fish host population or as indicators of local pollution (Overstreet, 1997; Marcogliese, 2005; Sasal et al., 2007; Thilakaratne et al., 2007). The control of fish health can be used as an indicator of possible changes in the aquatic ecosystem. In addition, the study of the effects of environmental factors allows a better understanding of host-parasite relationship and of the general ecology of the system (Sasal et al., 2004).

2.3.1. Pollution tags (bioindicators)

Historically, parasitological papers have dealt with parasites as a threat for the health of the fish, but since 1980 there has been a general concerning with the relationship between pollution and parasitism in the aquatic environment (see references in Sures, 2001). Parasites are indeed important components of any ecosystem that not only play key roles in population dynamics and community structure, but that can provide information on environmental stress, food web structure and function, and biodiversity (Marcogliese, 2003, 2004).

A bioindicator is a biological response that gives a measure of pollutant exposure or toxic effect, at the molecular, cellular, or population/ecosystem level. In general terms, responses at lower biological organization levels (e.g. molecular and biochemical responses) are more specific, sensitive, reproducible and easier to determine, but more difficult to relate to ecological changes and generally lack realism. On the other hand, responses at higher biological organization levels (e.g. population and community responses) are directly indicative of ecosystem health and hence, much more relevant to

environment management (Van der Oost et al., 2003; Au, 2004). Parasitic composition responses have an intermediate location, they are relatively easy to determine, and can be related to health and fitness of individuals (Blasco, 1999) which, in turn, allows further extrapolation to population/community effects. Thus, bioindicators are considered species that reflect environmental impact because they respond to habitat alterations with changes in physiology or chemical composition (Vidal-Martínez et al., 2009).

Interest in biological diversity has recently increased in response to the damage caused to ecosystems by anthropogenic activities. For conservation purposes, a big number of marine species has traditionally been used as a surrogate for monitoring species diversity (Mérigot et al., 2007). It has been widely demonstrated that fish from contaminated environmental can exhibit the degradation conditions through different alterations (Khan and Payne, 1997; Landsberg et al., 1998; Lafferty and Kuris, 1999; Sures, 2004; Khan and Billiard, 2007; Lafferty, 2008; Vidal-Martínez et al., 2009; Khan, 2010). The majority of these investigations have focused on the effects of pollution (eutrophication, pulp-mill effluent, oil, acid precipitation, sewage and heavy metals) on the parasites of fishes, mostly ciliates, monogeneans, cestodes, digeneans, acanthocephalans and nematodes. Following Lafferty (1997), ciliates and nematodes should be sensitive indicators of eutrophication and thermal effluent, while digeneans and acanthocephalans should make good indicators of heavy metals and human disturbances.

A typical parasite life cycle may include the fish definitive host and several intermediate invertebrate hosts, and for the parasite to survive, all hosts must co-occur in a stable community structure. Changes in environmental conditions that affect any of the hosts, directly or indirectly, will have a significant effect on the prevalence and intensity of the infection, and on the diversity of parasites which infect the fish (Steedman, 1994; Mackenzie et al., 1995; Marcogliese and Cone, 1997; Marcogliese, 2004). In this sense, parasite communities of fish have been used as comprehensive tags of ecosystem health, mainly focusing on the structure of parasite communities and their relationship to pollution (Woo, 1999; Dzikowski et al., 2003).

Environmental disturbance can have a positive, negative or neutral effect on parasites, depending on the type of pollution and parasite taxa (Hernández et al., 2006). Pollution can increase parasitism if, for example, host defense mechanisms are negatively affected, thereby increasing host susceptibility, or by simply increasing the population densities of suitable intermediate or final hosts (Lafferty and Holt, 2003). Furthermore, effects of pollution can vary between parasite species and between developmental stages because larval and adult parasites could be affected in different ways. As Lafferty (1997) supports, a pollutant may also kill sensitive free-living stages of the parasite, such as miracidia or cercarie, leading for example to a lower prevalence of a trematode in intermediate-host snails. However, pollution can also decrease parasitism provided that: *(i)* infected hosts suffer more from environmental exposure than do uninfected hosts (Khan and Thulin, 1991; Lafferty, 2008); *(ii)* parasites are more susceptible to the particular pollutant than their host (Stadnichenko et al., 1995; Sures et al., 1997; Sures, 2001); or *(iii)* pollution drives the necessary intermediate and final hosts to become extinct (Lansdberg et al., 1998). Thus, parasites may be key in elucidating the impact of disturbances on whole communities and ecosystems, and in understanding the response to environmental disturbance by animals in the food web (Marcogliese, 2003).

Since there are many natural factors affecting the prevalence, infection intensity and biodiversity of parasites, its potential usefulness for the monitoring of pollution has been widely and controversially discussed (Kennedy, 1997; Overstreet, 1997). The heterogeneous array of potential effects of stress on infectious disease makes it unclear how a particular stressor should affect the overall course of an epidemic in a host population, or endemic levels of a disease. Although stressed individuals should be more susceptible to infection if exposed, the stressor could simultaneously reduce opportunities for infection because the contact rate between infected and uninfected individuals will decline with the extent that the stressor reduces host density (Lafferty and Holt, 2003). Nevertheless, researches become increasingly aware of the utility of parasites as sensitive indicators in environmental impact studies (Dusek et al., 1998).

2.3.2. Ecological tags

Studies that connect host and parasite composition variables provide also identification of ecological stocks and natural environmental variability. They contribute to the understanding of biogeographic patterns of the host, habitat use, host food and feeding mechanisms, and their integration with other members of host community (George-Nascimento, 1996; Vickery and Poulin, 1998; Poulin and Morand, 2000). For these reasons, parasites have been used as population tags for marine fish species (George-Nascimiento, 2000). Such knowledge is also fundamental to understanding the population dynamics and ecology of species, which will lead to a greater comprehension of their resilience and possible responses when faced with environmental change (Moore et al., 2003, Moore et al., 2012).

A major field of application is the stock discrimination: spatial and/or temporal comparison of parasite burdens allows delimitation of ecological stocks (Ferrer-Castelló et al., 2007). The parasite specificity to their host or their strict environmental requirements has been used to separate stock populations (Overstreet, 1997; Moore, 2002). The basic principle underlying the use of parasites as tags in fish population studies is that fish become infected with a parasite when they are within a parasite endemic area, in which conditions are suitable for the transmission of the parasite (Mackenzie and Abaunza, 1998). Parasite communities are highly complex ecological systems, driven by multiple ecological and evolutionary processes that interact across different spatial and temporal scales to create intricate assemblages with many interrelating entities (Vales et al., 2010). These features make recurrent patterns and general mechanisms less likely or difficult to identify (Poulin, 2007). Many parasite species can live in many different hosts and the dynamics of parasite populations and of communities they form can be quite different depending on the host characteristics. Parasite species richness and abundance can vary geographically for the same host species (Vales et al., 2010) and it can be influenced by the characteristics of the local ecosystem and its trophic web (Luque and Poulin, 2004). Thus, the existence of spatial variability in the composition and abundance of fish parasite assemblages constitutes the basis

for the use of parasites as biological tags for fish stock discrimination (Power et al., 2005). Stocks may differ from one to another in having different nursery, feeding, or spawning grounds, or possibly in some other forms of behaviour (Williams et al., 1992).

The use of parasites for stock discrimination requires, first, selecting appropriate parasite species. Most authors agree that suitable candidate taxa should at least exhibit significantly different levels of infection between geographical areas, do not reproduce in or on their hosts, persist in or on them for long periods (more than a year) and maintain relatively constant levels of infection from year to year (see Ferrer-Castelló et al., 2007 and references therein). However, these requirements are rarely found in most cases, and, therefore, a compromise is usually sought. The traditional methodology focuses on the differences between parasite populations/communities between zones, but similarities are commonly ignored. Similarity in species composition among parasite communities is expected to decrease with increasing distance between them (Poulin, 2003). This negative relationship is the outcome of ecological and evolutionary phenomena shaping spatial patterns in biodiversity and biogeography.

On the other hand, parasite communities also experience temporal structural changes related to seasonal variations in biotic and abiotic environmental factors and these variations can be reflected in species composition and density over time (Zander, 2004; 2005). Most studies designed to examine the seasonal variation in parasite community composition have been carried out in fishes from temperate regions (see Violante-González et al., 2008 and references therein). Many processes have been suggested to influence the seasonal variation in parasite communities in these regions, for example, temperature and other abiotic factors, intermediate host abundance, changes in host abundance, reproduction and feeding behaviour, and immunity. In relation to this, understanding temporal patterns of parasite assemblages is critical when using parasites as biological tags, as temporal changes in parasites assemblages may also confound differences between locations if

sampling is conducted across different seasons and years (Timi and Lanfranchi, 2009).

Otherwise, examination of parasite assemblages may reflect alterations in food web structure and functions that result from the countless ecological disturbances to host distributions, water levels, eutrophication, stratification, oceanic currents, and resulting human interference that are predicted to accompany climatic change (Marcogliese, 2005). Thus, by the nature of their different life cycles, parasites in a host population provide information about the role of the host in the food webs (Marcogliese, 2003). Since many helminth parasites exhibit some degree of specificity to their intermediate hosts, the presence of such parasites in fish indicates predation on particular organisms (Williams et al., 1992). Whereas the examination of stomach contents show only the very recent food items eaten by the fish, parasites give an indication of the diet over a much longer period of time.

2.4. Habitat and distribution of the demersal fish species studied

Mullus barbatus L.

The red mullet *Mullus barbatus* L. (Perciformes, Mullidae) is distributed in the eastern Atlantic (from British Isles (occasionally Scandinavia) to Dakar, Senegal and Canary Islands), Mediterranean Sea, Black Sea, and it is also known from the Azores (Fig. 1). This benthic species is found grazing on gravel, sand and, mostly, on muddy bottoms of the continental shelf, in depth range of 5 to 350m, usually from 100 to 300m. This is one of the most common commercial species caught by trawling in the Mediterranean Sea and it is characteristic of the middle part of the continental shelf, within the local thermocline. Thus, it is a dominant species in the shallower 50m depth zone in the Greek waters but it also dominates the depth zone around 100m (Kallianotis et al., 2000). In the Catalan Sea its abundance is higher than 15% in the total fish abundance (F. Maynou, pers. comm.).

Figure 1. *Mullus barbatus* L.

Spicara maena (L.)

The blotched picarel *Spicara maena* (L.) (Perciformes, Centracanthidae) is distributed in the Eastern Atlantic (Portugal, Morocco, and Canary Islands), Mediterranean and Black Sea (Fig. 2). This pelagic-neritic species, with a depth range of 30 to 130m, usually lives in coastal waters, along a wide range of sandy and muddy bottoms. Although it is usually captured by trawling arts, it has a minor commercial importance since it has a scarce gastronomic value but it possess a distinguished value in game fishing. Abundance of *S. maena* in the north-western Mediterranean is outstanding: in the Ligurian Sea its abundance data accounted for 84% of the whole stock, being the most abundant species (Guidetti et al., 1998) whereas in the Catalan Sea, at 50-60m depth, its abundance is around 11% in the total fish abundance (F. Maynou, pers. comm.).

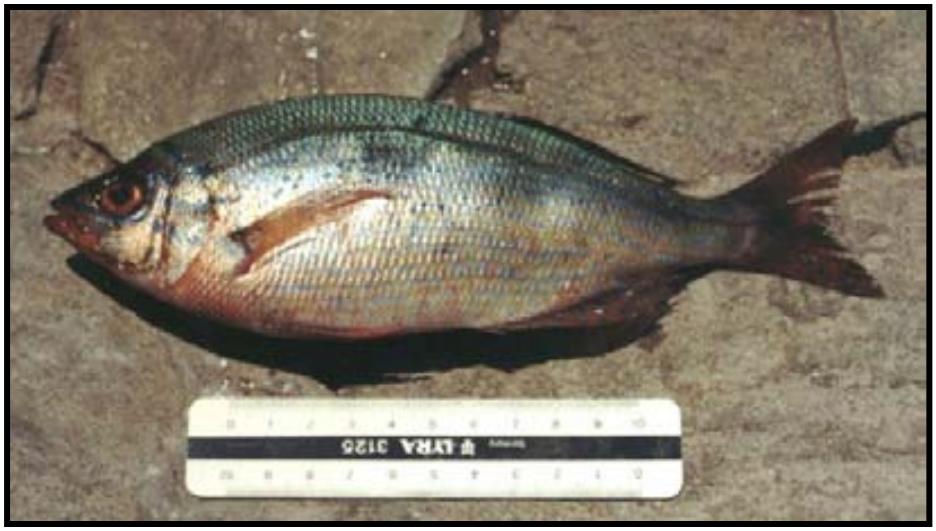

Figure 2. *Spicara maena* (L.)

Trachinus draco L.

The greater weaver *Trachinus draco* L. (Perciformes, Trachinidae) is distributed in Eastern Atlantic (from Norway to Morocco, Madeira and Canary Islands), Mediterranean Sea, Black Sea and it has been also reported from Mauritania (Fig. 3). This benthic species is found on sandy, muddy or gravelly bottoms, from a few meters to about 150m. The body is on the bottom, often buried with eyes and tip of first dorsal fin exposed, and at night it swims around pelagically. Although this species has a low commercial value, it possesses an important weight in the trawling arts along the Mediterranean fishing ports, as well as in fishing games and public aquariums exposition. Abundance of *T. draco* in the south-western Mediterranean reaches the 36% of the whole stock (Portillo et al., 2008) in captures from 50 to 164m, whereas in the Catalan Sea, its abundance in the continental platform at 60m depth do not reach the 1% of the total fish abundance, probably due to its deeper distribution range (F. Maynou, pers. comm.).

Figure 3.*Trachinus draco* L.

2. AIM AND OBJECTIVES

The general target of the present thesis is twofold: on the one hand we aimed to provide a better understanding of the composition and structure of parasite communities in the perciform teleosts *Mullus barbatus* L., *Spicara maena* (L.) and *Trachinus draco* L. from the north-western Mediterranean (specifically, the coasts of Catalonia); on the other hand we aimed to test whether variations in parasite community structure can be related to pollution loads and/or with natural variability (geographical and temporal).

To achieve these aims, we shall develop the following concrete three objectives:

1. Exhaustive revision of the parasite fauna communities of the three fish hosts along four seasons and two localities sampled and taxonomical description of some parasites interesting for science.

2. Assessment of the usefulness of the fish parasite populations to reflect small-scale differences in the pollution loads of PCBs in a recognized sentinel species, *Mullus barbatus*, collected in two close areas of the Catalan Sea.

3. Assessment of the geographical and seasonal variations in parasite community structure and discussion about their use as ecological tags, in the bentho-pelagic fish *Spicara maena* and the benthic fish *Trachinus draco*.

4. MATERIALS AND METHODS

4.1. Sampling area and fish collection

Sampling took place in 2007 in the north-western Mediterranean Sea, in front of the coast of Barcelona (Catalonia, north-eastern Spain) on the continental shelf at depth of 54-68m (Fig. 4), at the mouth of the Besòs River (further on referred as Besòs) and off the coast of Vilanova i la Geltrú (further on referred as Vilanova), at depths between 60-63m, within the framework of the Spanish Science and Technology Ministry project BIOMARE (CTM2006-13508-CO2-02/MAR). Fish samples from the Besòs locality were obtained at the four seasons along the year: winter (February-March), spring (April-May), summer (June-July) and autumn (October-November) in 2007. Fish sampled off the coast of Vilanova, situated further south (Fig. 4), were collected in summer (July 2007). The Besòs site is located near a large city (Barcelona) and a submarine emissary whereas Vilanova site is located near a smaller city devoted to fisheries.

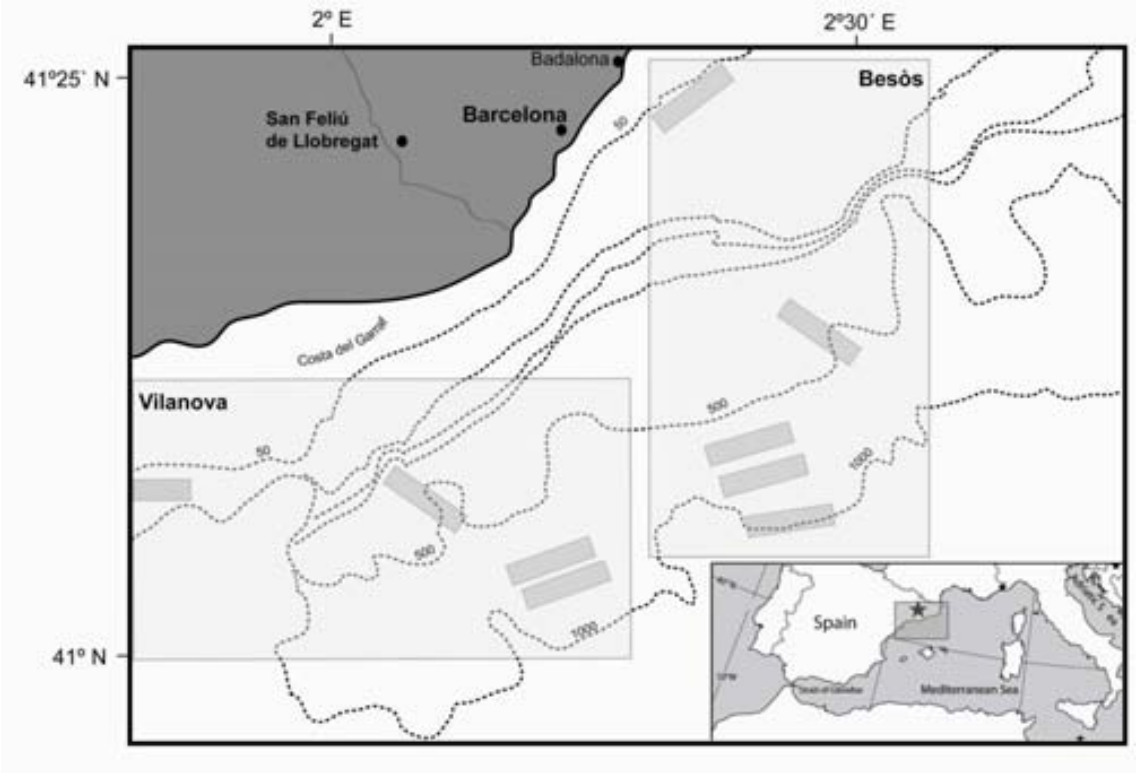

Figure 4. Map of the coast of Catalonia (Catalan Sea) indicating the two sampling areas (shaded rectangles) off Barcelona, Besòs and Vilanova.

4.2. Fish processing on board

Fish samples were collected by trawling using a research ("Garcia del CID", CSIC) and a commercial ("Stella Maris III") vessel. Sampling in the research vessel "Garcia del Cid" was carried out by means of a semi-balloon otter trawl (OTSB 14) (C. F. Merret and Marshal, 1981), whereas in "Stella Maris III", a bottom commercial trawling (BOU) was used. Table 1 shows the details of the captures during the Biomare sampling, as regards the study species of the present investigation.

Once on board, specimens were separated in three groups: (1) specimens of the first group were measured (total length) and weighted (total weight). Gall-bladder and a piece of liver were removed to biochemical procedures. The rest of the liver, together with the spleen and the left package of gills were removed and fixed in 4% formaldehid for histopathological procedures. External cavities and surface of the specimens were macroscopically examined for external parasites or gross pathologies to be registered. A piece of dorsal musculature was removed to chemical analyses. Finally, each individual was saved in an individual plastic bag and frozen at -20ºC for posterior inspection of parasite load; (2) half of the rest of the specimens captured were frozen directly to posterior parasitological analyses; and (3) the other half of the rest of the specimens were opened by the abdominal cavity and preserved in 10% buffered formalin for histopathological procedures.

Table 1. Data of the trawling collected during the seasonal sampling of the Biomare project in the coast of Catalonia. l (N), latitude north; L (E), longitude east. Trawling code abbreviations: B, Besòs; V, Vilanova; 1, winter; 2, spring, 3, summer; 4, autumn; OTSB, semi-balloon otter trawling; BOU, bottom commercial trawling. OTSB and BOU trawling were also coded following the fishing order of the survey.

Trawling	Locality	Date	Depth (m)	Initial situation		Final situation	
			Initial- Final	l (N)	L (E)	l (N)	L (E)
B1- OTSB1	Besòs	25-February-07	62,6- 62,6	41°26'37"	2°19'38"	41°25'07"	2°20'50"
B1- BOU3	Besòs	14-March-07	54,0- 52,0	41°23'86"	2°16'23"	41°25'43"	2°19'10"
B1- BOU4	Besòs	14-March-07	58,0- 68,0	41°25'33"	2°20'98"	41°25'16"	2°24'53"
B2- OTSB1	Besòs	28-April-07	61,9- 62,9	41°24'29"	2°19'19"	41°29'84"	2°21'73"
B2- BOU1	Besòs	09-may-07	65,0- 67,0	41°22'44"	2°18'33"	41°23'51"	2°21'97"
B2- BOU2	Besòs	09-may-07	66,0- 67,0	41°23'86"	2°22'04"	41°22'71"	2°18'95"
B3- OTSB1	Besòs	30-June-07	62,0- 61,0	41°24'53"	2°19'44"	41°26'21"	2°22'40"
B3- BOU3	Besòs	18-July-07	54,0- 52,0	41°37' 56"	2°30'78"	41°39'32"	2°36'58"
B3- BOU4	Besòs	18-July-07	61,9- 62,9	41°38'81"	2°35'72"	41°37'10"	2°30'25"
B3- BOU5	Besòs	18-July-07	58,0- 68,0	41°37'68"	2°32'08"	41°38'80"	2°38'05"
B4- OTSB1	Besòs	02-October-07	62,3- 61,5	41°41'16"	2°32'55"	41°43'86"	2°37'35"
B4- BOU1	Besòs	13-November-07	61,7- 62,9	41°37'77"	2°31'18"	41°39'43"	2°36'85"
B4- BOU2	Besòs	13-November-07	54,3- 52,0	41°35'22"	2°35'83"	41°36'93"	2°29'68"
B4- BOU3	Besòs	13-November-07	57,0- 68,5	41°37'37"	2°31'30"	41°38'62"	2°36'88"
V3- OTSB8	Vilanova	06-July-07	63,0- 62,0	41°08,686"	1°48,014"	41°08,636"	1°43,834"
V3- BOU8	Vilanova	20-July-07	61,4- 62,7	41°14'08"	1°78'97"	41°13'54"	1°72'67"
V3- BOU9	Vilanova	20-July-07	61,0- 62,5	41°13'54"	1°71'38"	41°14'52"	1°63'62"

4.3. Material examined

The relation of the material studied is shown in Table 2. A total of 117 specimens of *Mullus barbatus*, 81 of *Spicara maena* and 74 of *Trachinus draco* was collected. For each fish species it is given the number of specimens analyzed in each season period sampled and the minimum and maximum length of these specimens used.

Table 2. General data of the fish specimens captured for the present study. TL: total length.

Species	Nº of individuals	Season	Locality	Minimum TL (cm)	Maximum TL (cm)
Mullus barbatus	19	Winter	Besòs	11.9	22.1
	20	Spring	Besòs	10.7	15.6
	29	Summer	Besòs	13.3	24.8
	29	Summer	Vilanova	11.0	22.7
	20	Autumn	Besòs	13.1	24.1
Spicara maena	16	Winter	Besòs	9.1	16.0
	18	Spring	Besòs	10.1	15.2
	16	Summer	Besòs	10.0	16.3
	17	Summer	Vilanova	9.3	15.2
	14	Autumn	Besòs	10.4	18.2
Trachinus draco	14	Winter	Besòs	18.0	26.9
	10	Spring	Besòs	19.9	26.5
	11	Summer	Besòs	18.0	27.0
	19	Summer	Vilanova	17.4	27.4
	20	Autumn	Besòs	18.0	28.0

4.4. Parasite collection and identification

After thawing, individuals were dissected. Liver and gonads weight was recorded to the nearest 0.1mg. Thawed specimens were examined for metazoan ectoparasites, and all organs (including muscle and bones) were removed and carefully checked for endoparasites under the stereomicroscope. All parasites collected were counted and preserved in 70% ethanol. Digeneans and cestodes were stained with iron acetic carmine (Georgiev et al., 1986) and examined as permanent mounts in Canada balsam; nematode larvae were identified on temporary mounts in saline solution or glycerine. Parasites were identified to family, genus or species level when possible.

4.5. Data analysis

Fish condition was assessed by hepatosomatic index (HSI) calculated as liver weight (g)/ body weight (g) $\times$ 100, Fulton's condition factor (K) calculated as body weight (g)/ length $(cm)^3 \times 100$, and gonadosomatic index (GSI), only for females, calculated as gonad weight (g)/ body weight (g) $\times$ 100.

Parasitological terms (Prevalence (P) and Mean Abundance (MA)) were calculated according to Bush et al. (1997). We considered “total prevalence” the prevalence of the total sample, without taking into account the categorical spatial/temporal grouping done in the environmental studies of this thesis. Species with a “total prevalence” higher than 10% in the entire database are further referred to as common. Abundance is defined as the number of individuals of a particular parasite in/on a single host regardless of whether or not the host is infected. The mean abundance would be calculated by the sum of individuals of a given species in a sample divided by the total number of hosts examined.

Species richness (SR) is defined as the total number of taxa (species, when possible) found in each sample. Mean species richness (MSR) is the sum of the total taxa (species, when possible) present in each single host of the sample divided between the total hosts of that sample. Metazoan parasite diversity was estimated in terms of abundance of the parasite items, expressed as the Mean Diversity (MD), calculated with the Brillouin’s Index divided by the number of hosts examined (PRIMER v6 (Anderson et al., 2008)).

The concepts regarding to the nesting of parasite populations and parasite communities also followed Bush et al. (1997). A parasite infrapopulation includes all individuals of a species in an individual host at a particular time. A parasite component population refers to all of the individuals of a specified life history phase at a particular place and time. Parasite community data were gathered at two hierarchical community levels: infracommunity and component community. An infracommunity is a parasite community of parasite infrapopulations in a single host. A component community refers to all infrapopulations of parasites associated with some subset of a host species.

During the general parasitological surveys done in the north-western Mediterranean fish host species, we classified the parasites in specialist/specific (parasite which is specialized in parasitize one determined species, genus or family of host) and generalist (parasite which can reach a part or all its life cycle in a wide range of hosts, belonging to different species from different taxa).

5. DESCRIPTION OF PARASITES OF TAXONOMIC INTEREST OF MEDITERRANEAN BENTHOPELAGIC FISH SPECIES

5.1. A NEW CRYPTIC SPECIES OF *APONURUS* LOOSS, 1907 (DIGENEA: LECITHASTERIDAE) FROM MEDITERRANEAN GOATFISH (TELEOSTEI: MULLIDAE)

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A new cryptic species of *Aponurus* Looss, 1907 (Digenea: Lecithasteridae) from Mediterranean goatfish (Teleostei: Mullidae)

Marta Carreras-Aubets · Aigües Repullés-Albelda · Aneta Kostadinova · Maite Carrassón

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Abstract The status of the trematode *Aponurus laguncula* Looss, 1907 in the western Mediterranean is re-assessed by means of a comparative morphological study and rDNA sequences based on newly collected material. *A. laguncula* (*sensu stricto*) is redescribed from *Trachinus draco* L. and a new cryptic species of the '*A. laguncula* complex', *Aponurus mulli* n. sp., is described on the basis of abundant material from *Mullus barbatus* L. (type-host) and *M. surmuletus* L. off the Spanish Mediterranean coasts. The new species is differentiated from *A. laguncula* (*sensu stricto*) by its: significantly larger, elongate body, with maximum width at the level of the ventral sucker; shorter forebody; distinctly larger sinus-sac, seminal receptacle and seminal vesicle, with the latter also being more elongate; vesicular pars prostatica; more anteriorly located vitellarium, which consists of eight globular follicles; and distinctly smaller eggs, which are also smaller in relation to body size and have both their opercular and anopercular poles rounded. The variability and the allometric growth of the morphological characters in the new species were studied in detail, resulting in additional distinguishing features. Sequences of the large subunit rRNA (28S) gene (domains D1–D3) and ITS2 rRNA gene region for the new species have been submitted to GenBank in order to enhance future studies on species differentiation within the '*A. laguncula* complex'.

M. Carreras-Aubets · A. Repullés-Albelda · M. Carrassón
Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, Cerdanyola, 08193 Barcelona, Spain

A. Kostadinova (✉)
Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic
e-mail: Aneta.Kostadinova@uv.es; kostadinova@paru.cas.cz

A. Kostadinova
Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria

Introduction

Looss (1907) erected *Aponurus* Looss, 1907 for *A. laguncula* Looss, 1907, which he briefly described and figured on the basis of material from Adriatic fishes, i.e. *Belone belone* L. (type-host: first listed host and host of the specimens of the illustration by Looss, 1908), *Lichia amia* L., *Engraulis encrasicolus* L., *Merlangus merlangus euxinus* L., *Merluccius merluccius* L., *Trachinus draco* L. and *Mullus barbatus* L. He only provided the size ranges for the body (up to 1,000 × *c*.250 μm), the diameters of the oral and ventral suckers (100 and 200 μm, respectively) and the eggs (a single measurement of 27 × *c*.16 μm) (Looss, 1907, 1908). *A. laguncula* has since been recorded in more than 60 teleost fishes

worldwide [see Bray & MacKenzie (1990) and Bray et al. (1993) for host records and synonymies]. Nearly a third of these hosts represent fishes studied in the Mediterranean (18 host species of 14 genera, and 38 host-parasite-locality combinations; see Table 1). However, despite the poor description of *A. laguncula*, no morphological data exist from the Mediterranean basin where this species was originally described (see Bray & MacKenzie, 1990, for references to published redescriptions of the species worldwide), although there exist a few records of another species of the genus, *A. tschugunovi* Issaitschikoff, 1927, described from the Black Sea (ex *M. barbatus*; see Issaitschikoff, 1927; Vlasenko, 1931; Osmanov, 1940; Pogorel'tseva, 1952).

Bray & MacKenzie (1990) were the first to redescribe *A. laguncula* from European waters. These authors recorded, for the first time, this species from the north-eastern Atlantic (English Channel) and discussed several aspects of its taxonomy, synonyms, host-specificity and geographical distribution. They suggested that the very wide distribution of *A. laguncula* can be related either to the use of a number of related molluscs as the first intermediate hosts or the existence of a group of sibling species "which will not be separable until life-cycle or biochemical information is available" (Bray & MacKenzie, 1990). The material from the clupeid *Clupea harengus* L. was described by Bray & MacKenzie (1990) to exhibit a distinctive shape of the eggs, i.e. with narrow and more or less pointed anopercular pole; this feature of *A. laguncula*, although noted by several authors, has not been observed "in some of the Mediterranean material" (see Bray & MacKenzie, 1990, and references therein). This characteristic shape of the eggs of *A. laguncula* has recently been confirmed in the description of worms in the sparid *Boops boops* (L.), which represents the second record of this parasite in the north-eastern Atlantic (Pérez-del Olmo et al., 2006).

In a study of the biodiversity of parasite communities in *Mullus surmuletus* L., *M. barbatus* and *T. draco*, we found two forms of *Aponurus*: one rare parasite of *T. draco*, which exhibited the distinctive shape of the eggs described by Bray & MacKenzie (1990) and Pérez-del Olmo et al. (2006), and one with smaller eggs with normal oval shape, which had high prevalence in populations of both *Mullus* spp. from off the Spanish coasts of the western Mediterranean. This paper provides comparative descriptions

Table 1 Records of *Aponurus laguncula* Looss, 1907 (*sensu lato*) in the Mediterranean region

Host	Locality	Reference
Belone belone, *Engraulis encrasicolus*, *Lichia amia*, *Merlangus merlangus euxinus*, *Merluccius merluccius*, *Mullus barbatus*, *Trachinus draco*	off Trieste (Adriatic Sea)	Looss (1907)
Belone belone, *Engraulis encrasicolus*, *Lichia amia*, *Merlangus merlangus euxinus*, *Merluccius merluccius*, *Mullus barbatus*, *Spicara maena*, *Trachinus draco*	off Trieste (Adriatic Sea)	Looss (1908)
Mullus barbatus, *M. surmuletus*	off Italy	Mola (1928)
Belone belone	Black Sea	Osmanov (1940)
Serranus cabrilla	"Mediterranean Sea"	Nikolaeva & Parukhin (1969)
Belone belone	"Mediterranean Sea"	Parukhin et al. (1971)
Diplodus annularis, *D. puntazzo*, *Lithognathus mormyrus*, *Scomber japonicus colias*, *S. scombrus*	Saronic Gulf	Papoutsoglou (1976)
Alepes djedaba, *Trachinotus ovatus*, *Trachinus araneus*	off Israel	Fischthal (1980a)
Alepes djedaba, *Trachinotus ovatus*, *Trachinus araneus*	off Israel	Fischthal (1982)
Mullus surmuletus	off Corsica	Bartoli (1990); Bartoli et al. (2005)
Belone belone, *Mullus barbatus*	"Mediterranean Sea"	Naidenova & Mordvinova (1997)
Mullus surmuletus	off Algeria	Brahim et al. (2009)
Mullus surmuletus	off Sardinia	Figus et al. (2004)
Mullus surmuletus	off Corsica	Bartoli et al. (2005)
Mullus surmuletus	off Spain	Ferrer-Castelló et al. (2007)

of this material, which includes a description of a cryptic species that we believe, based on the available morphological evidence, is new to science, and a redescription of *A. laguncula* (*sensu stricto*) from the western Mediterranean.

Materials and methods

A total of 321 *Mullus* spp. were examined along the western Mediterranean coasts of Spain: 119 *M. barbatus* collected off Besòs (41°11′–41°26′N, 2°22′–2°25′E) and Vilanova (41°08′N, 1°38′–1°48′E) (Catalonia) and 268 *M. surmuletus* collected off Burriana (39°49′–39°50′N, 0°01′–0°40′E) and Santa Pola (38°00′–38°20′N, 0°10′–0°40′E) (Valencian Community). Comparative material of *Aponurus laguncula*, *sensu* Bray & MacKenzie (1990), was collected from *Trachinus draco* off Besòs (n = 61 fish; 2 infected). The trematodes were dissected out from fresh or freshly frozen fish, fixed by being pipetted into nearly boiling saline and 70% ethanol, respectively, stained with iron acetocarmine, dehydrated through a graded alcohol series, cleared in dimethyl phthalate and examined as permanent mounts in Canada balsam. The type- and voucher material is deposited in the British Museum (Natural History) Collection at the Natural History Museum, London, UK (BMNH). Measurements were taken from illustrations made using a drawing tube at high magnification. All measurements are in micrometres.

Allometric growth was calculated as a power function of body length (BL) for non-transformed data: $y = a\ BL^b$, where y is the dependent morphometric variable, BL is parasite body length, a the intercept, and b is the growth coefficient (Gould, 1971). The latter scaling exponent (b) indicates isometric growth ($b = 1$) and allometric growth (positive when $b > 1$ and negative when $b < 1$). The growth equations and the scaling exponent (corresponding to the slope of the ordinary least squares regression) were established from individual regressions performed on ln-transformed data (84 worms) for each morphometric variable using worm body length (BL) as the independent variable. Quantitative comparisons of infection parameters (Fisher's exact test for prevalence and bootstrap tests for abundance and intensity) were carried out with Quantitative Parasitology 3.0 (Rózsa et al., 2000).

Genomic DNA was extracted from specimens fixed live in molecular grade 100% ethanol using Qiagen® DNeasy™ tissue kit following the manufacturer's protocol. Partial (domains D1–D3; ~1149 nt) 28S rDNA sequences were amplified using primers LSU5 (5′-TAG GTC GAC CCG CTG AAY TTA AGC A-3′; Littlewood et al., 2000) and LSU1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′; Tkach et al., 1999). Complete ITS2 rDNA sequences were amplified using primers 3S (5′-GTA CCG GTG GAT CAC GTG GCT AGTG-3′; Anderson & Barker, 1993) and ITS2.2 (5′-CCT GGT TAG TTT CTT TTC CTC CGC-3′; Anderson & Barker, 1993). PCR amplifications run on a Veriti™ 96-well thermal cycler (Applied Biosystems) were performed in a total volume of 30 µl containing *c*.1.5 units of Thermoprime Plus DNA polymerase (Abgene, Epsom, UK) and 10X buffer containing 1.5 mM $MgCl_2$ (ABgene), 0.2 mM of each dNTP, 15 pmol of each primer and 50–80 ng of template DNA. The following thermocycling profile was used for rDNA amplification: denaturation of DNA (95°C for 2 min); 35 cycles of amplification [95°C for 50 s, 56°C (28S) or 53°C (ITS2) for 50 s and 72°C for 50 s); and 8 min extension hold at 72°C]. PCR amplicons were purified using Qiagen QIAquick™ PCR Purification Kit and cycle-sequenced from both strands using ABI BigDye™ Terminator v3.1 Ready Sequencing Kit, alcohol-precipitated and run on an ABI 3130*xl* automated sequencer. The PCR primers and, in the case of the 28S rDNA products, internal primers ECD2 (5′-CCT TGG TCC GTG TTT CAA GAC GGG-3′; Littlewood et al., 2000) and LSU1200R (5′-GCA TAG TTC ACC ATC TTT CGG-3′; Littlewood et al., 2000) were used for cycle sequencing. Contiguous sequences were assembled and edited using Bioedit v7.0.5. (©1997–2005, Hall, 1999) and submitted to GenBank.

Family Lecithasteridae Odhner, 1905
Subfamily Lecithasterinae Odhner, 1905
Genus *Aponurus* Looss, 1907

***Aponurus mulli* n. sp.**
Syn. *Aponurus* sp. of Pankov et al. (2006)

Type-host: *Mullus barbatus* L.
Other host: *Mullus surmuletus* L.
Type-locality: Off Besòs, Spain.

Other localities: Off Burriana, Santa Pola and Vilanova, Spain.
Site: Stomach.
Type-material: Holotype BMNH 2010.12.7.1; paratypes BMNH 2010.12.7.2-12.
Voucher material: BMNH 2010.12.7.13-15.
Etymology: The species name indicates the genus of the type-host.
Representative DNA sequences: Partial (D1-D3) 28S rRNA gene: GenBank accession nos DQ354368 (Pankov et al., 2006) and HQ713441 (present study). Partial 18S rRNA gene: DQ354372 (Pankov et al., 2006); the isolate ex *M. surmuletus* was referred to as *Aponurus* sp. DTJL-2006 by Pankov et al., (2006) to avoid nomenclatural problems. ITS2 rDNA: HQ713 442 (present study).

Description (Figs. 1, 3, 4b; Table 2)

[Based on 84 whole-mounted adult specimens. Measurements in description from holotype (Fig. 1); sample sizes, ranges and means given in Table 2.] Body elongate, 745 long; maximum width at level of ventral sucker, 171 (23% of body length), in hindbody at level of ovary 163 (21.9% of body length). Tegument smooth. Pre-oral lobe distinct, 11 long. Oral sucker subterminal, spherical, 78 × 82. Ventral sucker large, muscular, spherical, between the first and second body thirds, 152 × 162. Sucker-width ratio 1:1.97. Forebody 201 long (27% of body length). Prepharynx absent. Pharynx muscular, subglobular, overlaps posterior margins of oral sucker dorsally, 32 × 42; pharynx/oral sucker-width ratio 1:1.95. Two symmetrical glandular structures present dorsally at level of pharynx. Oesophagus very short or apparently absent. Small but distinct 'Drüsenmagen' present. Caeca thin-walled, terminate close to posterior extremity; ends obscured by uterine loops and difficult to observe.

Testes 2, subglobular (occasionally subtriangular), oblique, contiguous, very close to or contiguous with ventral sucker (overlapping it dorsally in 21 specimens); anterior testis 82 × 95; posterior testis 72 × 87. Seminal vesicle large, saccular, elongate-oval, in posterior forebody, reaches to or slightly overlaps anterior margin of ventral sucker dorsally, 53 × 38. Pars prostatica vesicular (Fig. 3a), 27 × 19 (paratypes: 30 × 18 in ventral aspect; width 21 in lateral aspect, Fig. 3b), postero-dorsal to sinus-sac, lined with anuclear blebs, appears as small dorsal loop in lateral aspect (Fig. 3b), surrounded by 2–3 layers of large external gland-cells which appear in ventral view to have large characteristic elongate-oval shape, 53 × 48. Sinus-sac elongate-oval, 36 × 29, with thin walls; gland-cells present in connective tissue (Fig. 3). Hermaphroditic duct short. Genital atrium absent. Genital pore a wide median slit at level of mid-pharynx or just posterior to it, at 87 from ventral sucker. Small sinus-organ observed in some specimens protruding through genital pore (Fig. 3b).

Ovary usually transversely oval, post-testicular, sinistral, contiguous with posterior testis and overlaps vitellarium dorsally, 67 × 72. Large, elongate-oval blind seminal receptacle (usually masked by uterine coils) observed in some specimens dorsally anterior to ovary. Mehlis' gland small, mostly dorsal to ovary (Fig. 3c). Uterus extensive in hindbody, with its main bulk dorsal to gonads and posterior to vitellarium; loops reach fairly close to posterior extremity. Eggs abundant, operculate, with both opercular and anopercular poles rounded (Fig. 4b), small in relation to size of body. Metraterm very short, enters sinus-sac ventrally to male duct (Fig. 3). Vitellarium well developed, 86 × 137, ventral and slightly overlapping to just posterior to ovary, composed of 8 closely located (frequently overlapping) but apparently not convergent globular follicles (Figs. 1, 3c), at 148 from posterior extremity (19.9% of body length).

Excretory pore terminal. Vesicle Y-shaped; arms unite dorsally to pharynx.

Differential diagnosis

The present material exhibits the diagnostic characteristics of the genus *Aponurus* and appears most close morphologically to *A. laguncula*, as redescribed by Bray & MacKenzie (1990), Pérez-del Olmo et al. (2006) and present study (see Table 2 and description below). However, *A. mulli* n. sp. is characterised by a much larger, elongate body, with the maximum width at the level of the ventral sucker [*vs* in the hindbody close to the posterior extremity; hindbody width as % of body length 14–25% (mean 21%) *vs* 25–30%, respectively], shorter forebody [21–32% (mean 28%) of body length *vs* 26–43%], distinctly larger sinus-sac, seminal receptacle and seminal vesicle (the latter is also more elongate; Table 2), and a somewhat more anteriorly located vitellarium which consists of

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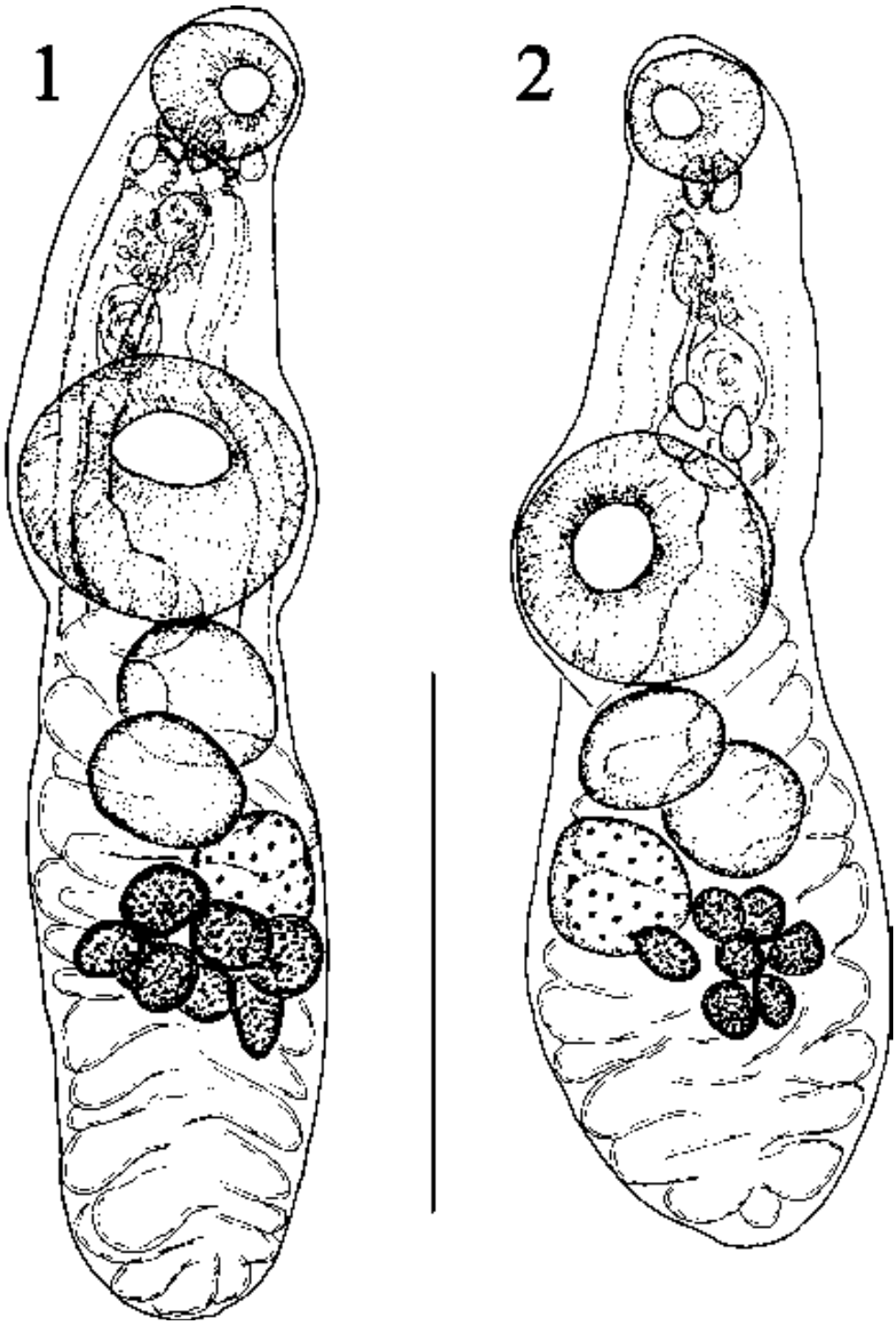

Fig. 1–2 1. *Aponurus mulli* n. sp. ex *Mullus barbatus*: holotype, ventral view, with uterus in outline. 2. *A. laguncula* (*sensu stricto*): voucher specimen ex *Trachinus draco*, ventro-lateral view, with uterus in outline. *Scale-bar*: 300 μm

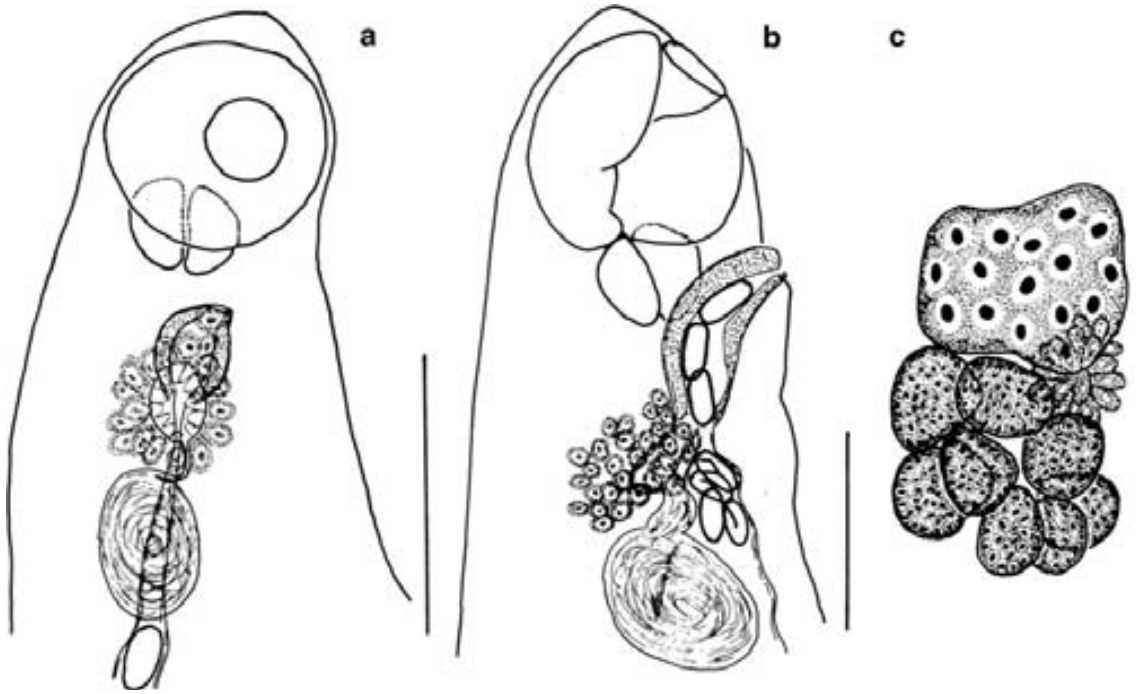

Fig. 3 *Aponurus mulli* n. sp. ex *Mullus* spp.: a, holotype, details of the terminal genitalia; b, voucher specimen ex *M. surmuletus*, details of the terminal genitalia; c, paratype, detail of ovarian complex. *Scale-bars*: 100 μm

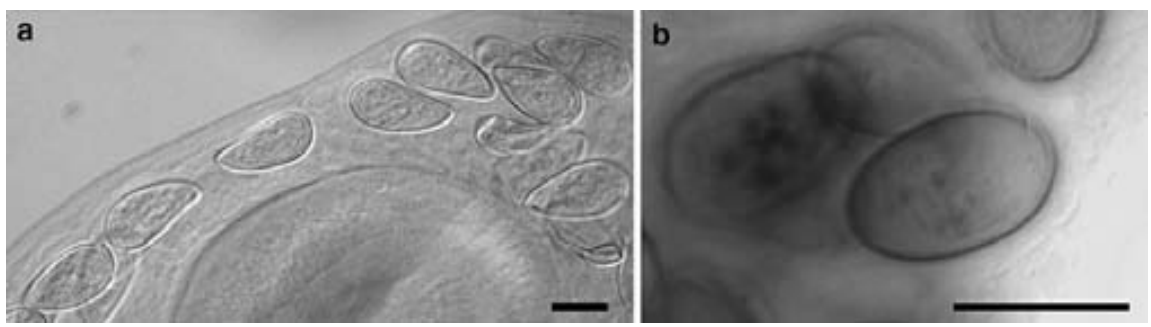

Fig. 4 Eggs of: a, *Aponurus laguncula* (*sensu stricto*), voucher material of Bray & MacKenzie (1990); b, *A. mulli* n. sp., paratype. *Scale-bars*: 20 μm

eight globular follicles (*vs* seven irregular or claviform follicles). Another difference, which we consider important, is the type of pars prostatica: vesicular in *A. mulli* (Figs. 1, 3a,b) and tubular in *A. laguncula* [Fig. 2; see also illustrations in Bray & MacKenzie (1990) and Pérez-del Olmo et al. (2006). Finally, although some range overlap for egg-length was detected in the newly-examined material of *A. laguncula*, the eggs in *A. mulli* are distinctly smaller [means 27 × 13 *vs* 30 × 16 (present material) and 38 × 18 μm (Bray & MacKenzie, 1990); see Table 2] and also smaller in relation to body size. Examination of a large number of non-collapsed eggs (n = 332) also revealed very low levels of variation in the size (CV 7.4% and 7.7% for length and width, respectively; see Table 2) and shape (i.e. both opercular and anopercular poles rounded) of the eggs in *A. mulli* (Fig. 4). These egg-size and shape differences between *A. mulli* and *A. laguncula* [present material and data from the

Table 2 Comparative measurements [ranges, means ± standard deviations (SD) and coefficients of variation (CV%)] for adult stages of *Aponurus mulli* n. sp., *A. laguncula* Looss, 1907 and *A. tschugunovi* Issaitschikoff, 1927

Species	*A. mulli* n. sp.					*A. laguncula*	*A. laguncula*		*A. laguncula*	*A. tschugunovi*
Region	Western Mediterranean					Western Mediterranean	North East Atlantic		North East Atlantic	Black Sea
Source	Present study					Present study	Bray & MacKenzie (1990)		Pérez-del Olmo et al. (2006)	Issaitschikoff (1927)
Host	*M. barbatus*	*M. surmuletus*	*Mullus* spp.			*Trachinus draco*	*Clupea harengus*		*Boops boops*	*Mullus barbatus*
	Range	Range	Global range	Mean ± SD	CV%	Range (n = 4)	Range	Mean	n = 1	Range
Measurements										
Body length	696–1,154	834–1,372	697–1,372	1,022 ± 122	11.9	642–812	500–705	620	390	2,769–3,784
Body width at ventral sucker	170–209	192–292	170–292	243 ± 32	13.2	181–187	–	–	–	–
Hindbody maximum width	106–248	146–309	106–309	211 ± 35	16.6	181–206	167–242	192	143	654–980
Pre-oral lobe	5–18	8–25	5–25	15 ± 4	26.7	3–15	<25	–	10	–
Oral sucker length	70–115	92–146	70–146	109 ± 12	11.0	73–88	68–81	74	56	160–202
Oral sucker width	64–121	92–129	64–129	108 ± 12	11.1	76–83	68–85	78	56	175–238
Pharynx length	30–45	33–67	30–67	45 ± 6	13.3	33–52	32–45	35	30	78–100
Pharynx width	30–58	42–67	30–67	51 ± 7	13.7	33–45	32–47	39	26	82–125
Ventral sucker length	161–236	163–267	161–267	205 ± 23	11.2	136–167	123–151	137	77	344–516
Ventral sucker width	155–203	163–263	155–263	207 ± 23	11.1	133–170	126–144	134	87	361–551
Sinus-sac length	42–88	42–92	42–92	61 ± 11	18.0	45–58	c. 35*	–	27	–
Sinus-sac width	24–42	25–50	24–50	35 ± 5	14.3	24–36	c. 22*	–	19	–
Prostatic cells (field length)	–	46–96	46–70	70 ± 12	17.1	–	–	–	47	–
Prostatic cells (field width)	42–97	38–83	38–97	57 ± 10	17.5	30–42	–	–	29	–
Seminal vesicle length	33–94	29–104	29–104	68 ± 17	25.0	45–70	45*	–	41	–
Seminal vesicle width	24–55	21–75	21–75	41 ± 11	26.8	36–42	40*	–	29	–
Anterior testis length	64–121	58–154	58–154	99 ± 19	19.2	68–98	38–110	72	69	176–292
Anterior testis width	64–121	71–188	64–188	107 ± 20	18.7	74–106	42–92	67	41	194–344
Posterior testis length	52–118	71–154	52–154	99 ± 18	18.2	67–98	50–108	73	41	176–327
Posterior testis width	52–106	71–183	52–183	98 ± 22	22.4	58–92	48–85	65	56	191–361
Ovary length	55–100	46–129	46–129	84 ± 16	19.0	58–97	54–95	67	34	147–292
Ovary width	55–115	58–142	55–142	101 ± 22	21.8	85–121	48–98	65	33	265–361
Seminal receptacle length	97–97	33–100	33–100	66 ± 16	24.2	–	40*	–	–	–
Seminal receptacle width	61–61	21–88	21–88	48 ± 13	27.1	–	30*	–	–	–

Table 2 continued

Species Region	*A. mulli* n. sp. Western Mediterranean					*A. laguncula* Western Mediterranean	*A. laguncula* North East Atlantic		*A. laguncula* North East Atlantic	*A. tschugunovi* Black Sea
Source	Present study					Present study	Bray & MacKenzie (1990)		Pérez-del Olmo et al. (2006)	Issaitschikoff (1927)
Host	*M. barbatus*	*M. surmuletus*	*Mullus* spp.			*Trachinus draco*	*Clupea harengus*		*Boops boops*	*Mullus barbatus*
	Range	Range	Global range	Mean ± SD	CV%	Range (n = 4)	Range	Mean	n = 1	Range
Vitellarium length	82–185	71–221	71–221	129 ± 30	23.3	82–142	–	–	79	189–258
Vitellarium width	64–133	108–242	64–242	149 ± 32	21.5	73–167	–	–	49	273–447
Egg length	22–29	21–32	21–32	27 ± 2	7.4	29–32 (30 ± 1)	32–42	38	–	17–24
Egg width	11–15	11–17	11–17	13 ± 1	7.7	14–19 (16 ± 1)	16–21	18	–	7–9
Distances										
Forebody length	161–348	217–442	161–442	288 ± 48	16.7	200–236	–	–	–	–
Genital pore to ventral sucker	76–170	67–250	67–250	147 ± 33	22.4	91–103	–	–	–	–
Ventral sucker to anterior testis	−9–55	−63–71	−9–71	90 ± 22	244	2–28	–	–	–	69–82
Post-vitelline field	100–285	138–271	100–285	193 ± 37	19.2	88–133	–	–	–	430–1,204
Ratios										
Body width at ventral sucker as % of body length	20–25	20–28	20–28	24.0 ± 2.1	8.8	22–25	23**	–	–	24**
Hindbody width as % of body length	14–25	15–25	14–25	21.0 ± 2.3	11.0	25–30	27**	–	–	26**
Forebody as % of body length	21–30	22–32	21–32	28.1 ± 2.5	8.9	29–33	26–38	32	42.6	23**
Post-vitelline field as % of body length	12–26	13–26	12–26	19.0 ± 3.5	18.4	11–17	14	–	–	17**
Anterior testis width as % of body length	7–13	8–15	7–15	10.6 ± 1.0	9.4	11–14 (12.7 ± 1)	–	–	–	6**
Ovary width as % of body length	6–13	6–14	6–14	9.9 ± 2.0	20.2	11–17 (14.2 ± 2)	–	–	–	5**
Sucker width ratio	1:1.66–2.16	1:1.62–2.27	1:1.62–2.27	1:1.92 ± 0.12	6.3	1:1.76–2.04	1:1.57–1.85	1:1.71	1:1.55	1:2.33**
Pharynx/oral sucker width ratio	1:1.62–2.80	1:1.86–2.64	1:1.62–2.80	1:2.14 ± 0.19	8.9	1:1.83–2.27	–	–	–	

* Longitudinal section; ** estimated from published drawing

re-examination of the material of Bray & MacKenzie (1990)] are graphically illustrated in Fig. 5. We conclude that, in addition to size, the distinctive shape of the eggs in *A. laguncula* described from European material [i.e. with narrow and pointed anopercular poles; see also Bray & MacKenzie (1990) and Pérez-del Olmo et al. (2006)] may serve as a reliable distinguishing feature.

The only other species of *Aponurus* from European waters is *A. tschugunovi*, described by Issaitschikoff (1927) from *M. barbatus* in the Black Sea. Although its description lacks important detail on the structure of the terminal genitalia and purports the existence of a rudimentary ecsoma (?) in larger specimens, *A. mulli* differs from *A. tschugunovi* in a number of morphological features. The latter is a substantially larger form (body and all organs more than twice the size) and widest at the first third of the hindbody (the latter also distinctly longer), with a forebody shorter in relation to body length and with much smaller eggs. There is an overlap in the upper range for egg-length, but the means for both length and width in *A. mulli* are well above the range for *A. tschugunovi* (see Table 2). Furthermore, the testes in *A. tschugunovi* are small in relation to the body size and well separated from the ventral sucker and ovary, and the uterus does not reach posterior to the vitellarium. *A. tschugunovi* has occasionally been recorded in Black Sea fishes [*M. barbatus*, *Sciaena umbra* L., *Scorpaena porcus* L., *Mesogobius batrachocephalus* (Pallas) and *Trachurus mediterraneus* (Steindachner); see Vlasenko (1931), Osmanov (1940), Pogorel'tseva (1952), Nikolaeva & Kovaleva (1966)]; of these authors only the former provided some descriptive elements. The measurements of Vlasenko (1931) show an overall agreement with the original description of *A. tschugunovi*, but the egg-size is outside the lower range for *A. tschugunovi* and, therefore, much lower than in *A. mulli*.

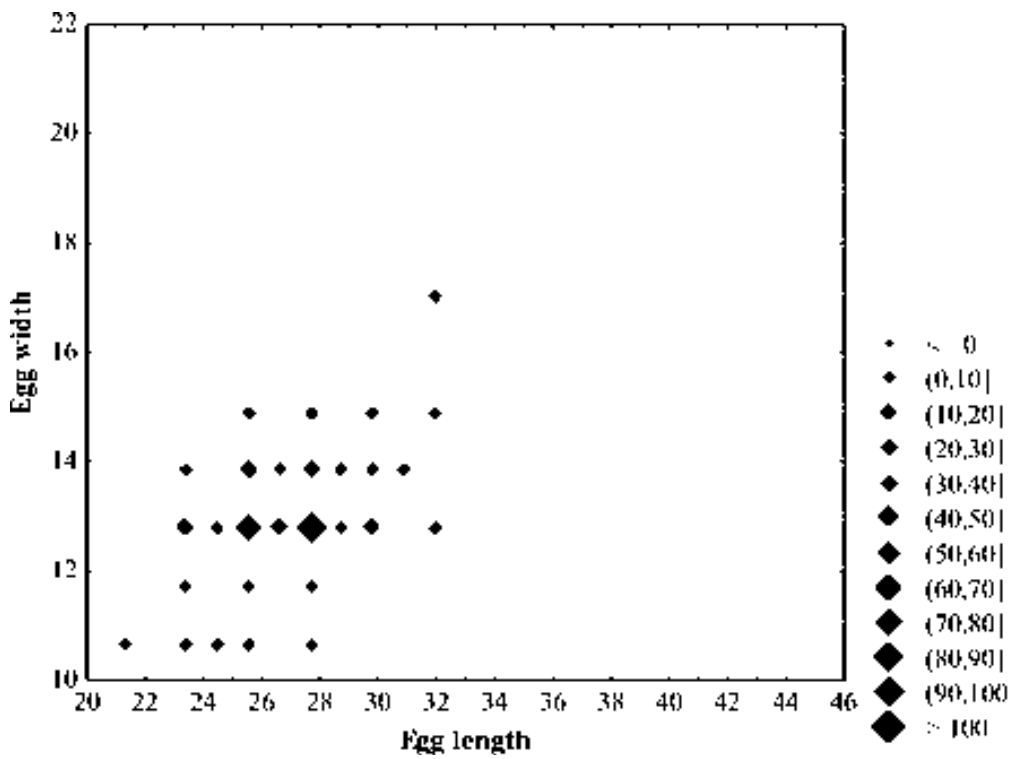

Fig. 5 Egg-size frequency distribution scatterplot for *Aponurus mulli* n. sp. (diamonds) and *A. laguncula* (*sensu stricto*) (squares). The size of the symbols indicates the relative frequencies of the number of points represented by a single plot position

The above comparisons, coupled with the consistently low morphometric variation, allometric growth patterns and stable host-parasite relationship (see below), support the distinct species status of *A. mulli* n. sp.

Morphometric variation and allometric growth

The coefficients of variation of the metrical features of *A. mulli* n. sp. not only indicate overall variable patterns but also a particularly high homogeneity within the two mullid hosts for the length of the body, the size of the suckers, the size of the eggs and virtually all ratios (CV values $< 12\%$ in Table 2). The scaling exponents of most morphometric variables (26 of 29) were associated with typically highly significant regressions (Table 3), their values ranging between 0.419 and 1.408. Most of the length-related variables exhibited negative allometry (9 variables) or isometric growth (3 variables). Notably, the dimensions of the attachment organs (the suckers) exhibited similar scaling exponents (Table 3), and scaled less steeply with body size than the dimensions of the worm features associated with reproduction, such as the size of the gonads and vitellarium. Of particular relevance to the morphometric discrimination between *A. mulli* and *A. laguncula* is the finding that the width of these latter organs plus the width of the hindbody, which helped distinguish these species, also exhibited an isometric growth in the large sample of the new species examined here (see Fig. 6 for a graphical illustration of the relationship of three of the variables with body length in *A. mulli vs* our newly-collected samples of *A. laguncula*).

Table 3 Allometric scaling exponents [estimated as the slope (b ± SE) in ln-ln regressions] of relationship between ln-transformed morphometric variables and body length in *Aponurus mulli* n. sp. and the coefficients of determination (R^2) of the regressions

Variable	b ± SE	P	R^2	Type of growth*
Body width at ventral sucker	0.884 ± 0.09	0.0001	0.584	N
Hindbody maximum width	1.060 ± 0.11	0.0001	0.529	I
Oral sucker length	0.718 ± 0.07	0.0001	0.555	N
Oral sucker width	0.743 ± 0.08	0.0001	0.537	N
Pharynx length	0.710 ± 0.10	0.0001	0.369	N
Pharynx width	1.009 ± 0.08	0.0001	0.650	I
Ventral sucker length	0.731 ± 0.06	0.0001	0.622	N
Ventral sucker width	0.762 ± 0.06	0.0001	0.656	N
Sinus-sac length	0.419 ± 0.18	0.0204	0.069	N
Sinus-sac width	0.523 ± 0.14	0.0003	0.159	N
Prostatic cells (field length)	0.590 ± 0.19	0.0026	0.129	N
Prostatic cells (field width)	0.573 ± 0.14	0.0001	0.179	N
Seminal vesicle length	0.766 ± 0.27	0.0069	0.117	N
Seminal vesicle width	0.628 ± 0.28	0.0289	0.080	N
Anterior testis length	0.829 ± 0.15	0.0001	0.281	N
Anterior testis width	1.076 ± 0.14	0.0001	0.463	I
Posterior testis length	0.956 ± 0.14	0.0001	0.363	I
Posterior testis width	1.045 ± 0.18	0.0001	0.328	I
Ovary length	0.786 ± 0.18	0.0001	0.206	N
Ovary width	1.010 ± 0.19	0.0001	0.277	I
Seminal receptacle length	0.979 ± 0.45	0.0378	0.161	I
Vitellarium length	1.065 ± 0.25	0.0001	0.231	I
Vitellarium width	1.365 ± 0.20	0.0001	0.434	P
Forebody length	1.234 ± 0.08	0.0001	0.744	P
Genital pore to ventral sucker	1.408 ± 0.16	0.0001	0.497	P
Post-vitelline field	0.834 ± 0.22	0.0003	0.190	N

*N, negative allometric growth; P, positive allometric growth; I, isometric growth

Sequence data

The partial sequence of the 28S rRNA gene region obtained from an isolate of *A. mulli* n. sp. ex *M. barbatus* (domains D1–D3, 1149 nt) was identical to that of the isolate from the voucher sample ex *M. surmuletus* described here (1303 nt; sequenced by Pankov et al., 2006). Unfortunately, we could not obtain material ex *T. draco* for sequencing and no comparative data are currently available to extend the molecular test to other isolates identified as *A. laguncula*. We, however, have made available a sequence of the ITS2 rRNA gene region of *A. mulli* (519 nt), which should provide enough resolution to aid distinguishing between putative cryptic species in future studies on the '*A. laguncula* species complex'.

Occurrence in *Mullus* spp.

The overall infection levels of *A. mulli* n. sp. were substantially higher in *M. surmuletus* (prevalence 63.8 *vs* 16.8%; mean abundance 2.99 *vs* 0.33; mean intensity 4.69 *vs* 1.95; all $p = 0.0001$). There were significant differences in the infection parameters in *M. surmuletus* sampled at the two locations. A larger proportion of the fish sample from off Santa Pola was infected (80.0 *vs* 54.9%; $p = 0.0001$), and the mean abundance and intensity of *A. mulli* at this locality was substantially higher than that in fish off Burriana (6.07 *vs* 1.30 and 7.59 *vs* 2.37, respectively; $p = 0.0001$). No differences in infection parameters of *A. mulli* in *M. barbatus* from the two localities Besòs and Vilanova were detected (all $p > 0.05$).

Aponurus laguncula Looss, 1907

Material studied: Ex *Trachinus draco* L., Perciformes, Trachinidae. Stomach. Mediterranean Sea: off Besòs, Spain. BMNH 2010.12.7.16-19.
Comparative material studied: Ex *Clupea harengus* L. Clupeidae. Eastern English Channel. BMNH 1989.8.24.1-8.

Description (Figs. 2, 4a; Table 2)

[Based on 4 whole-mounted adult specimens. Ranges for measurements given in Table 2.] Body small, widest at level of vitellarium (Fig. 2). Tegument smooth. Pre-oral lobe small. Oral sucker subterminal, spherical. Ventral sucker muscular, spherical, just anterior to mid-body. Prepharynx absent. Pharynx oval. Oesophagus very short or apparently absent. 'Drüsenmagen' present. Caeca thin-walled, obscured by uterine loops in hindbody.

Testes 2, subglobular to subtriangular, oblique, contiguous, very close to or contiguous with ventral sucker. Seminal vesicle saccular, elongate-oval, in posterior forebody, reaches to or overlaps ventral sucker dorsally. Pars prostatica tubular, surrounded by few external gland-cells. Sinus-sac oval, with thin wall. Genital atrium absent. Genital pore median at level of posterior margin of pharynx. Small sinus-organ observed in some specimens protruding through genital pore (Fig. 2).

Ovary oval to transversely oval, post-testicular, sinistral, contiguous with or overlapping posterior testis. Seminal receptacle and Mehlis' gland not observed. Uterus mainly in hindbody; main bulk dorsal to gonads and posterior to vitellarium; loops reach to posterior extremity. Eggs numerous, operculate, with narrower, pointed anopercular pole, large in relation to body size. Vitellarium ventral, just posterior to or slightly overlapping ovary, composed of 7 small closely located (frequently overlapping) globular to irregular follicles.

Details of posterior part of excretory system not observed; arms of excretory vesicle unite dorsally to pharynx.

Remarks

The specimens from *T. draco* agree well morphologically with the redescriptions of *A. laguncula* by Bray & MacKenzie (1990) and Pérez-del Olmo et al. (2006), based on material from the north-eastern Atlantic, and exhibit the characteristic egg-shape, with a narrow, pointed anopercular pole (Figs. 2, 4a). Both the range and the mean egg-length in specimens ex *T. draco* are lower than those reported from material ex *Clupea harengus* by Bray & MacKenzie (1990) (group of points on the upper-left in Fig. 5), but we did not have a chance to examine a large enough sample of well-positioned, uncollapsed eggs. The measurements for a single worm from *Boops boops* given by Pérez-del Olmo et al. (2006) are below the range observed in our material (Table 2). Although *T. draco* was reported as a host of *A. laguncula* by

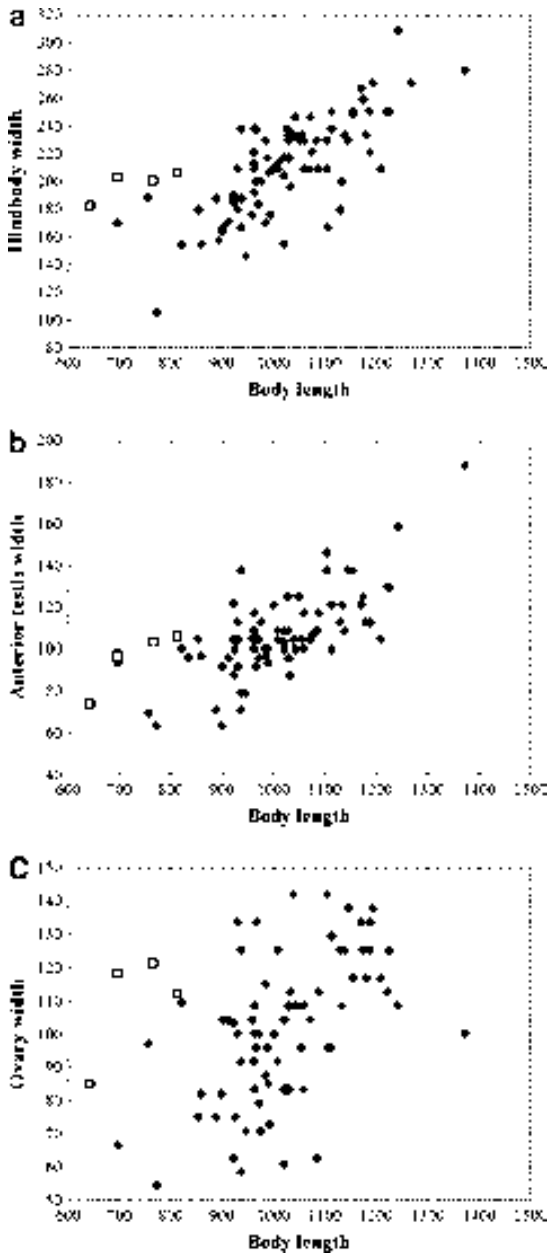

Fig. 6 The relationship between three morphometric variables showing isometric growth in *Aponurus mulli* n. sp. and body length: a, maximum width at hindbody; b, anterior testis width; c, ovary width. Data for individual worms given in micrometres and indicated by diamonds (*A. mulli*) and squares (*A. laguncula* (*sensu stricto*))

Looss (1907, 1908), our study provides the first description of *A. laguncula* (*sensu stricto*) in this host and from the Mediterranean.

Discussion

Our study provides the only detailed descriptions of species of *Aponurus* from the Mediterranean basin since the erection of *A. laguncula* by Looss (1907, 1908) and of *A. tschugunovi* by Issaitschikoff (1927). The euryxenic *A. laguncula* is one of the widespread digeneans with respect to both geographical distribution and host range (see e.g. Bray & MacKenzie, 1990), suggesting that this taxon probably consists of a number of cryptic species which exhibit considerable morphological similarity. The problematic current state of this species stems from the rather brief description of Looss (1907) and is further complicated by the lack of morphological information in nearly two thirds of the host-parasite records worldwide [see Bray & MacKenzie (1990) and Bray et al. (1993) for details]. As a result, the only character used in comparisons between existing descriptions of *A. laguncula*, the size-range for the eggs, has led to the conclusion that egg-length is not a useful taxonomic feature with regard to this species (Bray & MacKenzie, 1990; Bray et al., 1993). However, the extent of intraspecific variation in *A. laguncula* is virtually unknown, and this inspired the initial goal of our study, i.e. to provide a modern morphological description and assess the morphometric variability in the species frequently recorded as *A. laguncula* in Mediterranean *Mullus* spp. and *Trachinus* spp. (Table 1).

The results of this first detailed morphometric study of a species of *Aponurus* show a substantial morphological homogeneity of the material collected from different populations (and at different time periods) of the two *Mullus* spp. in the western Mediterranean and especially with regard to egg-size assessed on a very large sample (332 eggs; see Table 2). These low levels of variation, and comparisons with the newly-collected material from *T. draco* and the recent redescriptions of *A. laguncula* from European waters (Bray & MacKenzie, 1990; Pérez-del Olmo et al., 2006), clearly demonstrate that the material studied by us corresponds to two distinct cryptic species of the '*A. laguncula* species complex'.

Relating the taxon names to descriptions, however, posed a complicated nomenclatural problem. Looss (1907, 1908) did not specify the type-host and the hosts studied by us (i.e. *M. barbatus* and *T. draco*) are within the list of eight fish species in the original

Table 4 Infection parameters of *Aponurus mulli* n. sp. in the populations of the two species of *Mullus* studied

	Mullus surmuletus		*Mullus barbatus*	
	off Santa Pola	off Burriana	off Besòs	off Vilanova
Sample size	95	173	90	29
Prevalence (%)	80.0	54.9	20.0	6.9
Intensity (range)	1–38	1–8	1–6	1–4
Mean intensity ± SD	7.59 ± 7.09	2.37 ± 1.66	1.89 ± 1.45	2.50 ± 2.12
Mean abundance ± SD	6.07 ± 7.03	1.30 ± 1.71	0.38 ± 0.99	0.17 ± 0.76

description (Looss, 1907, 1908). It is, therefore, possible that either of the two species described here represent the genuine *A. laguncula*. Unfortunately, the type-specimens of *A. laguncula* were not available for examination (they may have been part of the Looss material lost in Egypt during the First World War, but are not amongst the Looss material, subsequently making its way to London, in the BMNH Collection). We have reached the pragmatic decision to maintain Bray & MacKenzie's (1990) conception as *A. laguncula* (*sensu stricto*) for the sake of stability and to describe the form parasitising Mediterranean *Mullus* spp. as new based on: (i) the distinct morphological differentiation between the forms from *Mullus* spp. and *T. draco* (both listed as hosts in the original description by Looss, 1907); and (ii) the agreement of the latter form with the recent redescriptions of *A. laguncula* of Bray & MacKenzie (1990) and Pérez-del Olmo et al. (2006).

Although the dependence of the shape of the organs on body size is recognised as a factor affecting the proportions among the various dimensions used in trematode taxonomy, only a few studies have addressed within-species allometry in this group (Thomas, 1967; Rohde, 1966; Fischthal, 1978a,b, 1979, 1980b; Fischthal et al., 1982; Saad-Fares & Combes, 1992; Swarnakumari & Madhavi, 1992; Kostadinova et al., 2000) and parasitic flatworms in general (Poulin, 2009). In addition to important information, e.g. useful additional ratios for variables showing isometric growth for future comparative morphology studies on *Aponurus* spp., our study on the allometric growth in *A. mulli* n. sp. provides support at the intraspecific level for a general pattern of trematode growth as suggested by Poulin (2009), i.e. that attachment structures do not scale more steeply with body size than structures associated with ingestion and reproduction.

The overall infection levels of *A. mulli* in *M. surmuletus* fall within the range of the highest levels ever recorded for *Aponurus* spp. (as *A. laguncula* in fish hosts from the South China Sea; see Bray & MacKenzie, 1990, and references therein). The prevalence in distinct samples of *M. surmuletus* studied by us surpasses the values for the latter (67.0–96.9 *vs* 31.0–85.0%) and the maximum parasite intensity is comparable (38 *vs* 35–50; Bray & MacKenzie, 1990). Although geographical variation in infection levels exists (Table 4), overall data suggest that *M. surmuletus* is more frequently infected with *A. mulli*. The two sympatric *Mullus* spp. exhibit some spatial overlap in the western Mediterranean but also exhibit a tendency for bathymetric habitat partitioning and clear niche segregation with respect to the bottom type of the selected habitats (Lombarte et al., 2000). The infection levels in *Mullus* spp. appear, therefore, to reflect the degree of overlap in habitat selection.

In conclusion, the detailed comparative morphology study revealed the presence of at least two cryptic species of the '*A. laguncula* species complex' in the Mediterranean. Further sequence data, especially of more variable genes and from well-described morphological isolates are essential in addressing the diversity, host-specificity and geographical distribution of the taxa within *A. laguncula* (*sensu lato*) and the genus *Aponurus*.

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5.2. REDESCRIPTIONS OF TWO FREQUENTLY RECORDED BUT POORLY KNOWN HEMIURID DIGENEANS, *LECITHOCHIRIUM MUSCULUS* (LOOSS, 1907) (LECITHOCHIRIINAE) AND *ECTENURUS LEPIDUS* LOOSS, (DINURINAE), BASED ON MATERIAL FROM THE WESTERN MEDITERRANEAN

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Redescriptions of two frequently recorded but poorly known hemiurid digeneans, *Lecithochirium musculus* (Looss, 1907) (Lecithochiriinae) and *Ectenurus lepidus* Looss, 1907 (Dinurinae), based on material from the western Mediterranean

Marta Carreras-Aubets · Francisco E. Montero · Aneta Kostadinova · David I. Gibson · Maite Carrassón

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Abstract Two frequently reported but poorly known hemiurid digeneans, *Lecithochirium musculus* (Looss, 1907) (Lecithochiriinae), from the stomach of *Trachinus draco* and *Citharus linguatula*, and *Ectenurus lepidus* Looss, 1907 (Dinurinae), from the stomach of *Spicara maena,* are redescribed based on material from off the Barcelona coast of the western Mediterranean. The two species are commented upon, and *Lecithochirium israelense* Fischthal, 1980 is considered a synonym of *L. musculus*. Records of the two species in the Mediterranean Basin and North East Atlantic region are summarised.

M. Carreras-Aubets · F. E. Montero · M. Carrassón
Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain

F. E. Montero
Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, PO Box 22 085, 46071 Valencia, Spain

A. Kostadinova (✉)
Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic
e-mail: Aneta.Kostadinova@uv.es; kostadinova@paru.cas.cz

A. Kostadinova
Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria

D. I. Gibson
Department of Zoology, Natural History Museum, Cromwell Road, London SW7 5BD, UK

Introduction

Studies on fish parasites of the Mediterranean Basin were initiated as early as the 18th Century and have resulted in the description of a substantial number of species, predominantly digenean trematodes. However, although a number of regional parasite inventories have been completed over the past century (e.g. Sey, 1968, 1970; Papoutsoglou, 1976; Orecchia & Paggi, 1978; Fischthal, 1980, 1982; Renaud et al., 1980; Radujković et al., 1989; Anato et al., 1991; Le Pommelet et al., 1997; Bartoli et al., 2005), taxonomic efforts at clarifying the position of many of the species in the early studies have been at least patchy (see Bartoli et al., 2005, and references therein). Thus, for seven of the nine named species of the family Hemiuridae Looss, 1899 recorded in more than 40% of the fish species during a long-term study in the Scandola Nature Reserve off Corsica by Bartoli et al. (2005), these authors provided as recent references, with useful descriptions or illustrations, only that of Gibson & Bray (1986) from the Northeast Atlantic. Furthermore, they had to resort to the original

descriptions of Looss (1907) for the other two species, *Aphanurus stossichi* (Monticelli 1891) and *Lecithochirium grandiporum* (Rudolphi, 1819), which were thought to be restricted to the Mediterranean region, both of which have been redescribed recently by Kostadinova et al. (2004) and Bartoli & Gibson (2007).

Among these hemiurids are two described by Looss (1907, 1908) from the eastern Mediterranean, *Ectenurus lepidus* Looss, 1907 and *Lecithochirium musculus* (Looss, 1907) Nasir & Diaz, 1971, which have been often recorded, predominantly from the eastern Mediterranean, but rarely described (see Gibson & Bray, 1986). The latter authors provided extensive redescriptions and figures of these species based on materials from the North East (NE) Atlantic. However, data on the morphology of *E. lepidus* and *L. musculus* from the Mediterranean, especially in hosts different from the type-hosts studied by Looss (1907, 1908), are scarce.

In a recent study of parasites in marine fishes off the Barcelona coast of the western Mediterranean, we have collected specimens that enabled us to redescribe these two poorly known species from the Mediterranean Sea and to assess their morphological variation.

Materials and methods

Fish sampling was carried out within the framework of the project BIOMARE (CTM2006-13508-C02-01/MAR) offshore in the region of Barcelona at depths of 52–68 m: (i) off Besòs (41°11′–41°26′N, 2°22′–2°25′E); a total of 38 *Citharus linguatula* L., 61 *Trachinus draco* L. and 93 *Spicara maena* (L.); and (ii) off Vilanova (41°08′N, 1°38′–1°48′E); a total of 21 *C. linguatula,* 26 *T. draco* and 30 *S. maena*.

The trematodes were collected from fresh or freshly frozen fish, fixed by being pipetted into nearly boiling saline or in 70% ethanol, respectively, stained with iron acetocarmine, dehydrated through a graded alcohol series, cleared in dimethyl phthalate and examined as permanent mounts in Canada balsam. No significant variation was observed as a result of the two types of fixation. Voucher material is deposited in the British Museum (Natural History) Collection at the Natural History Museum, London, UK (BMNH). Measurements were taken from illustrations made using a drawing tube at high magnification. All measurements are in micrometres. Data on prevalence (in %) and abundance (mean ± standard deviation) are provided. The Host-Parasite Database maintained at the Natural History Museum, London (Gibson et al., 2005) was searched for host-parasite records. The distribution lists follow the FAO's 'Major Fishing Areas' (http://firms.fao.org/firms/data-coverage/en) expressed in numerical form. Host names and classification are according to Fishbase (Froese & Pauly, 2011).

Family Hemiuridae Looss, 1899
Subfamily Lecithochiriinae Lühe, 1901

Lecithochirium musculus (Looss, 1907) Nasir & Diaz, 1971

Syns *Sterrhurus musculus* Looss, 1907; *Brachyphallus musculus* (Looss, 1907) Skrjabin & Guschanskaja, 1955; *Lecithochirium branchialis* (Stunkard & Nigrelli, 1934) Manter, 1934 of Nikolaeva (1966) and Parukhin et al. (1971); *L. proterorhini* Naidenova, 1972; *L. ophiocephalus* Naidenova, 1972; *L. floridensis* (Manter, 1934) Crowcroft, 1946 of Naidenova (1974); *Hemiurus communis* Odhner, 1905 of Balozet & Sicart (1960a,b); *L. israelense* Fischthal, 1980 (new synonym)

Material studied

Host and locality: Ex *Trachinus draco* L., Perciformes, Trachinidae. Stomach. Off Besòs and Vilanova, Spain (Mediterranean). BMNH 2011.7.7.14.
Prevalence: 9.8% (off Besòs); 3.9% (off Vilanova).
Mean abundance: 0.16 ± 0.55 (off Besòs); 0.19 ± 0.98 (off Vilanova).
Host and locality: Ex *Citharus linguatula* L., Perciformes, Citharidae. Stomach. Off Besòs and Vilanova, Spain (Mediterranean). BMNH 2011.7.7.15-17.
Prevalence: 10.5% (off Besòs); 9.5% (off Vilanova).
Mean abundance: 0.11 ± 0.31 (off Besòs); 0.19 ± 0.68 (off Vilanova).

Records

References: 1. Looss (1907) (as *Sterrurus musculus*); 2. Looss (1908) (as *S. musculus*); 3. Vlasenko (1931) (as *S. musculus*); 4. Chulkova (1939) (as *S. musculus*); 5. Sproston (1939) (as *S. musculus*); 6. Osmanov (1940) (as *S. musculus*); 7. Pogorel'tseva (1952) (as *S. musculus*); 8. Balozet & Sicart (1960a) (as *Hemiurus communis*); 9. Balozet & Sicart (1960b) (as

H. communis); 10. Kurashvili (1960) (as *Brachyphallus musculus*); 11. Dollfus (1962) (as *S. musculus*); 12. Brinkmann (1966) (as *B. musculus*); 13. Nikolaeva (1966) (as *B. musculus* and *Lecithochirium branchialis*); 14. Nikolaeva & Kovaleva (1966) (as *B. musculus*); 15. Dolgikh & Naidenova (1968) (as *B. musculus*); 16. Kovaleva (1968) (as *B. musculus*); 17. Kovaleva (1969) (as *B. musculus*); 18. Nikolaeva & Parukhin (1969) (as *B. musculus*); 19. Kovaleva (1970) (as *B. musculus*); 20. Nikolaeva & Solonchenko (1970) (as *B. musculus*); 21. Sey (1970) (as *B. musculus*); 22. Parukhin et al. (1971) (as *L. branchialis*); 23. Naidenova (1972) (as *L. proterorhini* and *L. ophiocephalus*); 24. Tesch (1973); 25. Naidenova (1974) (as *L. proterorhini*, *L. ophiocephalus* and *L. floridensis*); 26. Nikolaeva (1975) (as *B. musculus*, *L. proterorhini*, *L. ophiocephalus* and *L. floridensis*); 27. Papoutsoglou (1976) (as *S. musculus*); 28. Fischthal (1980) (as *L. israelense*); 29. Collins (1981); 30. Jardas & Hristovski (1985) (as *S. musculus*); 31. Gibson & Bray (1986); 32. Orecchia et al. (1988); 33. Radujković & Raibaut (1989); 34. Radujković et al. (1989); 35. Gijon-Botella & Lopez-Roman (1989); 36. Bos et al. (1993); 37. Kennedy et al. (1997); 38. Santos (1998); 39. Borgsteede et al. (1999); 40. Berrilli et al. (2000); 41. Vilas et al. (2000); 42. Di Cave et al. (2001); 43. Outeiral et al. (2001); 44. Lozano et al. (2001); 45. Paniagua & Vilas (2001); 446. Álvarez et al. (2002); 47. Vilas et al. (2002); 48. Vilas et al. (2003); 49. Vilas & Paniagua (2004); 50. Vilas et al. (2004); 51. Bartoli et al. (2005); 52. Ternengo et al. (2005); 53. Bartoli & Gibson (2007); 54. Costa et al. (2009); 55. Freitas et al. (2009); 56. Present study.

Descriptions: 1, 2, 3, 28, 31, 34, 47 (some measurements), 56.

Definitive hosts: ACIPENSERIDAE: *Acipenser gueldenstaedtii* Brandt & Ratzeburg (4 as *S. musculus*, 10 as *B. musculus*, 26 as *B. musculus*); *A. sturio* L. (1 as *S. musculus*, 2 as *S. musculus*, 26 as *B. musculus*); ANGUILLIDAE: *Anguilla anguilla* (L.) (1 as *S. musculus*, 2 as *S. musculus*, 8 as *H. communis*, 9 as *H. communis*, 11 as *S. musculus*, 24, 36, 37, 39, 40, 41, 42, 43, 44, 47, 48, 50, 52); BELONIDAE: *Belone belone* (L.) (13 as *L. branchialis*; 22 as *L. branchialis*); BOTHIDAE: *Arnoglossus laterna* (Walbaum) (13 as *B. musculus* and *L. branchialis*, 18 as *B. musculus*, 32, 33, 34); *A. thori* Kyle (13 as *L. branchialis*); *Bothus podas* (Delaroche) (27 as *S. musculus*); CARANGIDAE: *Alectis alexandrina* (Geoffroy Saint-Hilaire) (28 as *L. israelense*); *Lichia amia* (L.) (1 as *S. musculus*, 2 as *S. musculus*); *Trachurus mediterraneus* (Steindachner) (14 as *B. musculus*, 16 as *B. musculus*, 17 as *B. musculus*, 18 as *B. musculus*, 19 as *B. musculus*; 26 as *B. musculus*); CENTRACANTHIDAE: *Spicara maena* (L.) (27 as *S. musculus*); CITHARIDAE: *Citharus linguatula* (L.) (22 as *B. musculus*, 32, 33, 34, 56); CONGRIDAE: *Ariosoma balearicum* (Delaroche) (21 as *B. musculus*); *Conger conger* (L.) (5 as *S. musculus*, 35, 45, 48, 49, 51, 54); CYNOGLOSSIDAE: *Symphurus nigrescens* Rafinesque (22 as *L. branchialis*); GADIDAE: *Merlangius merlangus* (L.) (1 as *S. musculus*, 2 as *S. musculus*); *Trisopterus luscus* (L.) (5 as *S. musculus*); GOBIIDAE: *Gobius cobitis* Pallas (25 as *L. floridensis*, 26 as *L. floridensis*, 51); *G. cruentatus* Gmelin (21 as *B. musculus*, 51); *G. niger* L. (1 as *S. musculus*, 2 as *S. musculus*); *G. paganellus* L. (51); *Gobiusculus flavescens* (Fabricius) (29, 31); *Mesogobius batrachocephalus* (Pallas) (25 as *L. floridensis*, 26 as *L. floridensis*); *Neogobius melanostomus* (Pallas) (25 as *L. floridensis*, 26 as *L. floridensis*); *Pomatoschistus microps* (Krøyer) (55); *P. pictus* (Malm) (31); *Proterorhinus marmoratus* (Pallas) (23 as *L. proterorhini*, 25 as *L. proterorhini*, 26 as *L. proterorhini*); *Zosterisessor ophiocephalus* (Pallas) (23 as *L. ophiocephalus*, 25 as *L. ophiocephalus*, 26 as *L. ophiocephalus*); LABRIDAE: *Symphodus tinca* (L.) (30 as *S. musculus*, 51); LOPHIIDAE: *Lophius piscatorius* L. (1 as *S. musculus*, 2 as *S. musculus*, 27 as *S. musculus*, 51, 53); LOTIDAE: *Gaidropsarus mediterraneus* (L.) (16 as *B. musculus*, 21 as *B. musculus*, 26 as *B. musculus*); MORONIDAE: *Dicentrarchus labrax* (L.) (1 as *S. musculus*, 2 as *S. musculus*, 38); MULLIDAE: *Mullus surmuletus* L. (51); MURAENIDAE: *Muraena helena* L. (51, 53); OPHIDIIDAE: *Ophidion barbatum* L. (1 as *S. musculus*, 2 as *S. musculus*, 3 as *S. musculus*, 4 as *S. musculus*, 6 as *S. musculus*, 7 as *S. musculus*, 21 as *B. musculus*); *O. rochei* Müller (26 as *B. musculus*); PHYCIDAE: *Phycis phycis* (L.) (51); PLEURONECTIDAE: *Platichthys flesus* (L.) (31); SALMONIDAE: *Salmo trutta* L. (31); SCIAENIDAE: *Sciaena umbra* L. (20 as *B. musculus*, 26 as *B. musculus*); SCOMBRIDAE: *Scomber scombrus* L. (21 as *B. musculus*); SCOPHTHALMIDAE: *Lepidorhombus whiffiagonis* (Walbaum) (46); *Scophthalmus maeoticus* (Pallas) (6 as *S. musculus*, 10 as *B. musculus*, 26 as *B. musculus*); *S. maximus* (L.) (1 as *S. musculus*, 2 as *S. musculus*); SCORPAENIDAE: *Scorpaena porcus* L. (20 as *B. musculus*, 26 as *B. musculus*, 51); *S. scrofa* L. (51); SERRANIDAE: *Serranus cabrilla* (L.) (1 as *S. musculus*, 2 as *S. musculus*, 27 as *S. musculus*);

S. scriba (L.) (6 as *S. musculus*, 26 as *B. musculus*, 51); SOLEIDAE: *Buglossidium luteum* (Risso) (13 as *B. musculus*, 18 as *B. musculus*); *Pegusa lascaris* (Risso) (46); *Solea solea* (L.) (46); *Solea* sp. (44); SPARIDAE: *Dentex dentex* (L.) (1 as *S. musculus*, 2 as *S. musculus*); *Diplodus annularis* (L.) (51); *Pagellus erythrinus* (L.) (1 as *S. musculus*, 2 as *S. musculus*); *Pagrus pagrus* (L.) (51); SYNODONTIDAE: *Synodus saurus* (L.) (12 as *B. musculus*, 27 as *S. musculus*, 51); TRACHINIDAE: *Trachinus araneus* Cuvier (28 as *L. israelense*); *T. draco* L. (1 as *S. musculus*, 2 as *S. musculus*, 6 as *S. musculus*, 7 as *S. musculus*, 26 as *B. musculus*, 27 as *S. musculus*, 56); TRIGLIDAE: *Chelidonichthys lucerna* (L.) (51); *Trigloporus lastoviza* (Bonnaterre) (27 as *S. musculus*).

Distribution: Area 37, subarea 1 (Western Mediterranean): Tyrrhenian Sea (14, 40); off Corsica (51, 52, 53); Étang de Thau (France) (8, 9, 11); off Besòs and Vilanova (Spain) (56); Italy (37, 39); Area 37, subarea 2 (Central Mediterranean): Adriatic Sea (1, 2, 13, 14, 21, 30, 33, 34, 40, 42); Area 37, subarea 3 (Eastern Mediterranean): Aegean Sea (off Rhodes) (12); Saronic Gulf (27); off Israel (28); Area 37, subarea 4 (Black Sea) (3, 4, 6, 7, 10, 15, 16, 17, 19, 20, 23, 25, 26); Mediterranean basin (no area specified) (13, 17, 18, 19, 22, 32, 44); Area 27 (NE Atlantic) (31); off the Canary Islands (35); off Madeira (54); off Cádiz Province (Spain) (44); off Portugal (36, 38, 55); off Galicia (Spain) (41, 43, 45, 46, 47, 48, 49, 50); off Brittany (France) (5); off Devon (England) (31); off Galway (Eire) (29, 31); off Donegal (Eire) (31); North Sea (24).

Description (Figs. 1–2; Tables 1, 2)

[Based on 12 whole-mounted, fully gravid specimens ex *Trachinus draco* and 10 whole-mounted specimens ex *Citharus linguatula* (5 fully gravid and 5 neogravid). See Table 1 for measurements of adult worms and Table 2 for mean measurements for 3 sets of specimens.]

Body fusiform, but more tapered anteriorly, with maximum width at level of ovary/vitellarium or just posterior. Forebody relatively short, occasionally with transverse fold, but pre-somatic pit absent. Ecsoma always present, extended in 8 specimens, variable in length. Tegument smooth, lacking plications. Pre-oral lobe prominent, with transverse ventral pit. Oral sucker subterminal, subspherical, lacks internal elevations. Ventral sucker subglobular, large. Sucker width ratio 1:1.86–2.29.

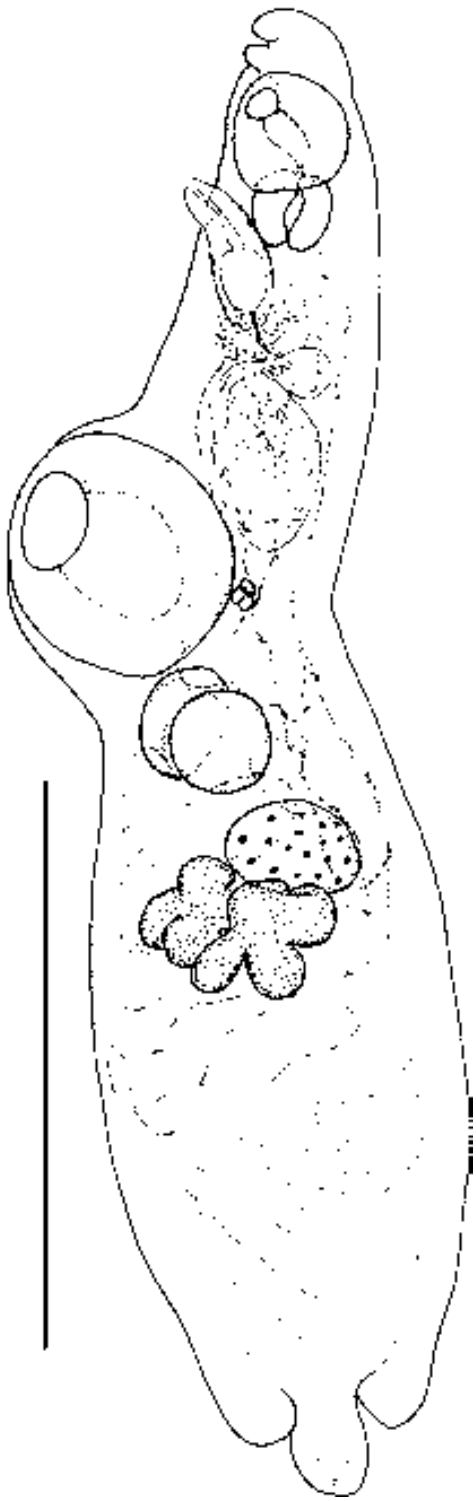

Fig. 1 *Lecithochirium musculus* ex *Trachinus draco.* Ventral view, with uterus in outline. *Scale-bar*: 500 μm

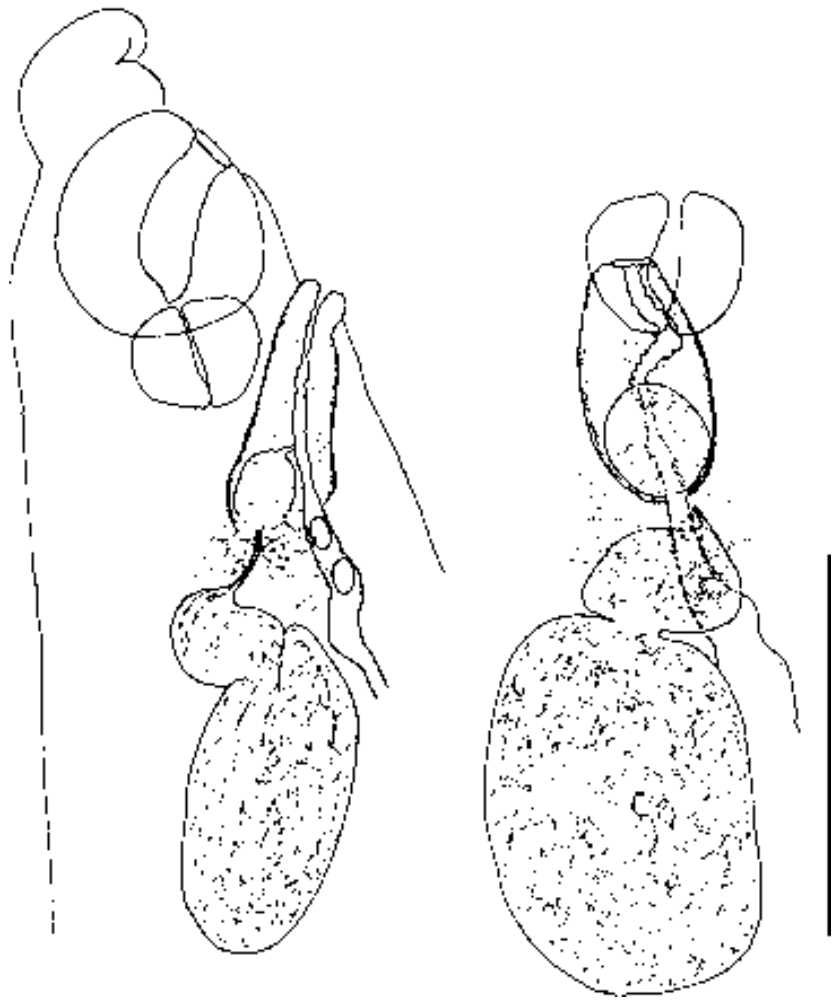

Fig. 2 *Lecithochirium musculus* ex *Trachinus draco.* Details of the terminal genitalia. *Scale-bar*: 200 μm

Prepharynx absent. Pharynx muscular, subglobular, overlaps posterior margin of oral sucker dorsally. Oesophagus short, wide. 'Drüsenmagen' distinct. Caeca lined with cells, reach to or just into ecsoma.

Testes 2, subglobular, occasionally almost rectangular or triangular in dorso-ventral plane (anterior testis with slight constriction in single specimen ex *T. draco*), symmetrical (15 specimens) or slightly oblique (7 specimens), contiguous, just posterior to ventral sucker or overlapping it postero-dorsally (distance from posterior margin of ventral sucker to anterior testis −173-88 and −48-5 in specimens ex *T. draco* and *C. linguatula*, respectively). Seminal vesicle large, saccular, distinctly bipartite; anterior chamber subglobular, much smaller than posterior; latter elongate-oval, overlaps ventral sucker antero-dorsally. Pars prostatica narrow, short, surrounded by large prostatic cells; prostatic cell field width 36–118. Sinus-sac elongate-oval, with rather thick, muscular wall, well anterior to ventral sucker. Ejaculatory vesicle subglobular, large, lined with anuclear 'blebs'. Hermaphroditic duct muscular, with irregular lining. Genital pore a wide median slit at level of pharynx or slightly anterior (2 specimens ex *T. draco* and 6 specimens ex *C. linguatula*), at 179–291 and 215–221 from ventral sucker in these hosts, respectively. Temporary sinus-organ protrudes through genital pore in some specimens.

Ovary transversely-oval to subglobular, just post-testicular but separated from testes by uterine coils. Mehlis' gland, Juel's organ and uterine seminal receptacle not observed. Bulk of uterus in anterior

Table 1 Comparative metrical data for *Lecithochirium musculus*

Species Host	*L. musculus* *Trachinus draco*	*L. musculus* *Citharus linguatula*	*L. musculus* *Ophidion barbatum*	*L. musculus* *Citharus linguatula*	*L. musculus* *Salmo trutta*	*L. musculus* *Anguilla anguilla*	*L. israelense* *Trachinus araneus*, *Alectis alexandrina*
Locality	Western Mediterranean (off Spain)	Western Mediterranean (off Spain)	Black Sea (off Crimean coast)	Adriatic Sea	NE Atlantic (North coast of Eire)	NE Atlantic (off Galicia)	Eastern Mediterranean (off Israel)
Source	Present study	Present study	Vlasenko (1931)	Radujković et al. (1989)*	Gibson & Bray (1986)	Vilas et al. (2002)	Fischthal (1980)
Soma length	1,176–1,700	982–1,821	c. 2,500	1,230–3,360**	900–2,100**	1,125–2,975**	1,340–1,555
Ecsoma length	52–891	567–1,194	850		0–150		510–650
Forebody length	285–503	351–488			200–400	270–580	330–410
Body width at ventral sucker	333–455	258–455					350–370
Hindbody width	303–573	276–561	600	480–950	420–800	270–660	–
Pre-oral lobe length	36–67	30–79		present			19–27
Oral sucker	97–155 × 97–145	97–139 × 97–142	? × 130–160	70 × 60–150	110–140 × 110–150	? × 90–150	109–119 × 114–119
Pharynx	58–82 × 58–82	61–85 × 41–85	? × 70	50–120 × 40–90			63–75 × 63–73
Ventral sucker	194–294 × 200–288	170–267 × 197–285	? × 260–330	170–390 × 160–390	200–300 × 200–300	? × 200–325	245–265 × 252–287
Sinus-sac	88–212 × 45–76	70–148 × 36–79	70–240 × 70–110				106–143 × 53–61
Ejaculatory vesicle	35–60 × 27–64	30–58 × 30–88					44–48 × 44–46
Anterior chamber of seminal vesicle	37–73 × 37–85	45–61 × 39–70					30–50 × 46–56
Posterior chamber of seminal vesicle	115–199 × 64–138	124–242 × 55–103					100–158 × 73–114
Testes	52–142 × 67–176	67–218 × 58–167	130–210 × 70–160	120–180 × 130–210	60–300 × 110–300	85–315 × 80–210	97–152 × 85–155
Ovary	79–147 × 97–194	64–206 × 76–152	190–240 × 120–200	110–170 × 160–220	120–270 × 150–320	95–215 × 105–240	102–116 × 138–172

Table 1 continued

Species Host	*L. musculus* *Trachinus draco*	*L. musculus* *Citharus linguatula*	*L. musculus* *Ophidion barbatum*	*L. musculus* *Citharus linguatula*	*L. musculus* *Salmo trutta*	*L. musculus* *Anguilla anguilla*	*L. israelense* *Trachinus araneus*, *Alectis alexandrina*
Locality	Western Mediterranean (off Spain)	Western Mediterranean (off Spain)	Black Sea (off Crimean coast)	Adriatic Sea	NE Atlantic (North coast of Eire)	NE Atlantic (off Galicia)	Eastern Mediterranean (off Israel)
Source	Present study	Present study	Vlasenko (1931)	Radujković et al. (1989)*	Gibson & Bray (1986)	Vilas et al. (2002)	Fischthal (1980)
Vitelline masses	88–258 × 76–182	82–173 × 61–170	190 × 160		100–300 × 120–200		105–165 × 90–128
Eggs	16–20 × 9–12	17–20 × 10–11	20 × 11	15–20 × 10–15	22–24 × 12–15		16–19 × 11–14
Width at ventral sucker as % of soma length	23–37	23–26	24***	25***	–		24***
Forebody as % of soma length	20–32	26–36	25***	18***	–		27***
Sucker width ratio	1: 1.86–2.18	1: 1.86–2.29	1:2.00***	1:2.00***	1:1.77–2.00		1: 2.18–2.47

* Perhaps extensively flattened specimens (see fig. 25 in Radujković et al., 1989); ** Total length; *** Calculated from published drawing

Table 2 Means for the morphometric features of *Lecithochirium musculus* ex *Trachinus draco* (12 adult specimens) and *Citharus linguatula* (5 adult and 5 neogravid specimens)

Host (parasite stage)	*Trachinus draco* (fully gravid worms)	*Citharus linguatula* (fully gravid worms)	*Citharus linguatula* (neogravid worms)
Measurements			
Soma length	1,344	1,364	817
Ecsoma length	422	797	167
Body width at ventral sucker	388	342	231
Hindbody width	428	411	255
Pre-oral lobe length	53	52	23
Oral sucker	117 × 117	114 × 120	93 × 90
Pharynx	69 × 69	67 × 66	49 × 55
Ventral sucker	232 × 239	221 × 246	161 × 176
Sinus-sac	122 × 59	101 × 58	62 × 20
Ejaculatory vesicle	46 × 40	47 × 48	25 × 16
Prostatic cells (field width)	86	88	–
Seminal vesicle total length	212 × 92	214 × 84	111 × 47
Anterior chamber of seminal vesicle	56 × 58	53 × 53	35 × 30
Posterior chamber of seminal vesicle	156 × 92	169 × 84	76 × 46
Testes	101–104 × 106–115	98–109 × 93–107	53–61 × 56–57
Ovary	110 × 138	108 × 115	61 × 79
Vitelline masses	128 × 111	122 × 104	78 × 52
Distances			
Forebody length	377	420	233
Genital pore to ventral sucker	215	217	107
Ventral sucker to anterior testis**	−50	−17	−9
Post-vitelline field length	443	347	228
Proportions			
Width at ventral sucker as % of soma length	0.28	0.25	0.27
Forebody as % of soma length	0.28	0.32	0.27
Post-vitelline field as % of soma length	0.32	0.24	0.28

** Negative values indicate overlap with ventral sucker

hindbody; loops reach to level of caecal extremities. Metraterm enters base of sinus-sac ventrally and unites with male duct closely anterior to ejaculatory vesicle. Eggs numerous, operculate, small. Vitellarium well developed, ventral, immediately post-ovarian; in 2 lobed (with 3 and 4 lobes) masses; postvitelline field (measured to end of soma) 212–606 (specimens ex *T. draco*) and 50–541 (specimens ex *C. linguatula*).

Excretory pore terminal. Excretory arms unite dorsally to posterior region of oral sucker. Other details of excretory system not observed.

Remarks

Since the original description by Looss (1907, as *Sterrhurus musculus*), *L. musculus* has been recorded in 60 fish species from the Mediterranean Basin and the NE Atlantic (157 host-parasite-locality records; see Gibson & Bray, 1986 for the treatment of records from elsewhere). It is apparent that the sampling effort has been higher in the Mediterranean than in the NE Atlantic (52 host species, 132 records *vs* 12 host species, 25 records). Nearly 20% of all records represent data from eels, mainly *Anguilla anguilla*

(type-host) and *Conger conger* but occasionally *Muraena helena*.

Although *L. musculus* is the most widely reported species of *Lecithochirium* and occurs in the most diverse range of hosts (e.g. compare with the data in Gibson & Bray, 1986), only a few published descriptions of *L. musculus* provide information on its morphological variation. The most recent and most detailed description of this species is that of Gibson & Bray (1986) based on ovigerous specimens from the body-cavities of gobies, *Gobiusculus flavescens* and *Pomatoschistus pictus*; these authors also provided data for worms found in the stomachs of *Salmo trutta* and *Platichthys flesus* in the NE Atlantic. Gibson & Bray (1986) suggested that gobiids represent an integral part of the life-cycle of *L. musculus*, the unencapsulated and in most cases gravid worms from gobiids being transferred to the definitive hosts, such as eels and other predatory fish species.

Our material, invariably recovered from the stomach of fishes, exhibited the diagnostic characteristics of *L. musculus*: (i) oral sucker without postero-lateral thickenings of the wall; and (ii) a vitellarium composed of two 3- and 4-lobed masses (Looss, 1907, 1908; Gibson & Bray, 1986). Additionally, the specimens studied by us exhibited a characteristic prominent pre-oral lobe with a transverse ventral pit (Figs. 1–2). Body size and the majority of metrical features were similar for the gravid specimens from both *Citharus linguatula* and *Trachinus draco*. However, specimens from *C. linguatula* exhibited higher mean values for the length of the forebody and the ecsoma and lower means for the length of the postvitelline field; the latter two are reflected in small differences in proportions (Table 2).

Morphologically, our specimens ex *T. draco* and *C. linguatula* correspond well to the brief original description of *L. musculus* by Looss (1907) subsequently detailed description of Looss (1908) but appear closest to the material ex *Ophidion barbatum* described by Vlasenko (1931), the latter having a higher approximate value for body length and a greater upper range for the width of the sinus-sac (Table 1). It is possible that the measurements of Radujković et al. (1989) are taken from extensively flattened specimens ex *C. linguatula* in the Adriatic Sea (see their fig. 25); this results in the higher upper limits for the size of the body, pharynx and ventral sucker, and the width of the testes and ovary (Table 1). Comparison with published data for material described from the NE Atlantic (ex *Salmo trutta* by Gibson & Bray, 1986, and ex *A. anguilla* by Vilas et al., 2002) reveals higher upper limits for the measurements of soma length, hindbody width and the sizes of the testes, ovary and vitelline masses in the NE Atlantic material. The egg-size for the specimens ex *S. trutta* also appears above the upper limits observed in other material of *L. musculus* (Table 1).

Fischthal (1980) described *Lecithochirium israelense* Fischthal, 1980 based on five adult worms from the trachinid *Trachinus araneus* Cuvier (type-host) and the carangid *Alectis alexandrina* (Geoffroy Saint-Hilaire) from the Eastern Mediterranean (off Israel). Morphologically *L. israelesis* is indistinguishable from our material (Table 1) and we consider it synonymous with *L. musculus*.

Subfamily Dinurinae Looss, 1907

Ectenurus lepidus Looss, 1907

Syns *Ectenurus trachuri* Nikolaeva & Kovaleva, 1966 *nec* (Yamaguti, 1934) Yamaguti, 1970; *E. virgulus* Linton of Parukhin et al. (1971) and Nikolaeva (1975)

Material studied

Host and locality: Ex *Spicara maena* (L.), Perciformes, Sparidae. Stomach. Off Besòs and Vilanova, Spain (Mediterranean). BMNH 2011.7.7.11–13.
Prevalence: 9.7% (off Besòs); 10.0% (off Vilanova).
Mean abundance: 0.11 ± 0.34) (off Besòs); 0.10 ± 0.31 (off Vilanova).

Records

References: 1. Looss (1907); 2. Looss (1908); 3. Nicoll (1913); 4. Vlasenko (1931); 5. Osmanov (1940); 6. Butskaya (1952); 7. Pogorel'tseva (1952); 8. Janiszewska (1953); 9. Chernyshenko (1955); 10. Williams (1960); 11. Mazza (1963); 12. Nikolaeva (1963); 13. Nikolaeva (1966); 14. Nikolaeva & Kovaleva (1966) (as *E. trachuri*); 15. Kovaleva (1968) (as *E. trachuri*); 16. Kovaleva (1969) (as *E. trachuri*); 17. Nikolaeva & Parukhin (1969) (as *E. lepidus* and *E. trachuri*); 18. Kovaleva (1970) (as *E. trachuri*); 19. Parukhin et al. (1971) (as *E. lepidus* and *E. virgulus* (= *E. trachuri*)); 20. Nikolaeva (1975) (as *E. lepidus* and *E. virgulus* (= *E. trachuri*)); 21. Papoutsoglou (1976); 22. Fischthal (1980); 23. Gaevskaya & Kovaleva (1980a); 24. Gaevskaya & Kovaleva (1980b); 25. Fischthal (1982); 26. Gaevskaya & Kovaleva (1982); 27. Gibson & Bray

Fig. 3 *Ectenurus lepidus* ex *Spicara maena.* Ventral view, with uterus in outline. *Scale-bar*: 500 μm

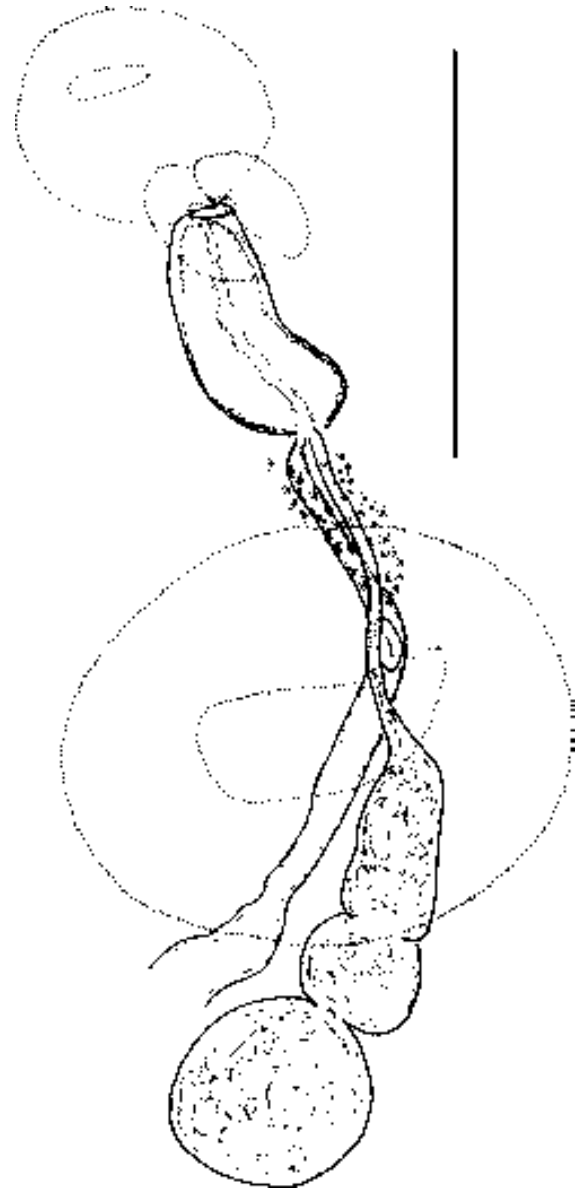

Fig. 4 *Ectenurus lepidus* ex *Spicara maena.* Details of the terminal genitalia. *Scale-bar*: 100 μm

(1986); 28. Carvalho-Varela & Cunha-Fereira (1987); 29. Orecchia et al. (1988); 30. Marinov & Golemansky (1989); 31. Radujković et al. (1989); 32. Radujković & Raibaut (1989); 33. Akmirza (1997); 34. Naidenova & Mordvinova (1997); 35. Lozano et al. (2001); 36. Akmirza (2003); 37. Bartoli et al. (2005); 38. Keser et al. (2007); 39. Present study.

Table 3 Comparative metrical data for *Ectenurus lepidus*

Host	*Spicara maena*	*Lichia amia*	*Spicara smaris*	*Trachurus mediterraneus*	*Trachurus trachurus*	*Trachurus trachurus*
Locality	Western Mediterranean	Off Trieste (Italy)	Mediterranean	Mediterranean	French Mediterranean	Kattegat
Source	Present study	Looss (1908)	Nikolaeva (1966)	Nikolaeva (1963)	Mazza (1963)	Gibson & Bray (1986)
Soma length	830–1,545	up to 2,000	420–1,020	1,680–2,280	1,720	2,200–2,700
Ecsoma length	152–274	–	–	–	–	450–500
Forebody length	112–200	–	–	–	–	300–400
Body width at ventral sucker	171–342	250–300	140–195	310–340	410	500–600*
Hindbody width	165–373	–	–	–	–	–
Pre-oral lobe length	3–16	–	–	–	–	–
Oral sucker	68–84 × 65–85	80–120	53–78 × 47–71	70–110 × 80–110	90 × 70	120 × 110–130
Pharynx	37–48 × 37–47	–	28–53 × 28–40	–	–	–
Ventral sucker	165–261 × 168–255	? × 200–300	118–177 × 99–167	170–220 × 200–320	? × 290	? × 350–360
Sinus-sac	44–53 × 19–37	–	–	–	–	–
Testes	53–112 × 59–143	–	62–93 × 31–62	70–130 × 80–310	140 × 150	170–230 × 200–250
Ovary	44–118 × 50–171	–	90 × 62	120–130 × 130–160	100 × 140	130–200 × 210–260
Egg-length	16–20 (mean 18 ± 1)	20	–	16–25	18–22	16–19 (usually 17–18)
Egg-width	8–11 (mean 9 ± 1)	10	–	10–12	11–13	7–10 (usually 8–9)
Sucker width ratio	1:2.58–3.00	> 1:2	1:2	1:3.4**	–	1:2.7–3.2

* Flattened specimens (see Gibson & Bray, 1986); ** Calculated from published drawing

Descriptions: 1, 2, 4, 11, 14, 31, 39.
Definitive hosts: ATHERINIDAE: *Atherina hepsetus* L. (1, 2); CARANGIDAE: *Alectis alexandrina* (Geoffroy Saint-Hilaire) (22); *Alepes djedaba* (Forsskål) (22); *Caranx rhonchus* Geoffroy Saint-Hilaire (22, 25); *Lichia amia* (L.) (1, 2, 25); *Pseudocaranx dentex* (Bloch & Schneider) (25); *Seriola dumerili* (Risso) (25); *Trachurus mediterraneus* (Steindachner) (4, 12, 14 as *E. trachuri*, 15 as *E. trachuri*, 16 as *E. trachuri*, 17 as *E. trachuri*, 18 as *E. trachuri*, 19 as *E. lepidus* and *E. virgulus* (= *E. trachuri*), 20 as *E. lepidus* and *E. virgulus* (= *E. trachuri*), 29, 30, 31, 32, 33); *T. picturatus* (Bowdich) (37); *T. trachurus* (L.) (1, 2, 3, 5, 7, 8, 9, 10, 11, 21, 23, 24, 26, 27, 34, 37, 38); *Trachynotus ovatus* (L.) (22, 25); CENTRACANTHIDAE: *Spicara maena* (L.) (1, 2, 21, 39); *S. smaris* (L) (1, 2, 13, 17, 33); CEPOLIDAE: *Cepola macrophthalma* (L.) (1, 2); LOPHIIDAE: *Lophius piscatorius* L. (1, 2);

POMATOMIDAE: *Pomatomus saltatrix* (L.) (6, 20); SCOMBRIDAE: *Scomber colias* Gmelin (1, 2, 33, 36, some as *S. japonicus* Houttuyn); SOLEIDAE: *Solea solea* (L.) (28); TRACHIPTERIDAE: *Trachipterus trachypterus* (Gmelin) (1, 2).

Distribution: Area 37, subarea 1 (Western Mediterranean): Étang de Berre, Provence (France) (11); off Corsica (37); off Manilva (Spain) (35); off Besòs and Vilanova (Spain) (39); Area 37, subarea 2 (Central Mediterranean): Adriatic Sea (1, 2, 8, 29, 31, 32); Area 37, subarea 3 (Eastern Mediterranean): Aegean Sea (21, 33, 36, 38); off Israel (22, 25); Area 37, subarea 4 (Black Sea) (4, 5, 6, 7, 9, 12, 15, 16, 18, 20, 30, 38); Mediterranean (area not specified) (13, 14, 17, 16, 18, 19, 34); Area 27 (NE Atlantic) (23, 26); Straits of Gibraltar (24); off Cádiz (35); Bay of Biscay (24, 27); off Scotland (3, 10); Kattegat (27).

Description (Figs. 3–4; Table 3)

[Based on 5 whole-mounted specimens (4 fully gravid and 1 neogravid). See Table 3 for measurements.]

Body elongate, subcylindrical, with maximum width (21–22% length of soma) at level of ventral sucker and/or in hindbody at level of ovary (22–30% length of soma). Ecsoma withdrawn (3 specimens) or partly extended (Fig. 1); extruded ecsoma variable in length but always shorter than 1/3 of soma. Forebody short (11–18% length of soma). Tegument smooth. Pre-oral lobe distinct (with 2 small dorso-lateral thickenings in 1 specimen). Oral sucker subterminal, spherical. Ventral sucker large, muscular, spherical, located close to oral sucker. Sucker width ratio 1:2.58–3.00.

Prepharynx absent. Pharynx muscular, subglobular, overlaps posterior margin of oral sucker dorsally. Oesophagus very short. 'Drüsenmagen' distinct. Caeca thick-walled, reach to mid-ecsoma (close to end of soma when ecsoma withdrawn).

Testes 2, subspherical, oblique, contiguous or slightly separated, located at about junction of first and second thirds of hindbody (ventral sucker to anterior testis 100–109). Seminal vesicle large, 121–197 × 45–85, saccular, clearly tripartite, with anteriormost part 47–61 × 24–36, middle part 28–45 × 33–58 and posteriormost part 44–91 × 45–85, situated between ventral sucker and anterior testis (overlaps ventral sucker dorsally in 2 specimens), connected to pars prostatica by long, narrow duct. Pars prostatica narrow, almost as long as sinus-sac, 36–57; prostatic cells few, small, with field width 28. Sinus-sac elongate-oval, muscular, anterior to (2 specimens) or overlapping ventral sucker postero-dorsally. Genital pore median, at level of pharynx (3 specimens) or slightly posterior, 3–28 from ventral sucker; genital atrium not seen.

Ovary transversely oval (4 specimens) to subglobular (1 specimen), post-testicular, submedian, close to or contiguous with posterior testis. Mehlis' gland, Juel's organ and uterine seminal receptacle not observed. Uterus mainly in hindbody, loops dorsally to gonads, reaches close to posterior margin of soma. Metraterm dorsal to ventral sucker, thin-walled, unites with male duct at base of sinus-sac. Eggs small, operculate. Vitellarium well developed, at level of and extending posterior to ovary, composed of 7 wide, tubular, sinuous lobes; post-vitelline field 251–554 (23–55% length of soma).

Excretory pore terminal. Excretory arms unite dorsally to posterior region of oral sucker. Other details of excretory system not observed.

Remarks

Our study represents only the second record, and the first detailed description, of *E. lepidus* from the western Mediterranean. Although *E. lepidus* has been recorded in a wide range of fish (20 spp.), carangids appear to act as main definitive hosts in the Mediterranean Basin and the NE Atlantic (50% of records) (see Gibson & Bray, 1986, for details on the taxonomy, synonymies and treatment of the records from outside these regions). Comparisons of the metrical data support this suggestion, since the specimens from the type-host, *Lichia amia*, and from *Spicara* spp. exhibit lower ranges for the size of body, suckers and gonads than those for material described from *Trachurus* spp., whereas egg-size varies within a narrow range (Table 3).

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6. PARASITE COMMUNITIES IN THE RED MULLET, *MULLUS BARBATUS* L., RESPOND TO SMALL-SCALE VARIATION IN THE LEVELS OF POLYCHLORINATED BIPHENYLS IN THE WESTERN MEDITERRANEAN

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Parasite communities in the red mullet, *Mullus barbatus* L., respond to small-scale variation in the levels of polychlorinated biphenyls in the Western Mediterranean

Marta Carreras-Aubets [a], Francisco Esteban Montero [b], Aneta Kostadinova [c,d], Maite Carrassón [a,*]

[a] Departament de Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma de Barcelona, Cerdanyola, 08193 Barcelona, Spain
[b] Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, P.O. Box 22 085, 46071 Valencia, Spain
[c] Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic
[d] Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria

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ABSTRACT

We examined parasite populations and communities in the Mediterranean sentinel fish species, *Mullus barbatus*, sampled at a small-scale PCB gradient at the shelf sediments off Catalonian coasts of the Western Mediterranean. A recurrent feature at both the population and community level was the differentiation of the samples along the increasing PCB levels simultaneously registered in the sediments. Both directly transmitted ectoparasites and endoparasites with complex life-cycles transmitted *via* food chains exhibited a decrease in abundance with the increase in PCB levels. Parasite numerical responses translated into significant differences in infracommunity structure with decreasing predictability associated with increasing PCB levels. The abundance of two species, the specialist *Opecoeloides furcatus* and the generalist nematode *Hysterothylacium fabri*, contributed substantially to the observed dissimilarity between infracommunity samples along the gradient. The observed parasite responses to moderate levels of pollution were simultaneously validated by both chemical monitoring and effect biomarkers.

1. Introduction

The marine environment is often viewed as the final repository of contaminants originating from human industrial, urban and rural activities that result in historical build up of persistent contaminants in the benthic habitats (Williams, 1996; Morrisey et al., 2003; Roberts et al., 2008). Recently, the application of a battery of exposure (i.e. chemical analysis of bioaccumulated xenobiotics e.g. Porte and Albaigés, 1993; Bocio et al., 2007) and effect biomarkers (i.e. biochemical and cellular responses) in conjunction with chemical monitoring, has been encouraged as an advanced approach in biomonitoring programs (reviewed by van der Oost et al., 2003; see also e.g. Zorita et al., 2007, 2008; Solé et al., 2009 and references therein). Benthic organisms such as the Mediterranean mussel *Mytilus galloprovinicalis* Lamarck and the Red mullet, *Mullus barbatus* L., have commonly been used as bioindicators of contamination in pollution hot-spots and sensitive areas as well as to establish baseline pollutant levels in the Mediterranean (e.g. Porte and Albaigés, 1993; Zorita et al., 2007, 2008 and references therein). *M. barbatus* is a valued commercial fish species with a wide distribution in the Mediterranean, found on gravel, sand and mud bottoms of the shelf within a depth range of 10–300 m (Froese and Pauly, 2012). This species has been categorised as belonging to the high hepatic xenobiotic-metabolising activity fish group and recommended as a sentinel species in the Mediterranean (FAO/UNEP, 1993, 1997; UNEP/RAMOGE, 1999). Due to its pronounced sensitivity and greater abilily to bioaccummulate different types of pollutants, *M. barbatus* has been used extensively as a bioindicator for pollution monitoring with the application of combined chemical and biomarker approaches (Zorita et al., 2008; Insausti et al., 2009; Solé et al., 2009; Della Torre et al., 2010 and references therein).

Parasites are increasingly being used as independent early warning bioindicators of environmental impact (reviewed in MacKenzie et al., 1995; Lafferty, 1997; Williams and MacKenzie, 2003; Sures, 2003, 2004, 2008; Marcogliese, 2005; Morley et al., 2006; Blanar et al., 2009; Vidal-Martínez et al., 2009) and there is an increasing frequency of multidisciplinary studies on effects of pollution and environmental stress in aquatic ecosystems that incorporate data on parasite populations and communities (Marcogliese, 2005 and references therein). Environmental pollution affects parasite population abundance, and consequently community structure, through direct exposure of adult and larval ectoparasites or the free-living stages of endoparasites and indirect effects on their intermediate and final hosts (Poulin, 1992; MacKenzie, 1999; Pietrock and

* Corresponding author. Tel.: +34 93 581 37 86; fax: +34 93 581 13 21.
E-mail address: Maite.Carrasson@uab.cat (M. Carrassón).

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Marcogliese, 2003). Because most of the parasites are trophically transmitted, the numerical responses of their populations to pollution stress may mirror potential alterations of the local food webs (Marcogliese, 2005). Due to the integrative nature of stress responses by parasites, an increasing number of studies use parasites and parasite communities to discriminate among host populations from environments with different levels of pollution impact. These can be generally grouped into one of two categories, studies on catastrophic events (e.g. Pérez-del Olmo et al., 2007; Pérez-del-Olmo et al., 2009) in which pollution effects are relatively easy to detect because of the magnitude of the impact but are difficult to assess due to the lack of reference data; and studies on adverse chronic exposure to toxicants based on planned comparisons between sites chronically exposed to pollutants and unpolluted reference sites (e.g. see recent meta-analyses by Blanar et al., 2009; Vidal-Martínez et al., 2009 and references therein).

This study is part of a monitoring program within the framework of the oceanographic project BIOMARE (Spanish Ministry of Science and Innovation) that applied a range of biomarkers and comparative approaches using a large spectrum of marine fish species as sentinels (Insausti et al., 2009; Solé et al., 2010a,b). Fish were sampled at the shelf and slope of two sites off Catalonian coasts of the Western Mediterranean, Besòs and Vilanova, which were selected based on *a priori* expected differences in chemical exposure, the former considered to be characterised by high pollution load (Castells et al., 2008; Solé et al., 2010a). However, this hypothesis was not confirmed by analyses of fish hepatic responses, bile PAH levels, muscular markers and the chemical analysis of the sediments (Insausti et al., 2009; Solé et al., 2010a,b). Instead, these studies revealed a small-scale pollution gradient with levels of pollutants falling within the lower range reported for the Mediterranean (Solé et al., 2010a and references therein).

Nevertheless, Solé et al. (2010a) reported elevated activities of carboxylesterases (CbE; EC 3.1.1.1) in *M. barbatus* from both localities thus indicating higher hepatic metabolism generally associated with exposure to sediment pollution. In a multivariate global analysis based on data for 18 fish species common in the two localities, these authors have also revealed that CbE levels were positively related to feeding on zooplankton and benthos (Solé et al., 2010a). Furthermore, in a comparison of muscle cholinesterase (ChE) activities and lipid peroxidation (LP) levels in summer samples of *M. barbatus* from off Besòs and Vilanova, Solé et al. (2010b) revealed significantly inhibited activities of the acetylcholinesterase (AChE; EC 3.1.1.7) and the pseudocholinesterases butyrylcholinesterase (BChE) and propionylcholinesterase (PrChE) (EC 3.1.1.8) in the fish sampled off Vilanova that also exhibited higher LP levels. These results from the biomarker approaches of the project provide additional confirmation for the sensitivity of *M. barbatus* as a sentinel species. On the other hand, the differential response detected by the muscular biomarkers indicates chemical stress situation off Vilanova associated with enhanced pollutant biodisponibility (Solé et al., 2010b).

Here we examine the composition and structure of the parasite communities in *M. barbatus* using the samples from off Besòs and Vilanova collected in the course of the project BIOMARE which encompass the subsamples examined for different biomarkers in order to test if variations in parasite community structure can be detected that might be related to small-scale differences in the pollution loads and levels of chemical stress at the two localities. In particular, we contrasted parasite population abundance and infracommunity structure in samples collected at three levels of PCB in sediments of the localities studied.

The red mullet-parasite system appears to be ideally suited for the use of parasites as indicators of pollution disturbance. *M. barbatus* is a demersal, sedentary species that exhibits fidelity to its benthic habitats (except for inter-depth migration related to reproduction), with a diverse diet comprising three major benthic invertebrate groups (polychaetes, decapods and small crustaceans) (Machias and Labropoulou, 2002). Due to this, *M. barbatus* hosts a large number of readily identifiable parasite species transmitted *via* food chains that occur commonly in the two species of *Mullus* in the Mediterranean, *Mullus surmuletus* L. and *M. barbatus* (Radujković and Raibaut, 1989; Le Pommelet et al., 1997; Bartoli et al., 2005 and references therein). A substantial proportion of these represent host specific parasites of *Mullus* spp. whose transmission stages (eggs, free-living larvae) are in contact with the sea sediments. The above host characteristics, combined with its sensitivity to chemical stress and the potential of parasite populations to reflect pollution impacts, served as the basis of our selection of the host-parasite system.

2. Materials and methods

2.1. Sampling area and parasite collection

A total of 97 specimens of *M. barbatus* was collected and examined for parasites in winter (February–March), summer (June–July) and autumn (October–November) 2007. Sampling was carried out on a research (García del Cid – CSIC) and a commercial trawling vessel (Stella Maris III) from the continental shelf at depths of 50–62 m off the mouth of River Besòs and off Vilanova (see coordinates in Table 1). The two localities are c. 60 km apart and thus representative of similar abiotic and biotic conditions for parasite transmission. Pollution loads at Besòs are under the influence of River Besòs and those at Vilanova are influenced by River Llobregat, the latter enhanced by a north–south water currents. Spatio-temporal trends associated with enhanced water turbidity at the two localities have created a small-scale pollution gradient with PCB levels falling within the lower range of those observed in the Western Mediterranean (Solé et al., 2010a). Data for PCB concentrations (means of five independent measures of the sum of seven congeners, IUPAC Nos. 28, 52, 101, 118, 138, 153, 180, recommended by the ''Bureau Communautaire pour la Reference'') in sediments obtained in the course of sampling showed the highest levels off Vilanova (Solé et al., 2010a). The difference in PCB levels associated with parasite samples was three- to more than 10-fold (Table 1).

On board, fish were measured and frozen at −20 °C in individual plastic bags. In the laboratory fish were defrosted, weighed (liver, gonad and eviscerated body weight) and dissected. External surfaces were examined for ectoparasites, and all organs and body musculature were carefully checked for endoparasites under stereomicroscope. Digeneans and cestodes were stained with iron acetocarmine and examined as permanent mounts in Canada balsam. Nematode larvae and crustaceans were identified on temporary mounts in saline solution or glycerin. All parasites were identified and counted.

2.2. Data analysis

Ecological terms used follow Bush et al. (1997). Prevalence (expressed in per cent) represents the proportion of the hosts in the sample infected with a given species. Species with prevalence higher than 10% in the entire dataset are further referred to as common. Abundance is defined as the number of individuals of a particular parasite in/on a single host regardless of whether or not the host is infected. Mean abundance is the sum of individuals of a given species in a sample divided by the total number of hosts examined. Fish condition was assessed by hepatosomatic index (HSI) calculated as liver weight (g)/eviscerated body weight

Table 1
Levels of polychlorinated biphenyl (PCB) concentrations in sediments off Besòs and Vilanova and summary data for the samples of *Mullus barbatus* Means ± standard deviations are provided for fish total length (TL); condition factor (*K*); and hepatosomatic index (HSI).

PCB[a](ng g^{-1} d.w.)	PCB factor levels	Locality	Season	Trawling coordinates (range)	Sample size	TL (cm)	K	HSI
0.5	1	Besòs	Autumn	41°21′–41°25′N, 2°19′–2°20′E	20	17.9 ± 3.6	1.17 ± 0.08	1.72 ± 0.51
2.4	2	Besòs	Winter	41°24′–41°25′N, 2°17′–2°22′E	19	16.7 ± 3.5	1.15 ± 0.15	2.02 ± 0.89
1.6	2	Besòs	Summer	41°22′–41°25′N, 2°19′–2°21′E	29	17.9 ± 3.4	1.13 ± 0.09	2.38 ± 1.16
5.8	3	Vilanova	Summer	41°08′N, 1°40′–1°45′E	29	16.6 ± 3.3	1.08 ± 0.25	1.94 ± 1.12

[a] Data (means of five site/season independent measures) from Solé et al. (2010b). Concentrations expressed as a Σ of PCB 28, 52, 101, 118, 138, 153 and 180.

(g) × 100 and Fulton's condition factor (*K*) calculated as eviscerated body weight (g)/length $(cm)^3 \times 100$.

Parasite abundance data were ln (x+1) transformed and those for fish total length (TL), HSI and K were ln-transformed prior to General Linear Model (GLM ANOVA) analyses. To visualise the patterns in parasite abundance in relation to spatial/temporal PCB variation we first applied Factorial Correspondence Analysis (FCA) on a data matrix comprising component population abundance for 11 common parasite species (out of the 22 species recovered) and three sediment PCB levels: (i) $\leqslant$0.5 ng g^{-1} dry weight (referred to as PCB1); (ii) 1.5–2.5 ng g^{-1} dry weight (referred to as PCB2); and (iii) >5.5 ng g^{-1} dry weight (referred to as PCB) (Table 1).

Secondly, using individual fish as replicate samples, we tested for differences in abundance and prevalence among the parasite populations associated with the three PCB levels (ANOVA and Fisher's exact test, respectively). Finally, we used permutational multivariate analyses of similarity (PERMANOVA) to test the null hypothesis of no differences in parasite community structure due to sediment PCB levels using parasite infracommunities (i.e. populations of all species in individual fish) as replicate samples. Two community level analyses were carried out with PERMANOVA+ for PRIMER v6 (Anderson et al., 2008) on Bray-Curtis similarity matrices derived from the square root transformed abundance data, one based on all four fish samples examined (i.e. at the three PCB levels) and one restricted to the summer samples from off Besòs and Vilanova (i.e. at two PCB levels, PCB2 and PCB3, Table 1). Permutation *P*-values were obtained under unrestricted permutation of raw data (9,999 permutations).

3. Results

All individual fish analysed were infected with at least one parasite species. A total of 1,973 parasites corresponding to 22 species were identified (Table 2): 12 digeneans, two nematodes, two larval cestodes and two crustaceans. Of these, eight species (indicated by a star in Table 2) represented parasites specific to Mediterranean *Mullus* spp.: the digeneans *Aponurus mulli* Carreras-Aubets et al., 2011, *Lasiotocus mulli* (Stossich, 1883), *Opecoeloides furcatus* (Bremser in Rudolphi, 1819), *Poracanthium furcatum* Dollfus, 1948 and *Proctotrema bacilliovatum* Odhner, 1911; the nematodes *Ascarophis mullusi* and *Paracapillaria* sp. (probably a new species; F. Moravec, personal communication); and the copepod *Hatschekia mulli* (van Beneden, 1851). Parasite component communities in *M. barbatus* sampled from off Besòs comprised 20 parasite species; of these, 10 were rare (prevalence <10% in the entire dataset). The component community sampled from off Vilanova consisted of 18 parasite species and included 10 rare species (Table 2). Species lists of the two close localities largely overlapped. The digenean *L. mulli* and the larval tetraphyllidean cestode reported under the collective name *Scolex pleuronectis* Müller, 1788 were only found in fish sampled off Vilanova and the digenean *P. furcatum* was found in one sample off Besòs only. Two species, the larval nematode *Hysterothylacium fabri* (Rudolphi, 1819) and the adult digenean *O. furcatus*, showed consistently high prevalence and abundance in all samples (Table 2). Fish length did not differ statistically among the samples representing the three PCB levels (ANOVA $F_{(2,94)} = 0.932$, $P = 0.397$) and no significant differences were found for fish hepatosomatic index (ANOVA $F_{(2,94)} = 1.702$, $P = 0.188$) or condition factor (ANOVA $F_{(2,94)} = 1.654$, $P = 0.197$).

Fig. 1 presents a plot of the first factorial plane of co-inertia analysis explaining 89% of the variance, predominantly on the first axis (69% of the total inertia) of the FCA carried out using component population data for the 11 common species in *M. barbatus*. The first plane of the FCA illustrates a clear gradient differentiating the samples in relation to the three PCB levels in the sediments. This pattern was associated with the abundance of the component parasite populations of five, four and two common species, respectively, that characterised the multivariate relationship among the samples examined at the increasing PCB gradient (Fig. 1). Component populations of four species exhibited the strongest correlations with the first FCA axis. These were the nematodes *H. fabri* (squared cosine value, $Cosine^2 = 0.952$) and *Contracaecum* sp. ($Cosine^2 = 0.915$); the isopod *Gnathia* sp. ($Cosine^2 = 0.921$); and the digenean *P. bacilliovatum* ($Cosine^2 = 0.834$). An additional suite of four species was strongly associated with the second FCA axis which differentiated the samples taken at intermediate PCB levels (PCB2): the nematodes *Paracapillaria* sp. ($Cosine^2 = 0.883$) and *Hysterothylacium aduncum* (Rudolphi, 1802) ($Cosine^2 = 0.592$); and the digeneans *Prosorhynchus crucibulum* Rudolphi, 1819 ($Cosine^2 = 0.641$) and *O. furcatus* ($Cosine^2 = 0.574$).

Univariate analyses of levels of parasitism revealed that although only two of the above species (*Paracapillaria* sp. and *Gnathia* sp.) showed significant variation in prevalence along the PCB gradient studied, five species (the specialist parasites of mullets *O. furcatus* and *Paracapillaria* sp., and the generalist larval parasites *H. fabri*, *Contracaecum* sp. and *Gnathia* sp.) exhibited a significant decrease in abundance at increased PCB levels: (Table 3, Analysis 1). There were no significant differences in infracommunity richness and abundance in relation to the three PCB concentration levels in sediments. Comparisons restricted to the samples examined in the same season (summer, PCB levels 2 and 3) revealed significant differences in both infracommunity richness and abundance and in the abundance of four of the species that have shown significant variation in Analysis 1, plus the specialist copepod *H. mulli*. The two species depicted in Analysis 1 also exhibited significant differences in prevalence in relation to PCB levels 2 and 3 (Table 3, Analysis 2). In all contrasts infection parameters had much lower values in the fish samples taken at the highest PCB level (Table 2).

These differences in parasite distributions among individual fish was reflected in the differentiation of community structure in relation to PCB levels in sediments which we assessed in two permutational multivariate analyses (PERMANOVA). The first analysis based on the replicate infracommunity samples from all three PCB levels revealed highly significant effect of 'treatment' (i.e. PCB levels) on community structure ($Pseudo\text{-}F_{(2,94)} = 3.86$; $P_{(perm)} = 0.0001$; 9924 unique permutations; all *post hoc* comparisons significant). We observed a marked decrease in the predictability of parasite infracommunities with increasing levels of PCB (mean similarity

Table 2
Prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites of *Mullus barbatus* and parasite community parameters associated with the three levels of polychlorinated biphenyl (PCB) concentrations in sediments off Besòs and Vilanova.

PCB level (Season)	PCB1 (Autumn)		PCB2 (Winter)		PCB2 (Summer)		PCB3 (Summer)	
Locality	Besòs						Vilanova	
	P%	MA ± SD	P%	MA ± SD	P%	MA ± SD	P%	MA ± SD
Digenea								
Aphallus tubarium met.	–	–	10.5	0.11 ± 0.31	–	–	3.5	0.03 ± 0.19
Aponurus mulli[*]	10.0	0.10 ± 0.31	42.1	0.84 ± 1.46	13.8	0.41 ± 1.15	6.9	0.17 ± 0.76
Derogenes latus	–	–	–	–	3.5	0.07 ± 0.37	6.9	0.07 ± 0.26
Diphtherostomum brusinae	–	–	–	–	3.5	0.03 ± 0.19	6.9	0.07 ± 0.26
Lasiotocus mulli[*]	–	–	–	–	–	–	3.5	0.07 ± 0.37
Lecithochirium musculus	5.0	0.05 ± 0.22	5.3	0.05 ± 0.23	–	–	–	–
Lecithocladium excisum	20.0	0.25 ± 0.55	5.3	0.05 ± 0.23	–	–	13.8	0.24 ± 0.69
Opecoeloides furcatus[*]	60.0	1.95 ± 2.16	68.4	4.37 ± 9.15	89.7	10.27 ± 16.59	69.0	4.79 ± 8.45
Phyllodistomum sp.	5.0	0.15 ± 0.67	10.5	0.16 ± 0.50	20.7	0.48 ± 1.15	6.9	1.21 ± 6.31
Poracanthium furcatum[*]	5.0	0.05 ± 0.22	–	–	–	–	–	–
Proctotrema bacilliovatum[*]	15.0	0.15 ± 0.37	21.1	2.26 ± 8.46	20.7	0.76 ± 2.20	31.0	3.83 ± 9.73
Prosorhynchus crucibulum met.	5.0	0.05 ± 0.22	5.3	0.05 ± 0.23	13.8	0.17 ± 0.47	6.9	0.07 ± 0.26
Nematoda								
Ascarophis mullusi[*]	30.0	0.60 ± 1.10	10.5	0.11 ± 0.32	34.5	0.62 ± 1.05	20.7	0.38 ± 0.82
Contracaecum sp. larva	35.0	1.10 ± 2.55	10.5	0.11 ± 0.32	24.1	0.55 ± 1.30	10.3	0.10 ± 0.31
Cucullanus longicollis	5.0	0.05 ± 0.22	–	–	–	–	3.5	0.07 ± 0.37
Hysterothylacium aduncum larva & adult	20.0	0.35 ± 0.81	5.3	0.05 ± 0.23	13.8	0.14 ± 0.35	13.8	0.17 ± 0.47
Hysterothylacium fabri larva	95.0	10.40 ± 8.92	78.9	3.74 ± 3.81	96.6	9.52 ± 8.93	86.2	4.00 ± 3.31
Paracapillaria sp.[*]	10.0	0.10 ± 0.31	15.8	0.21 ± 0.54	24.1	0.38 ± 0.82	–	–
Cestoda								
Scolex pleuronectis larva	–	–	–	–	–	–	6.9	0.17 ± 0.76
Larval Trypanorhyncha	–	–	10.5	0.11 ± 0.32	–	–	10.3	0.10 ± 0.31
Copepoda								
Hatschekia mulli[*]	–	–	5.3	0.16 ± 0.69	13.8	0.17 ± 0.47	–	–
Isopoda								
Gnathia sp. (praniza larva)	85.0	1.45 ± 1.10	21.1	0.26 ± 0.56	31.0	0.83 ± 2.27	3.5	0.10 ± 0.56
Total species richness	15		16		15		18	
Mean species richness		4.05 ± 1.47		3.26 ± 1.48		4.03 ± 1.70		3.10 ± 1.26
Mean abundance		16.80 ± 9.21		12.63 ± 11.95		24.41 ± 21.19		15.66 ± 17.23

[*] Indicate specialist parasites of mullets (*Mullus* spp.).

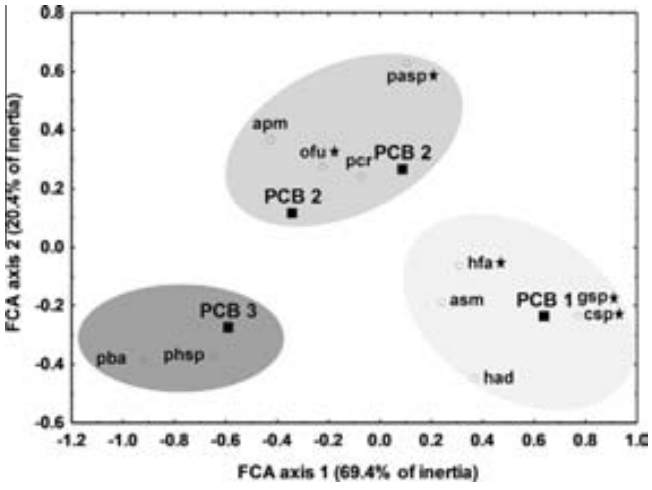

Fig. 1. Plot of the first factorial plane of co-inertia analysis of the factorial correspondence analysis (FCA) on component population data for the 11 common species in *Mullus barbatus*. Numbers indicate the three PCB levels in sediments. Abbreviations for species names: apm, *Aponurus mulli*; asm, *Ascarophis mullusi*; csp, *Contracaecum* sp.; gsp, *Gnathia* sp.; had, *Hysterothylacium aduncum*; hfa, *Hysterothylacium fabri*; ofu, *Opecoeloides furcatus*; pasp, *Paracapillaria* sp.; pba, *Proctotrema bacilliovatum*; pcr, *Prosorhynchus crucibulum*; phsp, *Phyllodistomum* sp.

from 50.9% to 38.6%, Table 4) which was coupled with an increase in dissimilarities among PCB levels (Table 4). Application of SIMPER analysis revealed that as a rule, the parasite species which contributed most to the similarity of infracommunities within the replicate samples of the three PCB levels also contributed substantially to the differentiation in community structure observed in the contrasts among them (see Table 4 for a breakdown into species percent contributions to the total average similarity/dissimilarity within/among PCB levels, respectively). The second PERMANOVA analysis restricted to the summer samples at the higher PCB levels (PCB2 and PCB3) confirmed the substantial parasite community differentiation in relation to the 'treatment effect' ($Pseudo\text{-}F_{(1,56)} = 3.52$; $P_{(perm)} = 0.0028$; 9942 unique permutations). Invariably, the mullet specialist digenean *O. furcatus* and the larval generalist nematode *H. fabri* were identified as 'key discriminating species' in all contrasts associated with PCB levels.

4. Discussion

The overall parasite diversity in *M. barbatus* studied in the PCB gradient was rather high since we identified 22 out of the 34 species recorded in the distributional range of this host (Kostadinova, unpublished database available upon request). These included one species new to science, *A. mulli* (see Carreras-Aubets et al., 2011), and a new host record, *Aphallus tubarium* (Rudolphi, 1819). A characteristic feature of parasite communities in *M. barbatus* was that specialist parasites of *Mullus* spp. accounted for more than half of the adult forms recovered whereas all larval parasites were host generalists completing their life-cycles in a range of fish definitive hosts.

A recurrent feature at both the population and community level was the differentiation of the samples along the increasing PCB gradient simultaneously registered in the sediments. At the population level, this was depicted by the multivariate relationship

Table 3
ANOVA statistics for the parasites of *Mullus barbatus* exhibiting significant variations in abundance and infracommunity parameters in relation to the levels of polychlorinated biphenyl (PCB) concentrations in sediments. Similarities between the two analyses indicated in bold.

Species	Analysis 1: All samples (all three PCB levels)				Analysis 2: Summer samples (PCB2 *vs* PCB3)			
	Abundance			Prevalence	Abundance			Prevalence
	df	*F*	*P*[a]	*P*[a]	df	*F*	*P*[a]	*P*[a]
Opecoeloides furcatus	2, 94	3.45	**0.036**	**ns**	1, 56	5.66	**0.021**	**ns**
Hysterothylacium fabri larva	2, 94	4.71	**0.011**	**ns**	1, 56	13.82	**<0.001**	**ns**
Paracapillaria sp.	2, 94	3.84	**0.025**	**0.014**	1, 56	7.66	**0.008**	**0.010**
Contracaecum sp. larva	2, 94	3.52	0.034	ns	1, 56	3.33	ns	ns
Gnathia sp. (praniza larva)	2, 94	17.60	**<0.001**	**<0.0001**	1, 56	5.64	**0.021**	**0.012**
Hatschekia mulli	2, 94	2.49	ns	ns	1, 56	4.24	0.044	ns
Mean species richness	2, 94	2.58	ns	–	1, 56	5.48	0.023	–
Mean abundance	2, 94	1.32	ns	–	1, 56	7.83	0.007	–

[a] *P*, significance of differences, *P*-values >0.05 indicated by ns.

Table 4
Mean similarity/dissimilarity for parasite infracommunities sampled within and among the three levels of polychlorinated biphenyl (PCB) concentrations in sediments and a breakdown into contributions from individual parasite species.

Species/PCB level/contrast	PCB1	PCB2	PCB3	PCB1 *vs* PCB2	PCB2 *vs* PCB3	PCB1 *vs* PCB3
Mean similarity/dissimilarity (%)	50.9*	42.3*	38.6*	57.1†	60.8†	62.0†
Hysterothylacium fabri	57.5	52.9	61.2	20.5	18.0	20.5
Opecoeloides furcatus	14.5	35.4	28.9	20.3	23.0	17.1
Gnathia sp.	20.8	–	–	12.2	–	13.7
Proctotrema bacilliovatum	–	–	–	–	13.1	10.7
Cumulative contribution (%)	92.8	88.3	90.1	53.0	54.1	62.0

Only species contributing to more than 10% of the mean community similarity (indicated by a *) within- /dissimilarity (indicated by a †) between PCB levels are included.

among the samples and supported by the consistently lower abundances of five of the 11 common parasite species in *M. barbatus* associated with the highest PCB levels off Vilanova. Furthermore, the restricted analysis considering possible temporal variation as a potential confounding factor (see e.g. Pérez-del-Olmo et al., 2009), reinforced the validity of these differences and revealed significantly lower infracommunity richness and abundance in fish sampled at the higher PCB levels.

Both directly transmitted ectoparasites and endoparasites with complex life-cycles transmitted *via* food chains exhibited a decrease in abundance with the increase in PCB levels. Whereas the data for endoparasites support the predictions on the effects of environmental stress on abundance, the numerical responses of the two ectoparasitic crustaceans in *M. barbatus* appear in contrast with the general expectation that infections with ectoparasites with direct single-host life-cycles would tend to increase, with increasing levels of pollution (MacKenzie, 1999). However, this prediction was based on data for trichodinid ciliates and monogeneans on gills, the mechanism implied being immunosupressive effects of environmental stressors (Broeg et al., 1999; Moles and Wade, 2001; Khan, 2003).

A common feature of both ectoparasitic crustaceans studied by us is the biphasic life-cycle i.e. comprising free-living adult and parasitic larval stages (*Gnathia* sp.) and free-living larval and parasitic adult stages (*Hatschekia mulli*). In both species the parasitic stage is temporary and the benthic dweller phases represent a considerable proportion of the life-span; they also undergo a series of moults (e.g. Smit et al., 2003). It is therefore possible that the decrease in prevalence and abundance of the parasitic stages of the generalist crustaceans on *M. barbatus* are associated with the prolonged contact of the free-living phases with contaminated sediments (*via* e.g. effect of pollutants on moulting; see Rinderhagen et al., 2000 for a review). Free-living crustaceans offer excellent opportunities to derive sensitive and ecologically relevant indicators of environmental stress (Rinderhagen et al., 2000) and recent meta-analysis on the effect of pollutants on aquatic parasites, demonstrated significant effects on parasite levels and negative interactions with environmental impact for parasitic crustaceans under a range of stressors such as PCBs, pesticides, heavy metals, pulp-mill effluents and eutropication (Vidal-Martínez et al., 2009).

Parasite numerical responses to the PCB gradient studied translated into significant differences in infracommunity structure with decreasing predictability associated with increasing PCB levels. This community differentiation depicted by both similarity analyses was due to the significant effect of the PCB gradient on the population abundance of two host specialist (*O. furcatus* and *Paracapillaria* sp.) and three generalist species (*H. fabri*, *Contracaecum* sp. and *Gnathia* sp.). The abundance of two species, the specialist digenean *O. furcatus* and the generalist larval nematode *H. fabri*, contributed substantially to the observed similarity within- and dissimilarity between infracommunity samples along the gradient. Both species have complex life-cycles and are transmitted to definitive hosts *via* food chains but utilise different pathways and intermediate hosts.

O. furcatus (currently reported solely from *M. barbatus* and *M. surmuletus*) uses the carnivorous gastropod *Mitrella scripta* (L.) (Jousson et al., 1999; Jousson and Bartoli, 2000). The second intermediate host could be, by analogy with the life-cycle of its sibling species *Opecoeloides columbellae* (Pagenstecher, 1863), a shrimp of the genus *Hippolyte* (Leach, 1814) (Decapoda) characterised by an extended larval development (Jousson and Bartoli, 2000; Guerao et al., 2011). Many aspects of the life-cycle of *H. fabri* still remain unknown. To date, *Trisopterus minutus* (L.) and *Uranoscopus scaber* L. were suggested to act as definitive hosts of this species in the Mediterranean (Nikolaeva and Naidenova, 1964; Petter and Radujković, 1986) but it is possible that the definitive host range is much wider. No data exist for the first intermediate hosts of this species, but inferring from the life-cycle of the closely related *H. aduncum*, it might be expected that eggs embryonate in the water to the second larval stage and that the development to a third stage larvae occurs upon ingestion by various invertebrates (mysids, copepods, isopods) and fish (Anderson, 2000). At least seven fish

species have been reported to act as intermediate and/or paratenic hosts of *H. fabri*: *M. surmuletus*, *M. barbatus*, *Trachurus trachurus* (L.), *Uranoscopus scaber*, *Pagellus erythrinus* (L.), *Phycis blennoides* (Brünnich), *Phycis phycis* (L.) (Petter et al., 1984; Petter and Maillard, 1988; Martín-Sánchez et al., 2003; Valero et al., 2006).

These different life-cycle strategies and host specificity indicate different possibilities for transmission failure resulting from pollution stress for the two key species in parasite communities in *M. barbatus*. Both species have a free-living stage in contact with the sea sediments. However, the local populations of *O. furcatus* are supported solely by the two *Mullus* spp. acting as definitive hosts and are strictly dependent on infection levels in the population of a single gastropod host (and perhaps a single decapod) intermediate host. On the other hand, the abundance of *H. fabri* in *M. barbatus* would be associated with the abundance levels in the local fish community and dependent upon infection levels in a wide range of crustacean intermediate hosts. Our initial expectation was that generalist parasites would have higher chances for transmission and persistence in stressed conditions due to their association with a multiple array of hosts. The fact that two specialist and three generalist parasites in *M. barbatus*, including the two key discriminating species in the multivariate similarity contrasts, exhibited the same direction of change along the PCB gradient, may therefore indicate a wider impact on benthic communities with consistent effects on parasite transmission.

Perhaps the most important result of our study is that the observed numerical and structural parasite community responses to moderate levels of pollution are in agreement with the significantly inhibited muscle cholinesterase (ChE) activities and the higher lipid peroxidation (LP) levels in the individual fish hosts studied off Vilanova (Solé et al., 2010b). Taken collectively, these data support the idea of stronger chemical stress situation with a detectable impact on parasite transmission off Vilanova. To the best of our knowledge, this is the first study providing data on parasite population responses to pollution that are simultaneously validated by both chemical monitoring and effect biomarkers, and the first examining small-scale variations in environmental pollutant concentrations in the Mediterranean.

Two mutually non-exclusive hypotheses may be suggested for the significant decrease in levels of parasitism associated with the increase in PCB levels in sediments. A range of physiological and behavioural processes in fish with a cholinergic-mediated neural control such as respiration, swimming capacity, feeding, predator–prey relationships can be drastically affected by an inhibition of brain and muscle AChE activity (e.g. Sanchez-Hernandez, 2001; Peakall et al., 2002 and references therein). It is therefore possible that substantially lower parasite abundance levels in the fish sampled from off Vilanova may have reflected reduced food consumption and/or abolished food search behaviour associated with the significantly inhibited activities of the acetylcholinesterases detected by Solé et al. (2010b) in the same sample.

Our second hypothesis is that impoverishment of the parasite communities along the gradient and of those sampled at the highest PCB levels off Vilanova in particular, is associated with reduced transmission success of the common parasites of *M. barbatus*. Vast evidence has been accumulated from experimental exposure studies that parasite free-living transmission stages are susceptible to different types of pollution (reviewed in Morley et al., 2003; Pietrock and Marcogliese, 2003; Williams and MacKenzie, 2003; Blanar et al., 2009). Therefore, direct effects to the sensitive to toxicants free-living transmission stages of the parasites (eggs, digenean miracidia and cercariae) that are largely constrained to habitats in close proximity with sediments may account for reduced transmission success with increased PCB gradient. For example, studies on the common whelk, Buccinum undatum L., along a marked spatial concentration gradient at an sewage-sludge dump-site in the Firth of Clyde, (Scotland) have shown that the contaminant levels in snails reflect those in the sediments (Halcrow et al., 1974) and that the prevalence of larval digenean parasites decrease substantially towards the dump site (Siddall et al., 1993). These authors associated the gradient in parasitism principally with toxic effects on the infective miracidium, reducing parasite transmission to the molluscan host (Siddall et al., 1993).

However, the small scale of the gradient studied by us may rather reflect indirect impact of moderate chronic pollution on parasite transmission due to effects on the benthic intermediate hosts in the parasites' life-cycles. The contamination of the benthic fauna (e.g. molluscs, amphipods, isopods) reflects that in the sediments (Halcrow et al., 1974; Knickmeyer and Steinhart, 1989; Bavel et al., 1995). Deleterious effects on lipid metabolism and reproduction caused by PCBs have been well documented in aquatic organisms of various trophic levels and a direct relationship between PCB exposure at environmentally realistic concentrations and reduced reproductive potential in oysters has been recently demonstrated (Chu et al., 2003 and references therein). Toxicity of xenobiotics to the intermediate mollusc host has been associated with the significantly lower prevalence and abundance of the digenean *Cryptocotyle lingua* in flounder examined near a PCB-contaminated naval facility than at farther sites (Khan, 1999). Parasite infection can further affect molluscan host fitness thus rendering it more susceptible to toxicants (Lafferty and Holt, 2003) the end result being reduced host population abundance. On the other hand, contaminated sediments can influence the patterns of recruitment of macroinvertebrates at small spatial scales and thus affect the structure of benthic communities (e.g. Roach et al., 2001; Roberts et al., 2008 and references therein). Both mechanisms, direct toxicity and reduced recruitment can affect synergistically the abundance of populations of a range of benthic invertebrate intermediate hosts and thus alter transmission rates of parasites transmitted via food webs in stressed marine environment.

In conclusion, small-scale differences in PCB levels may have resulted in disturbance of host-parasite relationships leading to detectable alterations in the parasite community structure in *M. barbatus*. The consistency of the direction of changes in population abundance of both host specific and generalist parasite species indicates that these alterations may have affected infracommunities in the sentinel fish species but also parasite populations supported by other local fish species. Our results suggest that consideration of the abundance of *O. furcatus* and *H. fabri* and the structure of parasite communities in biomonitoring studies using *M. barbatus* as a sentinel species, would lead to a more comprehensive evaluation of the ecological effects of anthropogenic pollution in the shelf habitats in the Mediterranean.

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7. PARASITE COMMUNITY COMPOSITION AND STRUCTURE OF THE BLOTCHED PICAREL *SPICARA MAENA* (L.) FROM NORTH-WESTERN MEDITERRANEAN: EFFECTS OF SEASON AND LOCALITY ON THE INFECTION PARAMETERS

Parasite community composition and structure of the blotched picarel *Spicara maena* (L.) from north-western Mediterranean: effects of season and locality on the infection parameters

Carreras-Aubets, M.[a*], Montero, F. E.[b], Carrassón, M.[a]

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

[b]Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, PO Box 22 085, 46071 Valencia, Spain.
*Corresponding author. Tel.: +34 93 581 28 17; fax: +34 93 581 13 21.

E-mail address: marta.carrerasau@e-campus.uab.cat (M. Carreras-Aubets).

ABSTRACT

We described the parasite communities of *Spicara maena* (L.) off the north-western Mediterranean, captured during a seasonal survey at Besòs River mouth and during a summer cruise near the city of Vilanova i la Geltrú, with a view of using parasite species as environmental tags. Over the seasonal survey, a total of 33 different taxa of parasites were identified. The raphidascarid nematodes *Hysterothylacium fabri* and *H. aduncum* were the most prevalent and abundant parasites. Nine common species with a prevalence higher than 10% were identified and considered as potential tag species: the myxozoan *Unicapsula pflugfelderi*, a tetraphyllidean metacestode (traditionally reported under the collective name *Scolex pleuronectis*), the digeneans *Cardiocephaloides longicollis*, *Aphanurus stossichii* and *Ectenurus lepidus*, the nematodes *H. fabri*, *H. aduncum*, *Contracaecum* sp., and the isopod *Ceratothoa oestroides*. A recurrent pattern at both the infracommunity and component community levels was the differentiation of the samples along the spatial/temporal groups. The abundances of *H. fabri* and *H. aduncum*, two generalist parasites, contributed substantially to the observed dissimilarity

between infracommunities along the seasons. The high abundance of *C. oestroides* characterized the Vilanova summer group whereas that of the metacercaria of *C. longicollis* characterized the Besòs autumn, both of them due to their life cycles, respectively. This study underlines the potentiality of the study of the parasites of *S. maena* from the Catalan Sea for ecosystem monitoring, suggesting, in this particular case, the usefulness of these four parasite species as biological tags for environmental studies.

Key words: *Spicara maena*, Mediterranean, component community, infracommunity, season, locality, environmental tags.

Running tittle: Parasite community of *Spicara maena* from the north-western Mediterranean.

INTRODUCTION

The blotched picarel *Spicara maena* (L.) is a pelagic-neritic fish species common in the Western Mediterranean Sea, usually found at a depth range of 30m to 170m. *S. maena* belongs to the Centracanthidae, a family of fishes distributed throughout the Atlantic Ocean and the Mediterranean Sea, with three very abundant Mediterranean species: *S. maena*, *S. smaris* and *Centracanthus cirrus* (Fischer et al., 1987; Froese and Pauly, 2012). Many authors have pointed out that the species of the genus *Spicara* could really belong to the Sparidae, based on morphological and molecular evidences (Jordan and Fesler, 1893; Day, 2002; Orrell and Carpenter, 2004; Chiba et al., 2009). *S. maena* lives in coastal waters, along a wide range of sandy and muddy bottoms and it is usually captured by trawling arts. In September and June, it is usually collected in small shoals around the surface and coastal waters. Abundance of *S. maena* in the north-western Mediterranean is very high; for example, in the Ligurian Sea its abundance data accounted for 84% of the whole stock, being the most abundant species (Guidetti et al., 1998), whereas in the Catalan Sea

its abundance at 60m depth is around 11% of the total fish abundance (F. Maynou, pers. comm.). This fish has minor commercial importance since it has a scarce gastronomic value but it possesses a distinguished value in game fishing, due to its capability to enter in estuaries or lagoons.

The biology and ecology of *S. maena* are widely well-known (Dulcik et al., 2000; Cicek et al., 2007; Soykan et al., 2010 and references therein). It is a relatively long-lived species, being the range of age of one to three years for females, and two to four years for males (Cicek et al., 2007). It feeds mainly on zooplankton but also benthic animals, especially at night; the importance of the plankton and benthos organisms involved is dependent on the season (Lipskaya and Salekhova, 1980). In the west coast of Sicilia, copepods are by far the most important food type (Vizzini and Mazzola, 2009), whereas in the Catalan Sea the main prey groups are Chaetognatha (30%), Mysidacea (29%) and Copepoda (22%) (J. Cartes, pers. comm.). Both seasonal and sexual dimorphism are marked in this species; reproduction happens between March and June in Mediterranean (Cicek et al., 2007; Soykan et al., 2010) and it is a protogynous hermaphrodite (Lepori, 1969).

Parasites have been widely used as indicators of various aspects of fish biology. They have been used as biological tags for stock discrimination of marine fish (MacKenzie and Abaunza, 1998; Moore et al., 2003; Oliva and González, 2004), as well as for zoogeographical patterns assessment to obtain information on the geographic distribution and migration routes of marine fishes (Ferreira-Marques et al., 2009; Vales et al., 2010). They have also been used in studies of host-parasite co-evolutionary processes, since parasites are ideal candidates for testing evolutionary and phylogenetic concepts (Sasal et al., 1998; Sasal et al., 1999). Another interesting aspect of parasite communities is that they experience temporal changes in structure and species composition in response to seasonal variations in environment, which involve the determination of the roles of different parasite species within a community (Valles-Ríos et al., 2000; Violante-González et al., 2008). From an ecological and evolutionary perspective, parasites are an important feature of their host's selective environment (Thomas et al., 2002; Kvach and Skóra, 2007).

Information on the parasite communities of *S. maena* is limited and fragmentary. The only existing investigation in the Mediterranean is a brief description of the parasite fauna of *Spicara* spp. from Italy (Figus et al., 2006). Moreover, some records of parasites in *S. maena* appeared in some general Mediterranean surveys (Desdevises et al., 2002a; Desdevises et al., 2002b; Horton and Okamura, 2001; Solak et al., 2007; Benmansour and Ben Hassine, 2009; Azmirza, 2010) and in some morphological descriptions (Saad-Fares and Maillard, 1990; Kostadinova et al., 2004; Bartoli et al., 2005; Oguz and Öktener, 2007; Paradiznik and Radujkovic, 2007; Alas et al., 2008; Beveridge and Campbell, 2010; Carreras-Aubets et al., 2011b; Carreras-Aubets et al., 2012a; Marzoug et al., 2012).

The aim of this study is to provide detailed information on the parasite fauna of *S. maena* in the north-western Mediterranean. The composition and structure of the parasite communities are analyzed seasonally and for two different localities in summer. The influence of these factors on parasite communities of *S. maena* is discussed and the usefulness of some parasites for environmental condition management is assessed.

MATERIALS AND METHODS

A total of 123 specimens of *Spicara maena* were captured in 2007. Sampling area, fish collection, processing of samples and general data analysis are described in the chapter 4 “Materials and Methods” from the present thesis (see table 3, in chapter 4).

Processing of samples

All specimens of *Ceratothoa oestroides* were removed from the buccal cavity and weighted. Females and males were identified by morphological criteria following Trilles (1968) and their specific position in the buccal cavity was registered.

Data analysis

Abundance of *Unicapsula pflugfelderi* was calculated by means of a semi-quantitative counting of the cysts observed in the muscular tissue: the degree of infection was classified in *i)* no infection, *ii)* low (total number of cysts ranging from one to 50), *iii)* moderate (from 50 to 100 cysts), or *iv)* high (total number of cysts higher than 100 and lower than 200). A categorical mean value was given for each of the three infection levels: 0 for uninfected fish, 25 for low infections, 75 for medium and 125 for the highest ones. Due to the wide variability in the number of spores of each cyst, mean species abundance and mean diversity of each seasonal/locality sample was calculated not including data of *U. pflugfelderi*.

Abundance of the metacercariae of the digenean *Cardiocephaloides longicollis* was calculated by means of a counting of the cysts observed in the brain tissue. The cysts of *C. longicollis* can contain different number of metacercariae and, although all individuals were registered, numerical analyses were done with the number of cysts.

From the total 123 fish individuals processed, total length ranged from 8.7 to 22.0 cm. All fish individuals analyzed (n= 123) were used for prevalence and mean abundance calculations. In order to avoid possible effects of the length of the individuals in the patterns of parasite abundance, from the total of 123 fish sampled, we selected 81 fish individuals, with a total length range from 9.1 to 16.5cm, for the rest of univariate and multivariate analyses. Thus, the selected 81 individuals were used for calculations of Species Richness (SR), Mean Species Richness (MSR), Mean Abundance (MA) and Mean diversity (MD). These three infracommunity descriptors (MSR, MA, MD) followed a normal distribution whereas TL, K, HIS and GSI data were logarithmic transformed (ln (x)) prior to General Linear Model (GLM) analyses.

Differences in fish condition indexes (TL, K, HSI, GSI) and in infracommunity descriptors (MSR, MSA, MD) were tested to explore the effect of the factor “locality/season” by GLM (five categories that will be henceforth referred to as Besòs autumn, Besòs winter, Besòs spring, Besòs summer, Vilanova summer, see chapter 4), with post-hoc pairwise comparison.

To visualize the patterns in parasite abundance in relation to spatial/temporal variation (factor "locality/season") we first applied Factorial Correspondence Analysis (FCA) on a data matrix comprising component population abundance for eight of the nine common parasite species (without *U. pflugfelderi*) and the five spatial/temporal groups. Also a cluster of dissimilarity between the five spatial/temporal groups was executed based on the Eigen values given by the FCA analysis, and depending on the parasite abundance of eight of the nine common species. The common species *U. pflugfelderi* was removed from the analysis because of the semi-quantitative counting used. Based on FCA and cluster grouping, and using individual fish as replicate samples, we tested the differences in prevalence and abundance among the parasite populations for the five spatial/temporal categories by means of Generalized Linear Model (GZM), using logistic model for prevalence and log-binomial model for abundance.

Finally, we used permutational multivariate analyses of similarity (PERMANOVA) to test the null hypothesis of no differences in parasite community structure due to spatial/temporal factor using parasite infracommunities (i.e. populations of all species in individual fish) as replicate samples. Analyses were carried out with PERMANOVA+ for PRIMER v6 (Anderson et al., 2008) on Bray-Curtis similarity matrices derived from the square root transformed abundance data. Permutation *P*-values were obtained under unrestricted permutation of raw data (9,999 permutations). *U. pflugfelderi* was also removed from the analyses.

RESULTS

Fish condition

Biometrical data of the fish are shown in table 1. No significant differences were found for fish length (GLM ANOVA, $P > 0.05$) when comparing all seasons and localities. Fish sampled in Besòs winter and Besòs spring showed a significantly higher K than fish sampled in Besòs summer and Besòs autumn (GLM ANOVA, $F_{(4,74)} = 4.683$, $P = 0.002$). Individuals captured in Besòs autumn showed a

significantly lower HSI than fish sampled in the other 4 spatial/ temporal groups, and fish from Vilanova summer had significantly higher HSI than Besòs winter and Besòs spring (GLM ANOVA, $F_{(4,72)}$= 10.630, P< 0.01). Besòs summer and Vilanova summer were very similar, but Besòs summer did not show significant differences with Besòs winter and Besòs spring due to the high standard deviation (Table 1). Females captured in Besòs winter and Besòs spring appeared with a significantly higher GSI than fish captured in Besòs autumn, Besòs summer and Vilanova summer (GLM ANOVA, $F_{(4,56)}$= 5.831, P= 0.001).

Table 1. Means and standard deviations of total length (TL), condition factor (K), hepatosomatic index (HSI), and gonadosomatic index (only for females) (GSI); sample size (n) for *Spicara maena* along the spatial/temporal assessment. Different letters showed significant differences in spatial/temporal assessment. *Sample size of females in brackets.

Spatial/temporal groups	TL	K	HSI	GSI	n*
Besòs winter	11.50 ± 2.42^{a}	1.09 ± 0.12^{a}	1.13 ± 0.62^{a}	2.59 ± 3.17^{a}	16 (12)
Besòs spring	11.97 ± 1.53^{a}	1.12 ± 0.17^{a}	1.12 ± 0.47^{a}	1.83 ± 2.27^{a}	18 (15)
Besòs summer	12.91 ± 2.41^{a}	0.97 ± 0.08^{b}	2.40 ± 3.84^{ab}	0.37 ± 0.18^{b}	16 (9)
Besòs autumn	12.22 ± 2.09^{a}	0.97 ± 0.15^{b}	0.73 ± 0.42^{c}	0.29 ± 0.12^{b}	17 (14)
Vilanova summer	13.36 ± 2.59^{a}	1.03 ± 0.07^{ab}	2.38 ± 1.28^{b}	0.31 ± 0.10^{b}	14 (14)

Parasite fauna

All fish individuals analyzed were infected with at least one parasite species, except two individuals from Besòs, captured in summer and autumn respectively, that did not show any parasite. A total of 1,610 parasites and an undetermined number of cysts of *U. pflugfelderi* (from 1 to 200 cysts, in 52 of 123 fishes, with a mean value of 36 cysts/fish) corresponding to 33 categories of parasite taxa was identified: 20 digeneans, four monogeneans, one larval cestode, five nematodes, one copepod and one isopod (Table 2). From these 33 parasites, we found six ectoparasites belonging to Monogenea, Copepoda and Isopoda and 27 endoparasites belonging to Digenea, Nematoda, Cestoda and Myxozoa. The degree of specificity of the parasites is indicated in table 2, including information on their presence in sparid fishes.

Nine species were common (P> 10%) in the blotched picarels of this sample: the nematodes *H. fabri*, *H. aduncum*, *Contracaecum* sp., the tetraphyllidean

metacestodes (traditionally reported under the collective name of *Scolex pleuronectis*), the myxozoan *U. pflugfelderi*, the isopod *C. oestroides* and the digeneans *C. longicollis*, *Aphanurus stossichii* and *Ectenurus lepidus*.

Parasites were found in specific sites of the examined fish and with different infection levels. The most prevalent and abundant species were the raphidascarid nematodes *Hysterothylacium fabri* (Total P= 80%, Total MA= 4.34) and *H. aduncum* (P= 74%, MA= 4.22). *Hysterothylacium* spp. were found in third larval stage (L3), which were mainly encysted in connective tissues of oesophagus and intestine but also in gonads, stomach and kidney. Also the nematode *Contracaecum* sp., less prevalent (P= 14%) and abundant (MA= 0.17) was found as L3 stage, always encysted in the connective tissues of digestive tract (mostly in the intestine and pyloric caeca). From a total of 313 mobile metacestodes of Tetraphyllidea gen. sp. (*S. pleuronectis*), the majority were found within the intestine. Cysts of *U. pflugfelderi* were always observed within the body muscle, elongated and with different shapes and sizes. Normally, cysts were larger when abundances were higher. Metacercariae of *C. longicollis*, with a prevalence of 20%, were encysted within large cysts which were found in the brain. The total 46 cysts observed were rounded with fragile and brownish walls, finding a maximum of three metarcercarie per cyst. The polyopisthocotylean monogenean, *Cyclocotyla bellones*, appeared always associated to the parasite *C. oestroides*, normally attached to the pleon of the isopod. Not all the specimens infected with *C. oestroides* were infected by *C. bellones* (see prevalences and abundances in table 2) but all specimens infected with the monogenean were also infected by the isopod. Maximum number of *C. bellones* found in the oral cavity was three.

Table 2. Prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites of *Spicara maena* and parasite community parameters associated with the parasitological survey done off Besòs (along seasons) and Vilanova (in summer). Mean abundance of *Unicapsula pflugfelderi* is based on a semi-quantitave counting of the cysts. Data on the specificity was obtained from Pérez-del-Olmo (2008) and Gibson et al., (2005): G, generalist; Spa, present in Sparid fishes; Bo!,present mostly in *Boops boops*; na, not applicable.

Locality		TOTAL		BESÒS								VILANOVA	
Season				Winter		Spring		Summer		Autumn		Summer	
	Specificity	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD
Mixozoa													
Unicapsula pflugfelderi	G	42.3	36.59 ± 50.64	10.0	10.00 ± 31.83	25.0	16.25 ± 38.28	50.0	45.83 ± 54.57	52.2	50.00 ± 54.36	60.0	48.33 ± 52.90
Digenea													
Aphanurus stossichii	G, Bo!	14.6	0.25 ± 0.77	-	-	5.0	0.10 ± 0.45	40.0	0.63 ± 1.07	4.3	0.22 ± 1.04	13.3	0.17 ± 0.46
Aponurus laguncula	G	1.6	0.02 ± 0.13	-	-	-	-	3.3	0.03 ± 0.18	-	-	3.3	0.03 ± 0.18
Arnola microcirrus	Spa, Bo!	0.8	0.01 ± 0.09	-	-	-	-	-	-	-	-	3.3	0.03 ± 0.18
Bacciger bacciger	G	1.6	0.02 ± 0.13	-	-	5.0	0.05 ± 0.22	3.3	0.03 ± 0.18	-	-	-	-
Cardiocephaloides longicollis	G, Bo!	19.5	0.42 ± 1.08	20.0	0.55 ± 1.47	10.0	0.30 ± 1.13	20.0	0.37 ± 1.00	30.4	0.70 ± 1.26	16.7	0.23 ± 0.57
Derogenes sp.	G, Bo!	0.8	0.01 ± 0.09	-	-	-	-	3.3	0.03 ± 0.18	-	-	-	-
Ectenurus lepidus	G	9.8	0.11 ± 0.33	25.0	0.25 ± 0.44	5.0	0.05 ± 0.22	6.7	0.07 ± 0.25	4.3	0.09 ± 0.42	10.0	0.10 ± 0.31
Galactosomum timondavidi	G	4.9	0.07 ± 0.31	-	-	-	-	3.3	0.03 ± 0.18	4.3	0.04 ± 0.21	13.3	0.20 ± 0.55
Hemiurus communis	G, Bo!	4.1	0.04 ± 0.20	-	-	-	-	6.7	0.07 ± 0.25	4.3	0.04 ± 0.21	6.7	0.07 ± 0.25
Lecithochirium musculus	G	1.6	0.02 ± 0.20	-	-	-	-	3.3	0.03 ± 0.18	-	-	3.3	0.07 ± 0.37
Lecithochirium sp.	G	0.8	0.01 ± 0.09	-	-	-	-	-	-	-	-	3.3	0.03 ± 0.18
Lecithocladium excisum	G, Bo!	8.9	0.20 ± 1.29	-	-	-	-	6.7	0.07 ± 0.25	4.3	0.04 ± 0.21	26.7	0.73 ± 2.56
Lepocreadidae	G, Bo!	3.3	0.03 ± 0.18	10.0	0.10 ± 0.31	-	-	3.3	0.03 ± 0.18	4.3	0.04 ± 0.21	-	-
Mesometridae	na	0.8	0.01 ± 0.09	-	-	-	-	-	-	-	-	3.3	0.03 ± 0.18
Didimozoid larval	na	0.8	0.01 ± 0.09	-	-	-	-	-	-	-	-	3.3	0.03 ± 0.18
Monorchis monorchis	G	2.4	0.02 ± 0.15	-	-	5.0	0.05 ± 0.22	3.3	0.03 ± 0.18	-	-	3.3	0.03 ± 0.18
Opecoelidae	na	0.8	0.01 ± 0.09	-	-	-	-	3.3	0.03 ± 0.18	-	-	-	-
Opecoeloides furcatus	G	0.8	0.01 ± 0.09	-	-	-		3.3	0.03 ± 0.18	-	-	-	-
Proctotrema maculatus	G	0.8	0.01 ± 0.09	-	-	-		3.3	0.03 ± 0.18	-	-	-	-
Tormopsolus sp. met.	G, Bo!	2.4	0.03 ± 0.22	-	-	-		3.3	0.03 ± 0.18	-	-	6.7	0.10 ± 0.40
Monogenea													
Axine sp.	G	0.8	0.02 ± 0.18	-	-	5.0	0.10 ± 0.45	-	-	-	-	-	-
Bivagina sp.	G, Sp	4.9	0.06 ± 0.27	5.0	0.05 ± 0.22	15.0	0.15 ± 0.37	3.3	0.03 ± 0.18	-	-	3.3	0.07 ± 0.37
Cyclocotyla bellones	G, Bo!	7.3	0.16 ± 0.73	-	-	15.0	0.25 ± 0.72	10.0	0.33 ± 1.21	-	-	10.0	0.17 ± 0.59
Lamellodiscus knoeppfleri	Spa	3.3	0.05 ± 0.28	-	-	-	-	6.7	0.10 ± 0.40	-	-	6.7	0.10 ± 0.40
Cestoda													
Scolex pleuronectis larva	na	58.5	2.54 ± 5.64	60.0	2.05 ± 2.28	45.0	1.00 ± 1.21	66.7	4.97 ± 10.44	60.9	2.04 ± 2.58	56.7	1.87 ± 2.61
Nematoda													
Capillaridae gen. sp.	na	0.8	0.01 ± 0.09	-	-	-	-	-	-	-	-	3.3	0.03 ± 0.18
Contracaecum sp. larva	G, Bo!	13.8	0.17 ± 0.47	-	-	10.0	0.10 ± 0.31	20.0	0.27 ± 0.64	4.3	0.04 ± 0.21	26.7	0.33 ± 0.61
Cystidicolidae gen. sp.	na	0.8	0.01 ± 0.09	-	-	-	-	-	-	-	-	3.3	0.03 ± 0.18
Hysterothylacium aduncum larva	G, Bo!	74.0	4.22 ± 5.86	75.0	3.70 ± 2.96	70.0	2.70 ± 3.21	76.7	6.80 ± 9.34	78.3	2.61 ± 2.81	70.0	4.23 ± 5.31
Hysterothylacium fabri larva	G	79.7	4.34 ± 5.04	95.0	4.20 ± 5.77	55.0	1.90 ± 2.20	86.7	3.87 ± 3.99	73.9	3.22 ± 2.71	83.3	7.40 ± 6.73
Isopoda													
Ceratothoa oestroides	G, Bo!	14.6	0.19 ± 0.50	10.0	0.10 ± 0.31	10.0	0.10 ± 0.31	20.0	0.30 ± 0.65	-	-	26.7	0.33 ± 0.66
Copepoda													
Naobranchia cygniformis	Spa, Bo!	2.4	0.02 ± 0.15	-	-	-	-	-	-	-	-	10.0	0.10 ± 0.31

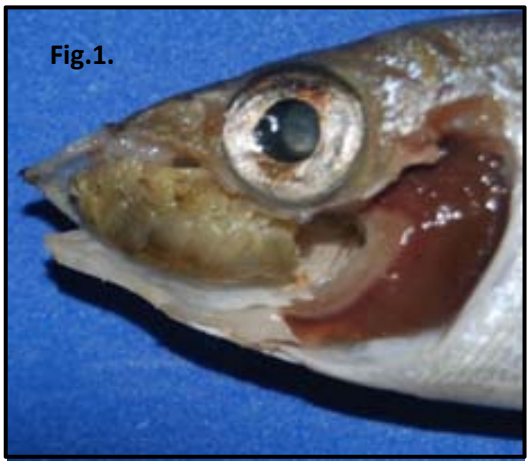
The isopod *C. oestroides* appeared attached to the palate of the host *S. maena*, as it is shown in figures 1 and 2. From a total of 22 specimens, 16 were females and 6 were males. From the total females, 6 of them were considered gravid and full of small larvae in development. The female isopods appeared with the cephalic part nearer to the mouth of the host and the pleon located inside the oral cavity. In all fish parasitized by that isopod species individuals, one female (mean weight females= 0.39g) was always found whereas the number of undetermined larval or males (which were smaller than females (mean weight males= 0.04g) could vary, being sometimes two or even three the number of males found around the female in the same infected host, and undetermined the number of larval isopods.

Parasite communities

The mean species richness (MSR) for the totality of the sample was 4.77 (Table 3). Regarding mean species richness for each sample (the five categorical groups, see materials and methods), significantly lower MSR was observed in Besòs spring than in other four groups (GLM, $F_{(4, 118)}= 6.087$, $p< 0.001$). MSR of Vilanova summer was the highest, being similar to that of Besòs summer and significantly higher than Besòs autumn, Besòs winter and Besòs spring (Table 3). Mean species abundance for each sample was also significantly lower in Besòs spring than in Besòs summer and Vilanova summer (GLM, $F_{(4, 118)}= 4.060$, $p= 0.004$). Besòs spring was the categorical group with lower parasite diversity (Brillouin Index= 0.55) and it was significantly lower than the other four groups (GLM, $F_{(4, 114)}= 2.639$, $p= 0.037$) (Table 3).

Table 3. Species richness, Mean species richness, mean abundance and mean diversity calculated for both the total sample and for each of the five categorical groups. All these infracommunity descriptors were calculated with 81 fish, and data of *Unicapsula pflugfelderi* was removed. Different letters showed significant differences between the five spatial/temporal groups assessed in *Spicara maena*.

Locality	TOTAL	Besòs				Vilanova
Season		Winter	Spring	Summer	Autumn	Summer
Species richness	33	9	14	26	12	26
Mean species richness	4.77 ± 2.13	3.10 ± 1.41^{b}	2.80 ± 1.64^{a}	4.60 ± 2.20^{bc}	3.26 ± 1.39^{b}	4.77 ± 2.12^{c}
Mean abundance	16.54 ± 10.87	11.00 ± 9.56^{abc}	6.85 ± 6.12^{a}	18.27 ± 19.19^{c}	9.09 ± 6.17^{ab}	16.54 ± 10.87^{bc}
Mean diversity (Brillouin Index)	0.71 ± 0.34	0.72 ± 0.26^{b}	0.55 ± 0.34^{a}	0.82 ± 0.33^{b}	0.62 ± 0.31^{b}	0.77 ± 0.36^{b}



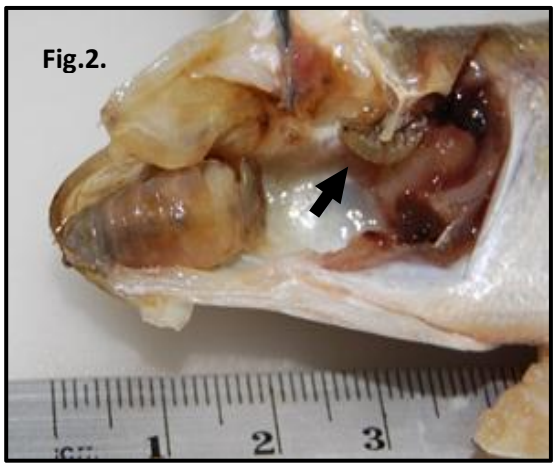


Figure 1. Attachment of the female of *Ceratothoa oestroides* on the palate of *Spicara maena*, with the cephalon in the most external part of the mouth and the pleon nearer the pharyngeal roof (lateral view; left mandible and operculum removed).

Figure 2. View of the infection of the female and male of *Ceratothoa oestroides*, located in the pharyngeal roof of the buccal cavity. Note that the male (black arrow) is also attached to the palate, not to the tongue (ventral view; mandible and left operculum removed).

Figure 3 presents a plot of the first factorial plane of co-inertia analysis explaining 89% of the variance, predominantly on the first axis (58% of the total inertia) of the FCA carried out using component population data for the eight of the nine common species in *S. maena*. The first axis of the FCA illustrates a clear gradient differentiating the samples in relation to the two localities sampled in this survey, the area off Besòs River mouth (groups B and C) and the area off Vilanova (group A). Regarding these Besòs groups, the second axis illustrates a less but enough clear gradient differentiating the samples in relation to the

seasons, being the group B characterized by cold seasons (Besòs autumn and Besòs winter) and the group C by the warmer seasons (Besòs summer and Besòs spring). Thus, the FCA showed clearly three groups and this grouping pattern was associated with the abundance of the component parasite populations of two, three and three common species, respectively, that characterized the multivariate relationship among the samples examined (Fig. 3). Component populations of three species exhibited the strongest correlations with the first FCA axis. These were *H. fabri* (squared cosine value, $Cosine^2$= 0.993), *H. aduncum* ($Cosine^2$= 0.803), and *C. oestroides* ($Cosine^2$= 0.544). An additional suite of three species was strongly associated with the second FCA axis which partially differentiated the samples taken at the seasons of 2007: *C. longicollis* ($Cosine^2$= 0.871), *Contracaecum* sp. ($Cosine^2$= 0.621), and *E. lepidus* ($Cosine^2$= 0.585).

From the FCA and cluster analysis of the five categories of locality/season, three different groups mentioned above were identified depending on their parasite load (Figs. 3 and 4). The samples off Vilanova (group A) were clustered separately from that off Besòs, indicating that locality is a determinant factor for parasite community structure. The samples of Besòs autumn and Besòs winter (group B) were clustered clearly separated from that of Besòs summer and Besòs spring (group C). Based on these results, the samples were separated into these three groups:

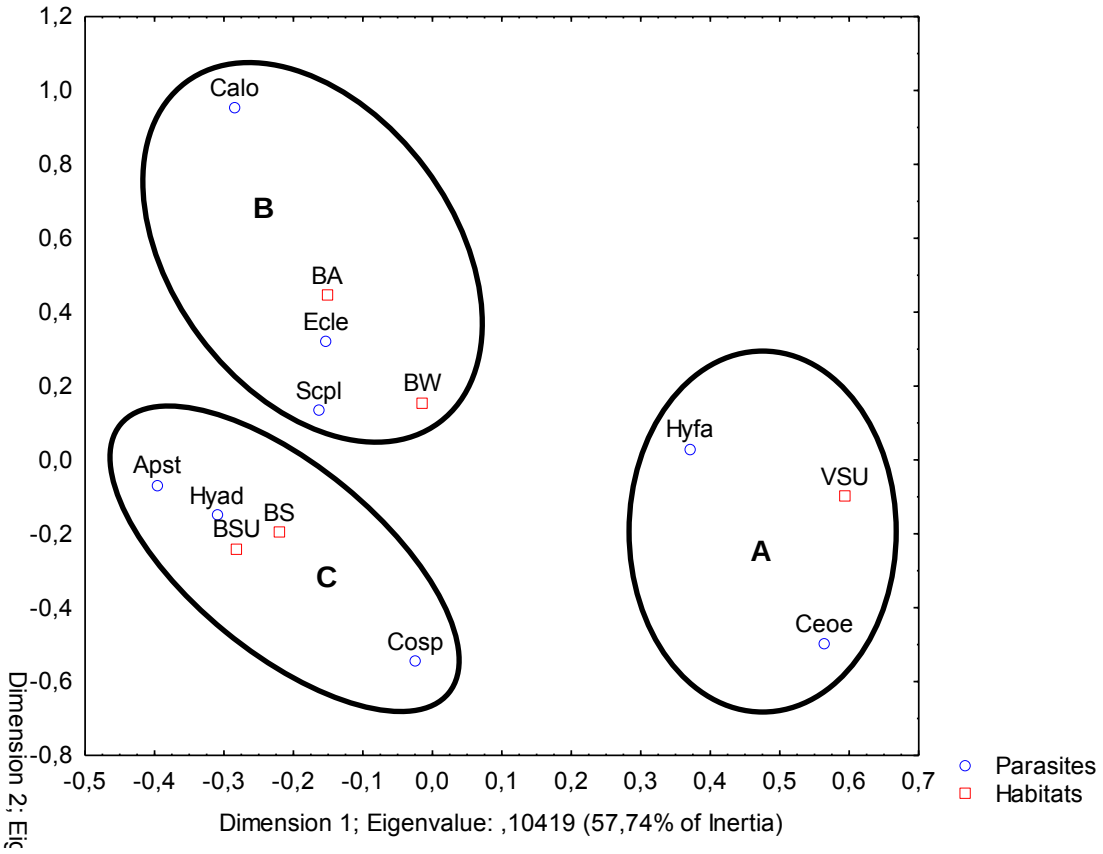


Figure 3. Plot of the first factorial plane of co-inertia analysis of the factorial correspondence analysis (FCA) on component population data for the 8 of the 9 common species in *Spicara maena* (data of *Unicapsula pflugfelderi* was removed from the analysis). A/B/C indicate the groups established in the parasite fauna description. Abbreviations for spatial/temporal groups: BW, Besòs winter; BA, Besòs autumn; BS, Besòs spring; BSU, Besòs summer; VSU, Vilanova summer. Abbreviations for species names: Apst, *Aphanurus stossichii*; Calo, *Cardiocephaloides longicollis*; Ceoe, *Ceratothoa oestroides*; Cosp, *Contracaecum* sp.; Ecle, *Ectenurus lepidus*, Hyad, *Hysterothylacium aduncum*; Hyfa, *Hysterothylacium fabri*; Scpl, tetraphyllidean metacestode (*Scolex pleuronectis*).

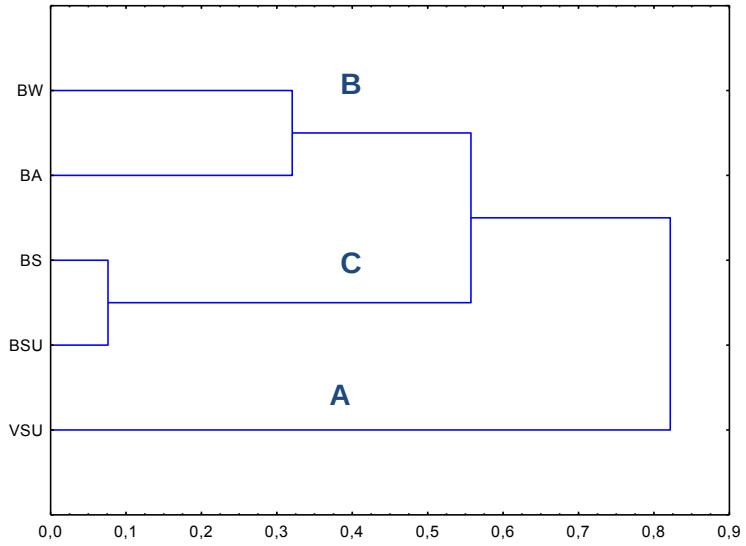


Figure 4. Dendrogram of dissimilarity between the five spatial/temporal groups assessed depending on the parasite abundance of 8 of the 9 common species in *Spicara maena*. Data of *Unicapsula pflugfelderi* was removed from the analysis. Abbreviations for spatial/temporal groups: BW, Besòs winter; BA, Besòs autumn; BS, Besòs spring; BSU, Besòs summer; VSU, Vilanova summer. Groups identified: A (Vilanova summer); B (Besòs winter and Besòs autumn); C (Besòs spring and Besòs summer).

Group A- Vilanova summer: it was characterized by *H. fabri* and *C. oestroides,* which showed the maximum mean abundance in this group (Table 2). Abundance of *H. fabri* from Vilanova summer was significantly higher than that of Besòs spring and Besòs autumn (GZM, Table 4). Another difference found in the GZM analyses between the five spatial/temporal groups were that *H. fabri* was significantly higher in fish captured in Besòs winter than in Besòs spring (Table 4). No significant differences in prevalences were found.

Group B- Besòs autumn and Besòs winter: it was characterized by *S. pleuronectis*, *E. lepidus* and *C. longicollis*. The last two reached the maximum abundance in autumn and winter (Table 2). *S. pleuronectis* characterized winter and autumn because of the similar values in those two seasons (Table 2). Significant differences in abundance were found in the spatial/temporal assessment for *C. longicollis* (GZM, χ^2= 12.234, *P*= 0.016): fish captured in Besòs autumn was significantly more infected than fish captured in Besòs summer, Besòs spring and Vilanova summer, and fish from Besòs winter was

significantly more infected than fish captured in Besòs spring and in Vilanova summer (Table 4). No significant differences in prevalences were found.

Group C- Besòs summer and Besòs spring: it was characterized by *H. aduncum* and *A. stossichi*, which reached maximum abundances in summer (Table 2). Significant differences were found for *H. aduncum* (GZM, χ^2= 13.937, *P*= 0.007) along the spatial assessment: abundance of *H. aduncum* was significantly higher in Vilanova summer than in Besòs summer and Besòs winter (Table 4). Regarding the temporal assessment, abundance of this nematode in Besòs summer was significantly higher than Besòs autumn and Besòs spring. No significant differences in prevalences were found.

Although *U. pflugfelderi* was not included in the general FCA analyses, the GZM analyses were also executed, independently, for the semi-quantitative data of this myxozoan on the five categorical spatial/temporal groups. Significant differences between abundances of the cysts were found: both Besòs winter and Besòs spring were significantly lower than Besòs summer, Besòs autumn and Vilanova summer, and Besòs autumn was significantly higher than Vilanova summer (Table 4). No significant differences in prevalence were found.

These differences in parasite distributions among individual fish were reflected in the differentiation of community structure in relation to seasons and localities which we also assessed in a permutational multivariate analysis (PERMANOVA). The analysis based on the replicate infracommunity samples from all five spatial/temporal groups revealed significant effect of the factor "locality/season" on community structure (*Pseudo- $F_{(2,94)}$*= 2.14; $P_{(perm)}$ = 0.0066; 9916 unique permutations; all *post hoc* comparisons significant except that between Besòs spring and Besòs summer). We observed a marked decrease in the predictability of parasite infracommunities when the season was assessed, observing a highly structured spatial/temporal pattern in Besòs winter (63.96%) whereas the samples from Besòs spring and Vilanova summer did not differ from a random distribution (37.15% and 33.55%, respectively, table 5). This pattern was coupled with an increase in dissimilarities among the five groups (Table 6).

Table 4. Significances (*) and no-significances (-) of Generalized Linear Model (GZM) executed on prevalences and abundances of the common parasite species found in *Spicara maena* from the five spatial/temporal groups defined in the sampling. Table 3 is only referred to abundances since no significant differences in prevalence were found. Common parasites without any significant difference in abundance have not been included in this table. Group A: Vilanova summer; Group B: Besòs summer; Group C: Besòs autumn, Besòs winter and Besòs spring.

	BW-BS	BW-BSU	BW-BA	BW-VSU	BS-BSU	BS-BA	BS-VSU	BSU-BA	BSU-VSU	BA-VSU
Unicapsula pflugfelderi	-	$\chi2$= 7.234 p= 0.007*	$\chi2$= 9.147 p= 0.002*	$\chi2$= 9.278 p= 0.002*	$\chi2$= 4.002 p= 0.045*	$\chi2$= 5.509 p= 0.019*	$\chi2$= 5.659 p= 0.017*	-	-	$\chi2$= 0.945 p= 0.005*
Cardiocephaloides longicollis	$\chi2$= 4.362 p= 0.037*	-	-	$\chi2$= 3.879 p= 0.049*	-	$\chi2$= 5.753 p= 0.016*	-	$\chi2$= 4.017 p= 0.045*	-	$\chi2$= 5.184 p= 0.023*
Hysterothylacium aduncum	-	-	-	$\chi2$= 4.546 p= 0.033*	$\chi2$= 5.334 p=0.021*	-	-	$\chi2$= 7.720 p= 0.005*	$\chi2$= 9.323 p= 0.002*	-
Hysterothylacium fabri	$\chi2$= 8.075 p= 0.004*	-	-	-	-	-	$\chi2$= 12.307 p<0.001*	-	-	$\chi2$= 6.127 p= 0.013*

Table 5. Mean similarity for parasite infracommunities sampled within the five spatial/temporal groups sampled in *Spicara maena* and a breakdown into contributions from individual parasite species. Only species contributing to more than 10% of the mean community similarity within spatial/temporal groups are included. BW: Besòs winter, BA: Besòs autumn, BSU: Besòs summer, BS: Besòs spring, VSU: Vilanova summer.

Species/ Spatial/temporal/ Contrast	BW	BA	BSU	BS	VSU
Mean similarity (%)	**63.96**	**47.31**	**42.57**	**37.15**	**33.55**
Hysterothylacium fabri	42.22	35.43	58.53	22.70	47.15
Hysterothylacium aduncum	33.30	43.25	19.13	58.82	22.96
Scolex pleuronectis	22.07	16.53	12.37	16.89	16.47
Ceratothoa oestroides	–	–	–	–	12.55
Cardiocephaloides longicollis	–	–	–	–	–
Cumulative contribution (%)	**97.58**	**95.21**	**90.03**	**98.39**	**99.13**

SIMPER analysis identified four parasite taxa that contributed most to the similarity/dissimilarity of infracommunities within/among the replicate samples of the five spatial/temporal groups. Invariably, *H. fabri*, *H. aduncum* and *S. pleuronectis* were identified as “key discriminating species” that contribute substantially to both similarity of infracommunities within the replicate samples and also to community distinctness with respect to spatial/temporal factor. However, the isopod *C. oestroides* solely contributed to infracommunity similarity within Vilanova summer sample and for the differentiation in community structure observed among this sample and the rest. The brain metacercariae of *C. longicollis* were also identified as “key discriminating species” associated with the differentiation of infracommnities off Besòs sampled in autumn (see tables 5 and 6 for a breakdown into species' percent contributions to the total average similarity/dissimilarity within/among seasons and locality groups, respectively).

Table 6. Mean dissimilarity for parasite infracommunities sampled among the five habitat groups sampled in *Spicara maena* and a breakdown into contributions from individual parasite species. Only species contributing to more than 10% of the mean community dissimilarity between spatial/temporal groups are included. BW: Besòs winter, BA: Besòs autumn, BSU: Besòs summer, BS: Besòs spring, VSU: Vilanova summer.

Species/ Spatial/temporal/ Contrast	BW *vs* BA	BW *vs* BSU	BW *vs* BS	BW *vs* VSU	BA *vs* BSU	BA *vs* BS	BA *vs* VSU	BSU *vs* BS	BSU *vs* VSU	BS *vs* VSU
Mean dissimilarity (%)	**47.14**	**51.26**	**53.61**	**56.42**	**58.13**	**56.85**	**62.4**	**62.98**	**63.59**	**65.95**
Hysterothylacium fabri	22.91	16.48	27.99	28.55	20.69	26.8	30.83	23.67	28.68	32.22
Hysterothylacium aduncum	25.65	30.77	25.11	25.49	27.96	23.42	21.2	28.79	24.06	23.52
Scolex pleuronectis	24.15	22.78	23.89	20.52	20,00	21.62	17.6	19.02	17.17	17,00
Ceratothoa oestroides	–	–	–	–	–	–	10.36	–	10.69	11.57
Cardiocephaloides longicollis	13.29	–	–	–	11.06	11.35	10.05	–	–	–
Cumulative contribution (%)	**86,00**	**70.03**	**76.99**	**74.56**	**79.7**	**83.19**	**90.04**	**71.48**	**80.6**	**84.27**

DISCUSSION

S. maena reproduction occurs between March and June in Mediterranean (Cicek et al., 2007; Soykan et al., 2010), when fish reaches maximum K values. During the previous months of winter and spring the individuals accumulate lipids in liver, which is related to higher HSI values, as it is observed in this study. Moreover, the gonads should mature during the reproductive period (winter and spring), reaching its maximum size at this period, as reflected in the highest values of GSI obtained.

Parasite fauna

The parasite fauna of Mediterranean *S. maena* showed endoparasite dominance, with only six ectoparasites identified. The relatively high parasite richness observed in the Catalan Sea (north-western Mediterranean) was in contrast to the one reported in the unique study on parasite fauna of *S. maena* carried out in the area of Sardinia (Italy), where the richness was half of the observed in the present study at the coast of Catalonia: of the four generalist parasites found in this study (*S. pleuronectis*, *H. fabri*, *C. longicollis* and *O. furcatus*), only the first two are common with Sardinia locality (Figus et al., 2006).

Interestingly *S. maena* shares almost half (42%) of the parasite fauna with other Mediterranean Sparidae fishes such as *Boops boops* (see table 2). This fact supports the evolutionary proximity of *Spicara* spp. with the Sparidae family, as is discussed in other both morphological (Jordan and Fesler, 1893; Day, 2002) and molecular (Orrell and Carpenter, 2004; Chiba et al., 2009) studies. Thus, new parasitological data are given in this study, which contributes to the discussion of the situation of *Spicara* genus inside or outside the Sparidae family. In particular, *S. maena* share an outstanding amount of parasites with *B. boops* from Mediterranean waters: the 45% of digeneans, 25% of monogeneans, 40% of nematodes, and all the cestodes, copepods and isopods that infect *S. maena* are also present in *B. boops* (see table 2). Surprisingly, *S. maena* is infected by two species which are specialists of Sparidae (*Arnola microcirrus* and *Naobranchia cygniformis*), one genus specific from *B. boops*, the digenean *Bacciger* and one genus specific of Sparidae

(*Lamellodiscus*). Moreover, the only *Lamellodiscus* species present in *Spicara* spp., *L. knoeppfleri*, also infects another sparid species, *Spondyliosoma cantharus* (Desdevises et al., 2002). The coincidences of the parasite fauna of *S. maena*, *B. boops* and *S. cantharus* agree with the strong genetic proximity of these 3 species reported by Chiba et al. (2009).

The increased presence of *C. oestroides* in summer months, specially characterizing the parasite community of Vilanova summer, agrees with the period of optimum isopod proliferation in Mediterranean (Trilles, 1964b), when sea temperature increases involving a raise of the parasitic infections (Sarusic, 1999). For this reason, *C. oestroides* could be suggested as useful tag for seasonal variation assessment in the host *S. maena* from the Catalan Sea. In the Mediterranean aquaculture, maximum values of prevalence of this species were also found in warmer months in some cases with similar values as the ones obtained in the present study (Mladineo, 2002) but it was even higher in others (Horton and Okamura, 2001). The position of *C. oestroides* in the pharingeal roof of the mouth is peculiar. Frequently, these large cymothoids invade the buccal cavity, attach to the fish tongue and limits the food range to smaller preys (Sarusic, 1999; Horton and Okamura, 2001; Mladineo, 2002). Our results noted that these cymothoid attach in the palate of *S. maena*, indicating a preference for that attachment surface, probably due to the morphological structure of the buccal cavity of the host. The preliminary results on the diet of the same north-western Mediterranean *S. maena* specimens used in this survey (mainly in juvenile specimens) point to a decrease in stomach contents related to the presence of *C. oestroides*, showing scarcity or absence of preys (L. Zucca, pers. comm.), probably due to the presence of this isopod, which act as a mechanical obstacle for prey's ingestion.

The increased presence and abundance of the metacercariae in *S. maena* of the bird parasite *C. longicollis* in the cold months of autumn and winter agrees with the feeding cycle of their final hosts, the Mediterranean gulls. Gulls are surface feeders, exploiting shoals of small epipelagic fish (Santoro et al., 2011). Because the gulls return to its breeding Mediterranean colonies from late February to early May (BirdLife International, 2009), it is possible that during the cold months, the intermediate fish host act as a reservoir of this

parasites to survive the winter, until they are ready to be transmitted to the final hosts. For this reason, *C. longicollis* could be suggested as useful tag for seasonal variation assessment in the host *S. maena* from the Catalan Sea. Some authors have reported the infection of this parasite in the brain of other Mediterranean fishes as *Diplodus annularis* or *B. boops* (Prévot and Bartoli, 1980; Osset et al. 2005; Pérez-del-Olmo et al., 2008).

The maximum number of the monogenean *C. bellones* observed in one host (n= 3), agree with that found in *S. maena* sampled in Aegean Sea (Solak et al., 2007). Most of the monogeneans of the genus *Cyclocotyla* adhere to fish surfaces but some species can hold on the cuticle of isopods. As Euzet and Combes (1998) suggested, change of the attachment surface could be due to a trial to avoid competition with other ectoparasites. Preliminary observations done in histological samples of some specimens of the present study found in the present investigation showed the presence of the fish blood serum and degraded erythrocytes within the gut of the monogeneans. These observations indicate that *C. bellones* is really an epibiont of the isopod and feeds on fish blood, rejecting the traditional assumption of the hyperparasitism (Bullard, 2000; Öktener and Trilles, 2004; Solak et al., 2007).

Parasite communities

A recurrent pattern at both the component community and infracommunity levels was observed, since the three habitat groups which were defined at the population level correlated with the gradient of dissimilarity observed in SIMPER analysis, being the cold seasons from group C that of highest predictability (meaning similarity) whereas warm seasons showed lower predictability values. It is widely known that parasite abundance can be influenced by the characteristics of the local ecosystem and the trophic web (Luque and Poulin, 2004; Marcogliese, 2001, 2002) so the distribution patterns of marine parasites are highly determined by temperature-salinity profiles (Esch and Fernández, 1993) being the seasonal effect marked. Parasite abundance responses to the seasonal effect studied translated into significant differences in infracommunity structure with decreasing predictability associated with the warm seasons (Pérez-del-Olmo et al., 2010).

Community differentiation was apparently due to the significant effect of the seasonality on the abundance of two generalist larval nematodes (*H. fabri* and *H. aduncum*). The abundance of those two nematodes contributed substantially to the observed similarity within- and dissimilarity between infracommunities along the seasonal assessment. Significant differences found in the GZM also supported these results. The dominance and high prevalence of *H. fabri* and *H. aduncum* in the parasite assemblages from both localities was responsible of the similarity within infracommunities. However, the differential abundance of these species was the main determinant of the observed seasonal and geographical differences. Both species of the same genus *Hysterothylacium* have complex life-cycles and are transmitted to definitive hosts *via* food chains but utilize different pathways and intermediate hosts, which include mysids, copepods, isopods and fish (Carreras-Aubets et al., 2012b). Given that larval helminths in fish hosts are transmitted to their definite hosts by predation, *S. maena* is an adequate fish species to act as intermediate hosts due to its small body size and its intermediate position in the food chain (Marcogliese, 2002). Both nematode parasites have a deep influence on the infracommunity structure, accounting for the highest percentage of infection. This larval nematodes greatly affect all infracommunity descriptors, and produce marked changes in the similarity among infracommunities, and thus, on the predictability of this system (Vales et al., 2010). Given the abundance and broad distribution of non-specific larval parasites in the component community of *S. maena*, infracommunities can be considered as subsets of the species available in the compound community, reflecting the structure of this higher-level assemblage. This pattern is also observed in the Atlantic Sea, in which the benthic species *Prionotus nudigula* was assessed in the same terms (Timi and Lanfranchi, 2009). It is necessary to take into account that some host features (feeding behaviour, habitat depth and geographical distribution) appeared to influence richness and abundance of larval heminths, highlighting the relevance of ecological factors in these patterns (Luque and Poulin, 2004).

Furthermore, the size of the pool of available species must differ from one geographical area to the next, limiting the number of parasite species that a host can acquire over time (Luque et al., 2004) so the grouping pattern between

localities showed in the first axis of the FCA agrees with the general knowledge of parasite distribution. Similarity in species composition among parasite communities is expected to decrease with increasing distance between them (Poulin, 2003; Pérez-del-Olmo et al., 2009) so the different parasite composition between Besòs and Vilanova, especially for digenean taxa, is explained.

In conclusion, both univariate and multivariate analyses revealed seasonal and locality changes in the community abundance and in the structure of parasite communities. Further, these differences in component community structure are reflected consistently in the change in infracommunity composition patterns which were reflected in statistical differences given by the GZM. The main factors structuring these infracommunities were the same ones responsible of structuring the component communities (Zander, 2004). These factors were host feeding behavior and the availability of free-swimming larvae, both influenced by seasonal changes. Moreover, at the parasite population level, significant differences in population descriptors as well as in infracommunity indices, between both localities for all parasite species demonstrate their potential for discriminating discrete stocks of *S. maena*, each having their own indicator species. The nematodes *H. fabri* and *H. aduncum* are able to mark some seasonality variations in the parasite community of *S. maena*, together with the digenean *C. longicollis* that could characterize the autumn season and *C. oestroides* that could characterize the summer period, due to the seasonal pattern present in their respective life cycles. This study underlined the usefulness of these fish host-parasite systems as a tool in ecosystem monitoring, and especially suggests four parasite species (*H. fabri*, *H. aduncum, C. longicollis* and *C. oestroides*) as useful tags for discrimination of *S. maena* populations from the Catalan Sea.

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8. SEASONAL OCCURRENCE AND LOCALITY INFLUENCE IN METAZOAN PARASITE COMMUNITY OF THE GREATER WEAVER *TRACHINUS DRACO* L. FROM NORTH- WESTERN MEDITERRANEAN: PARASITES AS ECOLOGICAL TAGS

Seasonal occurrence and locality influence in metazoan parasite community of the greater weaver *Trachinus draco* L. from north-western Mediterranean: parasites as ecological tags

Carreras-Aubets, M.[a*], Montero, F. E.[b], Carrassón, M.[a]

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

[b]Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, PO Box 22 085, 46071 Valencia, Spain.
*Corresponding author. Tel.: +34 93 581 28 17; fax: +34 93 581 13 21.

E-mail address: marta.carrerasau@e-campus.uab.cat (M. Carreras-Aubets).

ABSTRACT

Spatial and temporal variation of the parasite communities of the perciform *Trachinus draco* L. from two points of the north-western Mediterranean were studied at infracommunity and component community levels. In total, 87 fish were collected between October 2006 and August 2007. A total of 2.177 parasites corresponding to 22 categories of parasite taxa was identified. Nine species were common (prevalence higher than 10%): the myxozoan *Kudoa* sp., the digenean *Helicometra fasciata*, a tetraphyllidean metacestode (traditionally reported under the collective name *Scolex pleuronectis*), the nematodes *Hysterothylacium fabri*, *H. aduncum*, *Phyllometra globiceps*, *Contracaecum* sp. and *Ascarophis* sp., and the isopod *Gnathia* sp. A recurrent pattern at both the infracommunity and the component community levels was observed since some parasite species presented temporal changes in their infection levels as well as differences in infection parameters between Besòs and Vilanova were observed. Some parasite populations of *T. draco* from the north-western Mediterranean are suggested as ecological tags to discriminate its host populations and the seasonal influence in them. Especially, the nematodes *H.*

fabri, *P. globiceps* and *Ascarophis* sp. contributed most to the similarity and dissimilarity in community analyses, so they are purposed, in this particular case, for future studies in ecological biomonitoring for *Trachinus draco* from the Catalan Sea.

Key words: *Trachinus draco*, Mediterranean, component community, infracommunity, season, locality, environmental tags.

Running tittle: Parasite communities of north-western Mediterranean *Trachinus draco*.

INTRODUCTION

The Trachinidae is a family composed by nine littoral and benthic species, comprised in two genera: *Echiichthys* and *Trachinus* (Nelson, 1994). The greater weaver *Trachinus draco* L. is a common trachinid found in the Mediterranean Sea, Black Sea and north-eastern Atlantic, from Norway to Morocco, and Madeira. *T. draco* lives in a depth range from 1 to 150m, and it has shown a curious seasonal migratory behaviour: whereas in the Alboran Sea (Mediterranean) it shows a preference for shallower waters in autumn (up to 75 m depth) and falls to deeper waters (up to 160m) in warmer seasons (Portillo et al., 2008), in Atlantic Sea the tendency is the opposite, with a range of depth narrower (from 9 to 27m) (Bagge, 2004). Although this species has low commercial value, it possesses an important weight in the trawling arts along the Mediterranean fishing ports.

Information about the biology, habitat and distribution of *T. draco* is scarce both in the Mediterranean and in the Atlantic Sea (Olaso et al., 2002; Bagge, 2004; Portillo et al., 2008). Abundance of *T. draco* in the South-western Mediterranean reaches the 36% of the whole stock, in captures from 50 to 164 m (Portillo et al., 2008), whereas in north-western Mediterranean, its abundance in the continental platform at 60m depth (where the sampling of the present

study was done) do not reach the 1% of the total fish abundance, probably due to its abundance distribution center is under 60m (F. Maynou, pers. comm.) but no data of abundance has been found at depths upper than 60m. *T. draco* is a bottom-living fish which lies buried in the sandy sea-bed during daylight and emerges to forage at night (Wheeler, 1978). Its main diet comprises crustaceans (mysids and decapods) and teleosts (mainly gobiids and callionimids), being the seasonality and the size of the individuals factors which can influence the food habits of *T. draco* (Morte et al., 1999; Bagge, 2004). In the Catalan Sea, diet of *T. draco* comprises Polychaeta, Mysidacea (*Leptomysis gracilis*), Decapoda (*Alpheus glaber*, crangonids, *Ebalia tuberosa* and *Goneplax rhomboides*) and some undefined ostheychthyes (L. Zucca, personal comm.). The spawning occurs from June to September and the eggs are pelagics, whereas the larval stadium takes place in the benthic surface (Russell, 1976; Bagge, 2004).

The usefulness of the fish parasites as indicators of some variations in the environment is undisputable (MacKenzie et al., 1995; Broeg et al., 1999; Mackenzie, 1999: Dzikowski et al., 2003; Williams and Mackenzie, 2003; Sasal et al., 2007). Prevalences and abundances of parasites vary according to environmental conditions, parasite life cycles and presence of intermediate hosts (Janovy et al., 1997). One interesting field of study is the pollution biomonitoring, since fish parasites can reflect adverse effects of complex and variable environmental stresses (MacKenzie et al., 1995; Williams and MacKenzie, 2003; Sures, 2004; Marcogliese, 2005; Carreras-Aubets et al., 2011a; Carreras-Aubets et al., 2012b). The usefulness of the parasites as ecological tags is also widely known (MacKenzie and Abaunza, 2005, Timi, 2007): when seasonal variations happen, parasite communities which have perceived these changes respond to them and experience temporal changes in structure and species composition, thus determining the roles of different parasite species within a community (Valles-Ríos et al., 2000; Violante-González et al., 2008a). Moreover, the diminishing proportion of shared species between two communities with increasing geographical distance is an obvious feature of natural systems (Hubbell, 2001). Thus, in parasite communities there is evidence that distance between hosts population affects similarly in the

species composition of parasite communities (Poulin and Morand, 1999). Nevertheless, it has been recently demonstrated that other factors, such as specific environmental conditions of the localities as well as the displacement capacities of hosts and parasites could involve differences in parasite communities from close localities (Pérez-del-Olmo et al., 2009; Muñoz-Muga and Muñoz, 2010).

Information on the parasite communities of *T. draco* is limited and fragmentary. There only exists a brief description of its parasite fauna in spring in the Aegean Sea (Akmirza, 2004). Moreover, eleven records of parasites of *T. draco* appeared in some general Mediterranean surveys (López-Roman and Guevara Pozo, 1997; Orecchia and Paggi, 1978; Jardas and Hristovski, 1985; Petter and Maillard, 1988; Radujkóvic and Euzet, 1989; Hristovski and Jardas, 1991; Bruce et al., 1994; Sasal et al., 1997; Naidenova and Mordvinova, 1997; Belofastova and Korniychuk, 2000; Paradiznik and Radujkóvic, 2007) and in six morphological descriptions (Stossich, 1896; Sekerak and Arai, 1974; Reversat et al., 1991; Hartwich et al., 1998; Bray, 2001; Carreras-Aubets et al., 2012a). Otherwise, some other general references in Atlantic (Dillon and Hargis, 1965) and Indic (Nikolaeva, 1970) oceans are also reported.

In this paper we examine the metazoan parasites of *T. draco* from the north-western Mediterranean. The seasonal influence in the parasite community patterns is evaluated. The differences between two localities of the coast of Catalonia during the summer period are also tested in the present study. The usefulness of the fish parasite communities as ecological tags is discussed.

MATERIALS AND METHODS

A total of 87 specimens of *Trachinus draco* were captured in 2007. Sampling area, fish collection, processing of samples and general data analysis are described in the chapter of “Materials and Methods” from the present thesis (see table 3, in chapter 4).

Processing of samples

Cysts of *Kudoa* sp. were counted and measured under the stereomicroscope at 75x magnification. Adult nematodes of *Phyllometra globiceps* (females) and *Ascarophis* sp. (males and females) were classified by means of morphologically criteria, following Moravec (1994, 1998) and Moravec et al. (2006).

Data analysis

Abundance of the myxozoan *Kudoa* sp. was calculated by means of a counting of the cysts observed in the muscular tissue. The numerical analyses were done with the number of cysts.

From the total 87 fish individuals processed, fish total length ranged from 10.8 to 30.6cm. All fish individuals analyzed (n= 87) were used for prevalence and mean abundance calculations and descriptions of the parasite fauna. In order to avoid possible effects of the length of the individuals in the patterns of parasite abundance, from the total of 87 fish sampled, we selected 74 fish individuals, with a total length range from 18.0 to 28.0cm, for the rest of univariate and multivariate analyses. Thus, the selected 74 individuals were used for calculations of Species Richness (SR), Mean Species Richness (MSR), Mean Abundance (MA) and Mean diversity (MD). TL, K, HSI and GSI followed a normal distribution whereas the three infracommunity descriptors (MSR, MA, MD) data were square root transformed prior to General Linear Model (GLM) analyses. Differences in fish condition indexes (TL, K, HSI, GSI) and in infracommunity descriptors (MSR, MA, MD) were tested for effect of the factor “locality/season” (five categories that will be henceforth referred Besòs autumn, Besòs winter, Besòs spring, Besòs summer, Vilanova summer) by GLM, with *post-hoc* pairwise comparison.

To visualise the patterns in parasite abundance in relation to spatial/temporal variation (factor “locality/season”) we first applied Factorial Correspondence Analysis (FCA) on a data matrix comprising component population abundance for the nine common parasite species and the five spatial/temporal groups. Also a cluster of dissimilarity between the five spatial/temporal groups was executed

based on the Eigen values given by the FCA analysis, and depending on the parasite abundance of the nine common species. Based on FCA and cluster grouping, and using individual fish as replicate samples, we tested the differences in prevalence and abundance among the parasite populations for the three spatial/temporal groups given by the FCA and cluster analyses, by means of Generalized Linear Model (GZM), using logistic model for prevalence and log-binomial model for abundance.

Finally, we used permutational multivariate analyses of similarity (PERMANOVA) to test the null hypothesis of no differences in parasite community structure due to spatial/temporal factor using parasite infracommunities (i.e. populations of all species in individual fish) as replicate samples. Analyses were carried out with PERMANOVA+ for PRIMER v6 (Anderson et al., 2008) on Bray-Curtis similarity matrices derived from the square root transformed abundance data. Permutation *P*-values were obtained under unrestricted permutation of raw data (9,999 permutations). *U. pflugfelderi* was also removed from the analyses.

RESULTS

Fish condition

Biometrical data of the fish are shown in table 1. No significant differences were found for fish length (GLM ANOVA, $P > 0.05$) when comparing the five spatial/temporal groups. Fish sampled in Besòs spring showed a significantly higher K than fish sampled in Besòs summer and Vilanova summer (GLM ANOVA, $F_{(4,69)} = 3.708$, $P = 0.009$). Individuals captured in Besòs autumn and Besòs winter showed a significantly lower HSI than fish sampled in the other three spatial/temporal groups (GLM ANOVA, $F_{(4.69)} = 7.801$, $P < 0.001$). Significant differences were found in GSI of females (GLM ANOVA, $F_{(4.40)} = 3.543$, $P = 0.014$): females Besòs autumn showed a lower GSI than females of Besòs summer and Vilanova summer, and females from Vilanova summer showed a significantly higher GSI than Besòs winter and Besòs spring.

Table 1. Means and standard deviations of total length (TL), condition factor (K), hepatosomatic index (HSI), and gonadosomatic index (only for females) (GSI), sample size (n) for *Trachinus draco* along the spatial/temporal assessment. Different letters showed significant differences in the spatial/temporal assessment. *Sample size of females in brackets.

Spatial/temporal groups	TL	K	HSI	GSI	n*
Besòs winter	23.77 ± 2.64^{a}	0.60 ± 0.05^{ab}	0.73 ± 0.28^{a}	0.56 ± 0.11^{ab}	14 (9)
Besòs spring	22.59 ± 2.07^{a}	0.64 ± 0.06^{a}	1.18 ± 0.51^{b}	0.65 ± 0.11^{ab}	10 (7)
Besòs summer	22.63 ± 3.09^{a}	0.58 ± 0.06^{b}	1.13 ± 0.41^{b}	1.53 ± 1.07^{bc}	11 (10)
Besòs autumn	22.64 ± 3.14^{a}	0.63 ± 0.06^{ab}	0.63 ± 0.23^{a}	0.54 ± 0.17^{a}	20 (10)
Vilanova summer	22.01 ± 3.25^{a}	0.57 ± 0.05^{b}	1.16 ± 0.57^{b}	1.80 ± 1.48^{c}	19 (9)

Parasite fauna

All fish individuals analyzed were infected with at least one parasite species, except one individual fish from Besòs summer that did not show any parasite. A total of 2.177 parasites corresponding to 22 categories of parasite taxa were identified: one myxozoan, 11 digeneans, one monogenean, one larval cestode, seven nematodes and one isopoda (Table 2). Nine species were common (P> 10%) in the parasite fauna of *T. draco*: the myxozoan *Kudoa* sp., the digenean *Helicometra fasciata*, the tetraphyllidean metacestode (traditionally reported under the collective name *Scolex pleuronectis*), the nematodes *Hysterothylacium fabri*, *H. aduncum*, *Philometra globiceps*, *Contracaecum* sp., *Ascarophis* sp. and the isopod *Gnathia* sp. From the 22 taxa, 20 consisted in endoparasites whereas only two were ectoparasites. The degree of specificity of the parasites is indicated in table 2.

The most prevalent and abundant species were the raphidascarid *H. fabri* (Total P= 92%, Total MA= 9.37) and the philometrid *P. globiceps* (P= 82%, MA= 6.76). The two species of the genus *Hysterotylacium* (*H. fabri* and *H. aduncum*) were found in third larval stage (L3), mainly encysted in connective tissues of oesophagus and intestine but also in the gonad, stomach and kidney. From a total of 588 specimens of *P. globiceps*, 24 were females (one of them subadult). Females of *P. globiceps* were found in gonads of *T. draco*, mostly in fish ovaries (from the 24 females, 22 were found in 9 female hosts). In one gravid female of *P. globiceps* it was observed an intrauterine development of larval stages.

Males and larvae of *P. globiceps* were found with lower prevalence in gonads, most of them being in other organs and body cavity.

Parasites were found in specific sites of the examined fish and with different levels of infection. *Kudoa* sp. and *Ascarophis* sp., both with a prevalence of 38%, probably are new species for science and will be described in future studies. *Kudoa* sp. was found in elongated cysts of the body muscle (140 cysts). Size of the cysts varied, being the minimum length of 0.38mm and maximum of 5mm (mean value of 2.88mm) whereas width ranged from 0.13 mm to 5.25mm, being the mean value of 1mm. From a total of 140 *Ascarophis* sp., 33 specimens were considered L4 or subadults. The rest were 54 males and 53 females. *Ascarophis* sp. was mainly found within the stomach. Some specimens were also found in pyloric caeca and intestine tract. A total of 37 specimens of *Contracaecum* sp. was found as L3, encysted in connective tissues of the digestive tract. The digenean *Helicometra fasciata* was manily found as adult: from a total of 37 specimens only four were juveniles. They were mainly found within intestine and pyloric caeca. *S. pleuronectis* (P= 20%) was mainly found in the digestive tract, especially in the intestine whereas only four specimens were found in the pyloric caeca. *Gnathia* sp. was found in gills samples but the exact position of the attachment could not be registered since the defreezing process of the fish made the isopod detach from the gills.

Table 2. Prevalence (P%) and mean abundance (MA ± standard deviation, SD) of the parasites of *Trachinus draco* and parasite community parameters associated with the parasitological survey done off Besòs (along seasons) and Vilanova (in summer). Data on the specificity was obtained from Gibson et al., (2005): G, generalist; T*, specific of *T. draco* (*Kudoa* sp. and *Ascarophis* sp. are likely new species of *T. draco; Aspinatrium* sp. is probably *A. trachini*, specific of *T. draco* and *P. globiceps* is mostly specific of *T. draco* in the bibliography); na, not applicable.

Locality		TOTAL		BESÒS								VILANOVA	
Season				Winter		Spring		Summer		Autumn		Summer	
	Specificity	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD	P (%)	MA ± SD
Mixozoa													
Kudoa sp.	T*	37.9	1.61 ± 3.01	42.1	1.37 ± 1.92	40.0	2.00 ± 2.94	41.7	0.58 ± 0.90	45.0	2.90 ± 4.66	26.9	1.12 ± 2.50
Digenea													
Aponurus laguncula	G	2.3	0.03 ± 0.24	-	-	-	-	-	-	10.0	0.15 ± 0.49	-	-
Aphallus tubarium	G	1.1	0.02 ± 0.21	5.3	0.11 ± 0.46	-	-	-	-	-	-	-	-
Brachyphallus sp.	G	1.1	0.02 ± 0.21	5.3	0.11 ± 0.46	-	-	-	-	-	-	-	-
Brachyphallus crenatus	G	2.3	0.02 ± 0.15	10.5	0.11 ± 0.32	-	-	-	-	-	-	-	-
Derogenes varicus	G	1.1	0.07 ± 0.64	-	-	-	-	-	-	-	-	3.8	0.23 ± 1.18
Didymozoidae sp.	na	5.7	0.06 ± 0.23	5.3	0.05 ± 0.23	10.0	0.10 ± 0.32	8.3	0.08 ± 0.29	10.0	0.10 ± 0.31	-	-
Helicometra fasciata	G	23.0	0.43 ± 0.92	10.5	0.11 ± 0.32	20.0	0.40 ± 0.97	16.7	0.50 ± 1.24	30.0	0.50 ± 0.89	30.8	0.58 ± 1.06
Lecithocladium excisum	G	2.3	0.02 ± 0.15	-	-	-	-	-	-	5.0	0.05 ± 0.22	3.8	0.04 ± 0.20
Lecithochirium musculus	G	8.0	0.17 ± 0.70	-	-	20.0	0.40 ± 0.97	8.3	0.17 ± 0.58	15.0	0.20 ± 0.52	3.8	0.19 ± 0.98
Prosorhynchus squamatus	G	3.4	0.06 ± 0.32	-	-	-	-	8.3	0.17 ± 0.58	-	-	7.7	0.12 ± 0.43
Stephanostomum sp.	G	1.1	0.01 ± 0.11	5.3	0.05 ± 0.23	-	-	-	-	-	-	-	-
Monogenea													
Aspinatrium sp.	T*	3.4	0.03 ± 0.18	10.5	0.11 ± 0.32	-	-	8.3	0.08 ± 0.29	-	-	-	-
Cestoda													
Scolex pleuronectis larva	na	19.5	3.70 ± 12.50	-	-	-	-	25.0	7.33 ± 20.20	10.0	0.30 ± 0.98	46.2	8.77 ± 17.29
Nematoda													
Ascarophis sp.	T*	37.9	1.61 ± 3.17	26.3	1.16 ± 2.50	40.0	1.10 ± 1.73	41.7	0.58 ± 0.79	40.0	1.40 ± 2.84	42.3	2.77 ± 4.52
Capillaria sp.	na	1.1	0.03 ± 0.32	5.3	0.16 ± 0.69	-	-	-	-	-	-	-	-
Contracaecum sp. larva	G	32.2	0.43 ± 0.71	21.1	0.21 ± 0.42	40.0	0.40 ± 0.52	8.3	0.08 ± 0.29	55.0	0.85 ± 0.93	30.8	0.42 ± 0.76
Cucullanus sp.	na	1.1	0.01 ± 0.11	-	-	-	-	-	-	-	-	3.8	0.04 ± 0.20
Hysterothylacium aduncum larva	G	24.1	0.25 ± 0.46	36.8	0.42 0.61	40.0	0.40 ± 0.52	16.7	0.17 ± 0.39	15.0	0.15 ± 0.37	19.2	0.19 ± 0.40
Hysterothylacium fabri larva	G	92.0	9.37 ± 9.83	100.0	12.68 ± 16.43	100.0	8.60 ± 6.20	100.0	10.8 ± 7.14	95.0	12.65 ± 6.85	76.9	4.08 ± 4.32
Philometra globiceps	T*	81.6	6.76 ± 9.84	84.2	9.47 ± 12.06	90.0	7.20 ± 7.93	100.0	8.25 ± 9.03	100.0	9.40 ± 12.46	53.8	1.88 ± 3.85
Isopoda													
Gnathia sp.	G	14.9	0.30 ± 0.82	31.6	0.68 ± 1.16	20.0	0.30 ± 0.67	16.7	0.33 ± 0.89	15.0	0.30 ± 0.92	-	-

Parasite communities

The mean species richness (MSR) for the totality of the sample was 3.98. Regarding mean species richness for each sample (the five categorical groups), no significant differences between localities and seasons were observed (Table 3). The maximum value of total mean species abundance was 29.8 in Besòs spring, but no significant differences were observed with the other four categorical groups. Significant differences were found in mean diversity between the five spatial/temporal groups assessed (GLM ANOVA, $F_{(4.81)}$= 2.002, P= 0.042). Mean diversity from Vilanova summer was significantly lower than that observed in Besòs autumn (Table 3).

Table 3. Species richness, Mean species richness, mean abundance and mean diversity calculated for both the total sample and for each of the five categorical groups. All these infracommunity descriptors were calculated with 74 fish. Different letters showed significant differences between the five spatial/temporal groups assessed in *Trachinus draco*.

Locality	TOTAL	BESÒS				VILANOVA
Season		Winter	Spring	Summer	Autumn	Summer
Species richness	22	15	10	13	13	13
Mean species richness	3.98 ± 1.61	4.00 ± 1.67^{a}	4.20 ± 1.40^{a}	4.00 ± 1.13^{a}	4.45 ± 1.47^{a}	3.50 ± 1.90^{a}
Mean abundance	25.02 ± 21.11	26.79 ± 28.39^{a}	20.90 ± 12.69^{a}	29.08 ± 29.42^{a}	28.95 ± 15.42^{a}	20.42 ± 16.80^{a}
Mean diversity (Brillouin Index)	0.79 ± 0.32	0.80 ± 0.27^{ab}	0.85 ± 0.22^{ab}	0.84 ± 0.22^{ab}	0.88 ± 0.23^{a}	0.67 ± 0.45^{b}

The FCA carried out using component population data for the nine common species in *T. draco* (Fig. 1) showed a plot of the first factorial plane of co-inertia analysis which explained a total of 97% of inertia, being the 71% of the variance in the first axis and the 26% in the second. It was observed a clear gradient differentiating the samples in relation to the seasons sampled in this survey in the first axis of the FCA, separating the warmer season (Besòs summer and Vilanova summer) from the colder seasons (Besòs autumn, Besòs winter and Besòs spring). There existed a partial gradient between the two localities sampled in the survey, separating Besòs than Vilanova, reflected in the second axis. Thus, the FCA showed clearly three groups and this grouping pattern was associated with the abundance of the component parasite populations of two, one and six common species, respectively, that characterized the multivariate

relationship among the samples examined (Fig. 1). Component populations of three species exhibited the strongest correlations with the first FCA axis: *S. pleuronectis* (squared cosine value, $Cosine^2$= 0.975), *P. globiceps* ($Cosine^2$= 0.767) and. *H. fabri* ($Cosine^2$= 0.689). An additional suite of two species was heavily associated with the second FCA axis: *Ascarophis* sp. ($Cosine^2$= 0.861) and *Contracaecum* sp. ($Cosine^2$= 0.521).

From the FCA and cluster analysis of the five categories of locality/season, three different groups mentioned above were identified depending on their parasite load (Figs. 1 and 2). The samples of Vilanova summer (group A) were clustered separately from Besòs summer (group B) by the axis two, and the position of the group B in the negative part of the axis one indicates that locality is a partially determinant factor. The separation between groups of summer (A and B) and the rest of seasons indicates also a partial effect of the seasonality. Based on these results, the samples were separated into these three groups (A, B and C):

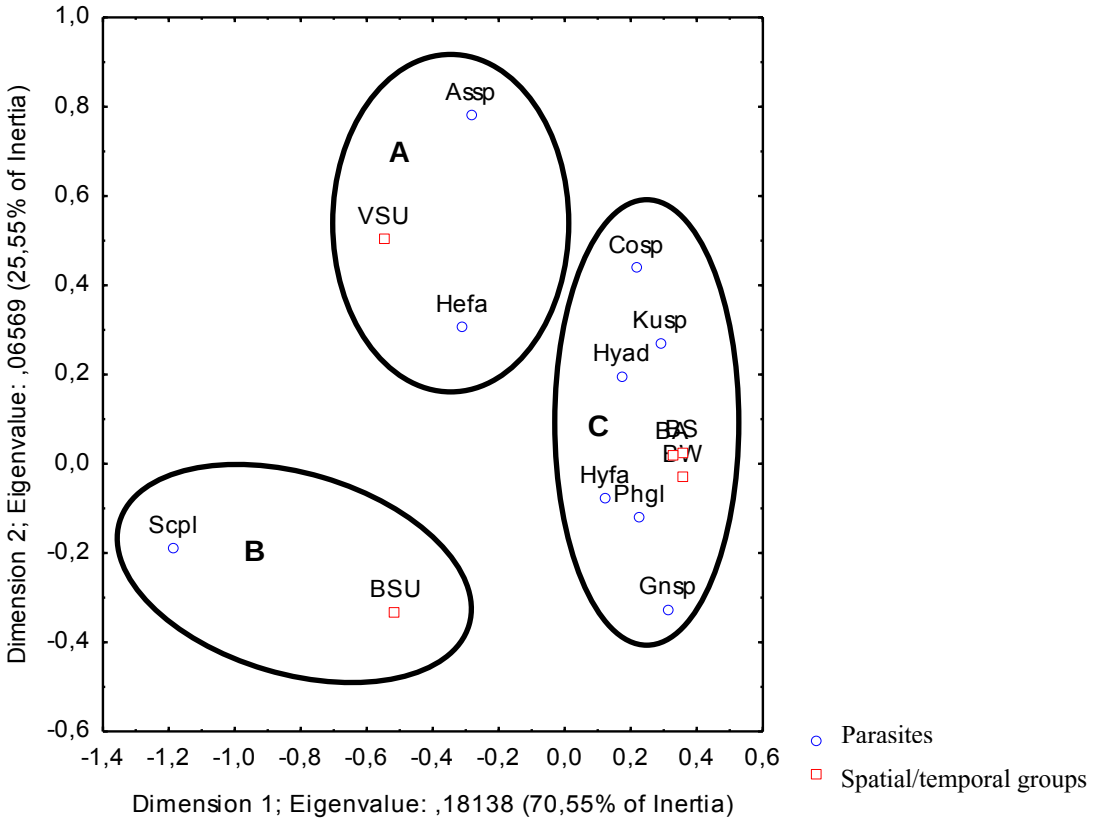

Figure 1. Plot of the first factorial plane of co-inertia analysis of the factorial correspondence analysis (FCA) on component population data for the 9 common species in *Trachinus draco*. A/B/C indicate the groups stablished in the parasite fauna description. Abbreviations for spatial/ temporal groups: BW, Besòs winter; BA, Besòs autumn; BS, Besòs spring; BSU, Besòs summer; VSU, Vilanova summer. Abbreviations for species names: Assp, *Ascarophis* sp.; Cosp, *Contracaecum* sp.; Gnsp, *Gnathia* sp.; Hefa, *Helicometra fasciata*; Kusp, *Kudoa* sp.; Hyad, *Hysterothylacium aduncum*; Hyfa, *Hysterothylacium fabri*; Phgl, *Phillometra globiceps*; Scpl, *Scolex pleuronectis.*

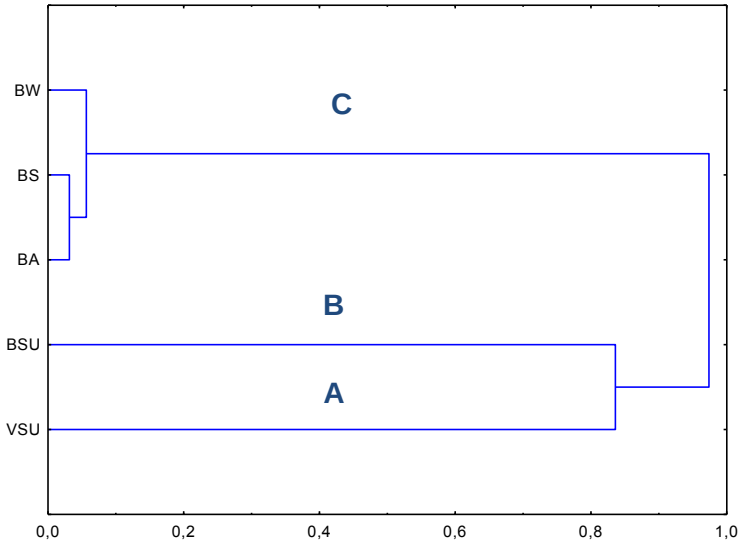

Figure 2. Dendrogram of dissimilarity between the five spatial/temporal groups assessed depending on the parasite abundance of the 9 common species in *Trachinus draco*. Abbreviations for spatial/temporal groups: BW, Besòs winter; BA, Besòs autumn; BS, Besòs spring; BSU, Besòs summer; VSU, Vilanova summer. Groups identified: A (Vilanova summer); B (Besòs summer); C (Besòs autumn, Besòs winter and Besòs spring).

Group A- Vilanova summer: it was the group with the lowest mean richness, mean abundance and mean diversity (Table 2) and it was characterized by *Ascarophis* sp. and *H. fasciata,* which showed the maximum abundance in this group (Table 2). Significant differences were found in the abundance of *Ascarophis* sp. (GZM, χ^2= 14.446, *P*= 0.001): abundance of this nematode from group A was significantly higher than that of group B and group C (Table 4). No significant differences in prevalences were found for any of the characteristic parasite species of the group A.

Group B- Besòs summer: it was characterized by *S. pleuronectis*, which showed significant differences in abundance (GZM, χ^2= 60.021, *P*< 0.001), being the abundance of this metacestode from group B and A significantly higher than C (Table 4). Moreover, another significant difference in prevalence were found for *S. pleuronectis* between the other groups given by FCA (GZM, χ^2= 6.723, *P*= 0.035) having the group A a significantly higher prevalence than group C (Table 4).

Group C- Besòs autumn, Besòs winter and Besòs spring: it was characterized by *Contracaecum* sp., *H. aduncum, H. fabri, Gnathia* sp., *P. globiceps* and *Kudoa* sp. *Contracaecum* sp. showed its maximum abundances in Besòs autumn and spring; *H. aduncum* in Besòs winter and Besòs spring, and *Gnathia* sp. in Besòs winter (Table 2). Besòs winter and Besòs autumn were the seasons in which the highest abundances of *H. fabri* were registered (Table 2). Significant differences in *H. fabri* and *P. globiceps* abundances were found (GZM, χ^2= 7.557, *P*= 0.023; GZM, χ^2= 15.244, *P*< 0.001): abundances of these nematodes in group B and C were significantly higher than group A (Table 4). *Kudoa* sp. showed significant differences in abundance (GZM, χ^2= 1.703, *P*= 0.037), since group C showed higher abundance than group B (Table 4). No significant differences in prevalence were found.

Table 4. Significances (*),marginally significances (**) and no- significances (-) of Generalized Linear Model (GZM) executed on prevalences and abundances of the nine common parasite species found in *Trachinus draco* from the three spatial/temporal groups given by the FCA. Since no significant differences in prevalence were found in comparisons between A and B and between B and C, columns of prevalences were removed. Common parasites without any significant difference in abundance were also removed from the Table 4. Group A: Vilanova summer; Group B: Besòs summer; Group C: Besòs autumn, winter and spring.

	Group A- Group B	Group B- Group C	Group A- Group C	
	Mean Abundance	Mean Abundance	Prevalence	Mean Abundance
***Kudoa* sp.**	-	χ2= 5.983 p= 0.014*	-	-
Scolex pleuronectis	-	χ2= 55.805 p<0.001*	χ2= 6.723 p= 0.010*	χ2= 44.355 p<0.001*
Hysterothylacium fabri	χ2= 3.800 p= 0.051**	-	-	χ2= 7.162 p= 0.007*
Philometra globiceps	χ2= 6.476 p= 0.001*	-	-	χ2= 15.023 p<0.001*
***Ascarophis* sp.**	χ2= 10.256 p= 0.001*	-	-	χ2= 9.583 p= 0.002*

The differences observed in parasite distributions among individual fish were again confirmed after the permutational multivariate analyses (PERMANOVA), which showed the differences in community structure in relation to the factor “locality/season”. The analysis based on the replicate infracommunity samples

from all five spatial/temporal groups revealed significant effect of the factor "locality/season" on community structure (*Pseudo-*$F_{(4.69)}$= 1.613; $P_{(perm)}$= 0.0439; 9918 unique permutations; only two *post hoc* comparisons significant: between Vilanova summer and Besòs winter (*t*= 1.6387; *P*= 0.022) and between Vilanova summer and Besòs autumn (*t*= 1 .8003, *P*= 0.0061). The highest predictability was observed in Besòs autumn (58.51%) and the lowest in Vilanova summer (39.12%, respectively, Table 5), so a marked decrease in the predictability of parasite infracommunities along the seasonal and locality assessment is outstanding.

Table 5. Mean similarity for parasite infracommunities sampled within the five spatial/ temporal groups sampled in *Trachinus draco* and a breakdown into contributions from individual parasite species. Only species contributing to more than 10% of the mean community similarity within habitat groups are included. BA: Besòs autumn, BS: Besòs spring, BW: Besòs winter, BSU: Besòs summer, VSU: Vilanova summer.

Species/ Spatial/temporal / Contrast	BA	BS	BW	BSU	VSU
Mean similarity (%)	**58.51**	**56.87**	**55.96**	**54.42**	**39.12**
Hysterothylacium fabri	49.18	52.69	54.47	52.22	46.91
Philometra globiceps	35.05	33.08	32.32	36.87	21.02
Ascarophis sp.	-	-	-	-	14.2
Kudoa sp.	5.19	5.09	6.31	4.37	-
Cumulative contribution (%)	**95.03**	**90.86**	**93.1**	**93.46**	**90.38**

Furthermore, application of SIMPER analysis indicated that the decrease in the similarity was generally coupled with an apparent increase in dissimilarities among the five groups (Table 6). The parasite species which contributed most to the similarity of infracommunities within the replicate samples of the five spatial/temporal groups also contributed substantially to the differentiation in community structure observed in the contrasts among samples (see tables 5 and 6 for a breakdown into species percent contributions to the total average similarity/dissimilarity within/among spatial/temporal groups, respectively). *H. fabri, P. globiceps* and *Kudoa* sp. were identified as "key discriminating species" in all contrasts associated with the spatial/temporal assessment (see tables 5 and 6). The nematode *Ascarophis* sp. could also be identified as "key discriminating species" only in Vilanova summer because it contributes to the

similarity of the Vilanova summer sample as well as in all the dissimilarities between categorical groups (see tables 5 and 6).

Table 6. Mean dissimilarity for parasite infracommunities sampled among the five spatial/temporal groups sampled in *Trachinus draco* and a breakdown into contributions from individual parasite species. Only species contributing to more than 10% of the mean community dissimilarity between habitat groups are included. BA: Besòs autumn, BW: Besòs winter, BS: Besòs spring, BSU: Besòs summer, VSU: Vilanova summer.

Species/ Spatial/temporal/ Contrast	BA *vs* BW	BA *vs* BS	BA *vs* BSU	BA *vs* VSU	BS *vs* BW	BS *vs* BSU	BS *vs* VSU	BW *vs* BSU	BW *vs* VSU	BSU *vs* VSU
Mean dissimilarity (%)	**43.22**	**41.6**	**43.74**	**55.44**	**41.53**	**43.6**	**54.15**	**44.44**	**56.67**	**55.71**
Philometra globiceps	23.7	21.48	18.43	20.38	26.64	20.13	21.07	23.71	22.9	18.17
Kudoa sp.	16.51	18.15	14.8	13.26	16.76	14.53	13.43	12.33	11.85	9.92
Hysterothylacium fabri	17.71	19.04	18.74	21.29	16.05	18.1	17.85	17.88	18.39	20.37
Ascarophis sp.	12.4	11.74	10.38	14.32	13.42	10.73	15.74	12.07	15.62	14.67
Cumulative contribution (%)	**70.31**	**70.41**	**75.01**	**79.67**	**72.87**	**75.23**	**78.22**	**77.44**	**68.76**	**80.15**

DISCUSSION

The present study adds some new data about the biology of *T. draco* from north-western Mediterranean Sea. *T. draco* spawning happens between June and September in both Mediterranean (Portillo et al., 2008) and Atlantic waters (Bagge, 2004), so the maximum GSI values given in our specimens agrees with the reproduction period, which reaches a peak in July. Maximum values of K found in spring agree with the data of the K presented in Atlantic Ocean (Bagge, 2004), being June the month in which females showed maximum body condition.

Parasite fauna

This is the first parasitological study about the spatial/temporal variations of the parasite communities of *T. draco* in the north-western Mediterranean. The high endoparasite richness observed in the Catalan Sea was not observed in the only other study done in the Mediterranean (Aegean Sea), where the richness was much reduced, since only five parasites were reported (one digenean, one nematode, one monogenean and two cestodes) (Akmirza, 2004). One specific parasite found in the present survey of *T. draco* (*Aspinatrium* sp., being probably *A. trachini*) and the generalist larval nematode *Contracacecum* sp. were shared with fish examined from the Aegean sea, whereas the generalist adult cestode described by Akmirza (2004) were not present in our survey. The results observed in *T. draco* coincide with studies done in other perciform fish from the same localities of the north-western Mediterranean. In *Spicara maena*, generalist parasites (mainly digeneans) also dominate the parasite composition whereas host-specific parasites were a minor element of the parasite communities (see chapter 7 of *S. maena* of the present thesis), as also happened in *T. draco*, which have predominance of generalist parasites and it only showed two specific parasites, *Aspinatrium* sp. and *P. globiceps*.

Two new species for science were found in parasite fauna of *T. draco*: the myxozoan *Kudoa* sp. and the nematode *Ascarophis* sp., but further studies are needed to describe them. Moreover, *T. draco* appears to be new host record for the following parasite species/genus: *A. laguncula*, *Aphallus tubarium*, *L. excisum*, *L. musculus*, *Prosorhynchus squamatus*,

Stephanostomum sp., *Cucullanus* sp., *H. aduncum*, *Capillaria* sp., and *Gnathia* sp.

The parasite fauna of Mediterranean *T.draco* showed clear endoparasite dominance, with only two ectoparasites identified. *S. pleuronectis*, Didymozoidae gen. sp. and three nematode species (*H. fabri*, *H. aduncum* and *Contracaecum* sp.) were exclusively found as larval stages. However, most of the 22 parasite species were found as adult, showing that *T. draco* is mostly a definitive host in its habitats in north-western Mediterranean Sea, indicating a high situation in the food web, despite its relatively small size. The infection site of gravid females of *P. globiceps* is variable depending on the fish host species, being these nematodes found in the host abdominal cavity (Moravec et al., 2010) or in the host ovaries (Gaglio et al., 2009). According to Molnar et al. (1982), during their development philometrids migrate to reach a final infection site, therefore they can be found in different locations until they reach the definitive target organ. In present study, gravid females of *P. globiceps* were only found in the ovaries of *T. draco*. *P. globiceps* is usually transmitted to the definite fish host by copepods which harbour larval stage. L3 penetrates the fish gut and migrates *via* the body cavity to their final site of infection (Moravec et al., 2008). Nevertheless, copepods are an accidental prey of Mediterranean *T. draco* (Morte et al., 1999; L. Zucca pers. comm.). The high prevalence and abundance of infection of these specimens found in this study suggest the possibility that *P. globiceps* could be also transmitted by another unknown invertebrate host for the perciform *T. draco*, as suggested Séguin et al. (2011), since copepods are not included in the definitive host diet. The alternative pathway of transmission could be related with the ingestion of other crustaceans or even teleosteans: fish infected by *P. globiceps* larvae could also be ingested by the host *T. draco* (Molnar et al., 1982; Séguin et al., 2011) by predation or because of the partial scavenger habits of this fish (Olaso et al., 2002). Further studies of the life cycle of *P. globiceps* in the Mediterranean are needed. Decapods are the main prey in the diet of *T. draco* (Morte et al, 1999), and they can act as intermediary hosts for the generalist cystidicolid *Ascarophis* sp. which is usually transmitted to the host by means of the ingestion of decapods, amphipods and isopods (Appy, 1981).

The digeneans appear to be the group with major species richness, although only one of them belongs to the common species, *H. fasciata*, reaching a total prevalence of 23%. The temporal variation pattern observed could depend on the transmission to the host. Second intermediate hosts of digeneans include crustaceans, chaetognaths and a few fish species (Bray, 1990). The seasonal variances in prevalence and abundance of some digeneans can reflect the feeding behavior of *T. draco*. Thus, seasonal variation together with the addition of organic matter favor the development of the crustacean population in winter, which are an important part of the diet of *T. draco* (Morte et al., 1999; L. Zucca, pers. comm.) and which moreover act as intermediate hosts for parasites such as digeneans. Specifically, the dominance of some digeneans could also be related with the high ingestion of natantid crustaceans during winter (Morte et al., 1999), which act as intermediate hosts for some digeneans, involving a recruitment of these species in the fish host by means of the prey ingestion. The reduced presence of some digeneans during spring and summer also agrees with the significantly lowest fullness of the stomach (average of prey per stomach) of *T. draco* during the warmest months (Morte et al., 1999), which coincides with the reproduction period. Nevertheless, other digeneans, as the hemiurids *L. excisum* and *L. musculus*, follow the usual seasonal patterns, predominating in warm periods (and early autumn), coinciding with the more favorable environmental conditions. The recruitment of these hemiuridids occurs from later spring until autumn, as also observed in other hosts such as *Coilia nasus* (Li et al., 2011). A similar pattern is observed in the tetraphyllidean *Scolex pleuronectis*: the increased consumption of crustaceans reptantia during spring (Morte et al., 1999) would cause the emergence of the cestode in the host in summer, acting *T. draco* as another intermediate host, as do other teleost fishes (Stunkard, 1977; Avdeeva and Avdeev, 1989).

The fact that the ectoparasite *Gnathia* sp. was not found in Vilanova locality could suggest a possible usefulness of this ectoparasite as ecological tag for stock differentiation between Besòs and Vilanova, but further information should be required.

Parasite communities

Our results indicate that even though total parasite species richness and diversity were rather similar throughout the annual period sampled, some parasite species presented temporal changes in their infection levels. The three habitat groups defined at parasite community level showed a marked seasonal separation between summer and the rest of the seasons, and we observed the same pattern in the infracommunity analysis. It is widely known that parasite communities experience temporal structural changes related to seasonal variations in biotic and abiotic environmental factors (Violante-González et al., 2008b). Many processes have been suggested to influence the seasonal variation in parasite communities, such as temperature and other abiotic factors (Granath and Esch, 1983), intermediate host abundance (Zander, 2004) and changes in host abundance, reproductive and feeding behavior (Simková, 2005). However, the effects of abiotic and biotic factors are difficult to distinguish, because they are interrelated and influence each other, since for example water temperature is connected with the amount of oxygen, the level of immunological response of the fish and with the movements and migration of fish (Valtonen et al., 1990). Our study has shown a relatively high variation in parasite abundance over time in response to alterations generated by the seasonality, which affects the parasite species recruitment process, or in response to seasonal increases in host feeding and reproductive activity influenced by seasonal water temperature fluctuations (Morte et al., 1999).

Another recurrent result in both types of community analyses (component community and infracommunity) was the differentiation between the two sampled areas, Besòs and Vilanova, partially observed in the component community analysis but reinforced with the results obtained by Permanova analyses at the infracommunity level. Since parasite species richness and abundance can vary geographically for the same host species (Vales et al., 2010), our results obtained from samples collected from Besòs and Vilanova during summer contributes to the discussion about the similarities between parasite communities from close areas. When geographical distance between two parasite communities increases, it is known that the proportion of shared species will diminish (Hubbell, 2001). However, this tendency is not so clear

when comparing close areas (Pérez-del-Olmo et al., 2009; Muñoz-Muga and Muñoz, 2010). In the present study, the total species richness value was the same in the two localities and moreover they shared ten species and differ only from five. Nevertheless, the rest of the infracommunity descriptors showed lower values in Vilanova locality, showing a slight impoverishment of that area, probably due to pollution effect (Carreras-Aubets et al., 2012b). The impoverishment of parasite fauna in more stressful conditions is in good agreement with the general theory of stressed ecosystems (Okland and Okland, 1986; MacKenzie, 1999). Component community analysis showed a partial differentiation between localities, which was also confirmed by differences in Permanova analysis. This result could partially agree with some studies, which suggested that some hosts collected from close localities may have different parasite communities, thus indicating that factors such as specific environmental conditions (of natural or anthropogenic nature) of each locality may produce differences in parasite communities in hosts from close localities (Muñoz et al., 2002; Carreras-Aubets et al., 2011a).

The groups given in the FCA partially correlate with the gradient of dissimilarity observed in SIMPER analysis, being the autumn from group C that of highest predictability (meaning similarity) whereas summer months of groups A and B showed lower predictability values. Parasite abundance responses to the seasonal and locality effects studied has been reflected on significant differences in infracommunity structure with a general tendency of decreasing predictability associated with the warmer seasons. At the parasite population level, the nematodes that contributed most to the separation of the samples agreed with those identified as potential biological tags in the multivariate analysis, being *H. fabri*, *P. globiceps* and *Ascarophis* sp. the best indicator species in similarity and dissimilarity analyses in this particular host-parasite system. The usefulness of the generalist *H. fabri* in the assessment environmental variations has been discussed for other perciform from the same area, *Spicara maena* (see the chapter 7 of *Spicara maena* from the present thesis), as well as the relationship between the infection of the other two nematodes and the diet of *T. draco* is also assessed above.

In conclusion, by means of both univariate and multivariate analyses which revealed seasonal and locality changes in the parasite community compositon and structure, this study demonstrates that some common parasites of *T. draco* could be used to reveal certains aspects of fish ecology and behavior as well as aspects about natural variability (seasonal and geographical) in a particular north-western Mediterranean system. Thus, we suggest the usefulness of some parasites of *T. draco* (the nematodes *H. fabri*, *P. globiceps* and *Ascarophis* sp.) as ecological tags to evaluate environmental (seasonal and locality) patterns in the Catalan Sea.

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9. CONCLUSIONS

The following conclusions can be drawn as a result of the present investigation:

1. The comparative morphological study of the "*Aponurus laguncula* species complex" reveals the presence of at least two cryptic species in the Mediterranean Sea, thus supporting the theory that this widespread digenean species consists of a number of cryptic species which exhibit considerable morphological similarity.

2. A new species to science, *Aponurus mulli* n. sp., is described for the first time on the basis of abundant material from goatfishes *(Mullus barbatus* and *M. surmuletus*) off the Mediterranean coasts. Specimens of both hosts are genetically indistinguishable.

3. The infection levels of *Aponurus mulli* n. sp. are higher in *Mullus surmuletus* than in *Mullus barbatus*, thus supporting the bathymetric habit partitioning tendency and clear niche segregation. There are significant differences in the infection parameters in *M. surmuletus* sampled at the two locations off the coast of Valencia but no differences in infection parameters in *M. barbatus* from the two localities of the coast of Catalonia.

4. The redescription of *Aponurus laguncula* from *Trachinus draco* provides the first detailed morphological description from the north-western Mediterranean Sea. The characteristic egg-shape, with a narrow and pointed anopercular pole, serves as a reliable distinguishing feature whereas egg-length is not a useful taxonomic feature with regard to this species.

5. The hemiurid *Lecithochirium musculus*, from the stomach of *Trachinus draco* and *Citharus linguatula* from the north-western Mediterranean Sea, exhibits a characteristic prominent pre-oral lobe with a transverse ventral pit. Moreover, *L. musculus* is morphologically indistinguishable from the congeneric *L. israelense* described by Fischthal (1980) so we consider them synonymous.

6. The description of the hemiurid *Ectenurus lepidus* in the stomach of *Spicara maena* from the north-western Mediterranean Sea represents the first detailed morphological description of this hemiurid and the only second record in this area. Specimens of *Spicara maena* exhibit lower ranges for the size of body, suckers and gonads than the material previously described from *Trachurus* spp.

7. The study of *Mullus barbatus* is the first study providing data on parasite communities' responses to moderate levels of pollution that are simultaneously validated by both chemical monitoring and effect biomarkers in north-western Mediterranean Sea.

8. *Mullus barbatus* from north-western Mediterranean showed a rather high overall parasite diversity, including 22 out the 34 species recorded in the distributional range of Mediterranean and also a new host record, the digenean *Aphallus tubarium*.

9. Endoparasites infecting *Mullus barbatus* from the coast of Catalonia exhibit a decrease in abundance with the increase in PCB levels, which supports the predictions on the effects of environmental stress on endoparasites abundance.

10. Parasite responses to the PCB gradient translate into significant differences in infracommunity parasite structure of *Mullus barbatus* from the north-western Mediterranean Sea, with decreasing predictability (meaning similarity) associated with increasing PCB levels.

11. The specialist digenean *Opecoeloides furcatus*, the generalist larval nematode *Hysterothylacium fabri* and the isopod *Gnathia* sp. contribute substantially to the observed similarity within and dissimilarity between infracommunity samples of *Mullus barbatus* from north-western Mediterranean Sea along the gradient of pollution. These "key species" are suggested as useful tags of pollution levels changes (bioindicators) for the host *Mullus barbatus* from north-western Mediterranean Sea.

12. Substantially higher parasite richness, with endoparasite dominance, is observed in *Spicara maena* from the coast of Catalonia compared with the only other study done in the Mediterranean, in the coasts of Sardinia.

13. The increased presence of *Ceratothoa oestroides* in summer months which characterized the parasite community of Vilanova in summer, together with the increased presence of *Cardiocephaloides longicollis* in Besós in autumn and in Besòs in winter, characterizing the parasite communities of the cold seasons, let us to suggest them as useful ecological tags in north-western Mediterranean environmental variations for the host-system *Spicara maena*.

14. In the seasonal assessment of parasite communities of *Spicara maena,* the cold seasons appear to be that of highest predictability (meaning similarity) whereas the warm seasons show lower predictability values. The high abundance of *Hysterothylacium fabri* and *H. aduncum* contribute substantially to the observed similarity within- and dissimilarity between infracommunity samples along the seasonal assessment, being also suggested as useful ecological tags to evaluate environmental patterns in the host *Spicara maena* from the Catalan Sea.

15. *Trachinus draco* from the north-western Mediterranean shows the highest endoparasite richness observed to date in the Mediterranean Sea.

16. Two new species for science are found in *Trachinus draco* from the Catalan Sea: the myxozoan *Kudoa* sp. and the nematode *Ascarophis* sp., but further studies are needed to describe them. *T. draco* appears to be new host record for the following parasite species/genus: *Aponurus laguncula*, *Aphallus tubarium*, *Lecithochirium excisum*, *Lecithochirium musculus*, *Prosorhynchus squamatus*, *Stephanostomum* sp., *Cucullanus* sp., *Hysterothylacium aduncum*, *Capillaria* sp., and *Gnathia* sp.

17. In the seasonal assessment in Besòs locality of parasite communities of *Trachinus draco*, autumn is the period of highest predictability (meaning

similarity) whereas summer months show lower predictability values. *Hysterothylacium fabri*, *Phyllometra globiceps* and *Ascarophis* sp. are the nematodes which contribute most to the separation of these samples, so they are also suggested as ecological tags to evaluate environmental variations in the host-system *T. draco* from the Catalan Sea.

18. The generalist endoparasite *Hysterothylacium fabri*, highly prevalent and abundant in three different fish hosts from the coasts of Catalonia, appear to be useful tag for both ecological and pollution assessments. For this reason, this nematode is highly suggested as the most potentially useful parasite for the assessment of the environmental condition in north-western Mediterranean for *Mullus barbatus*, *Spicara maena* and *Trachinus draco*.

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