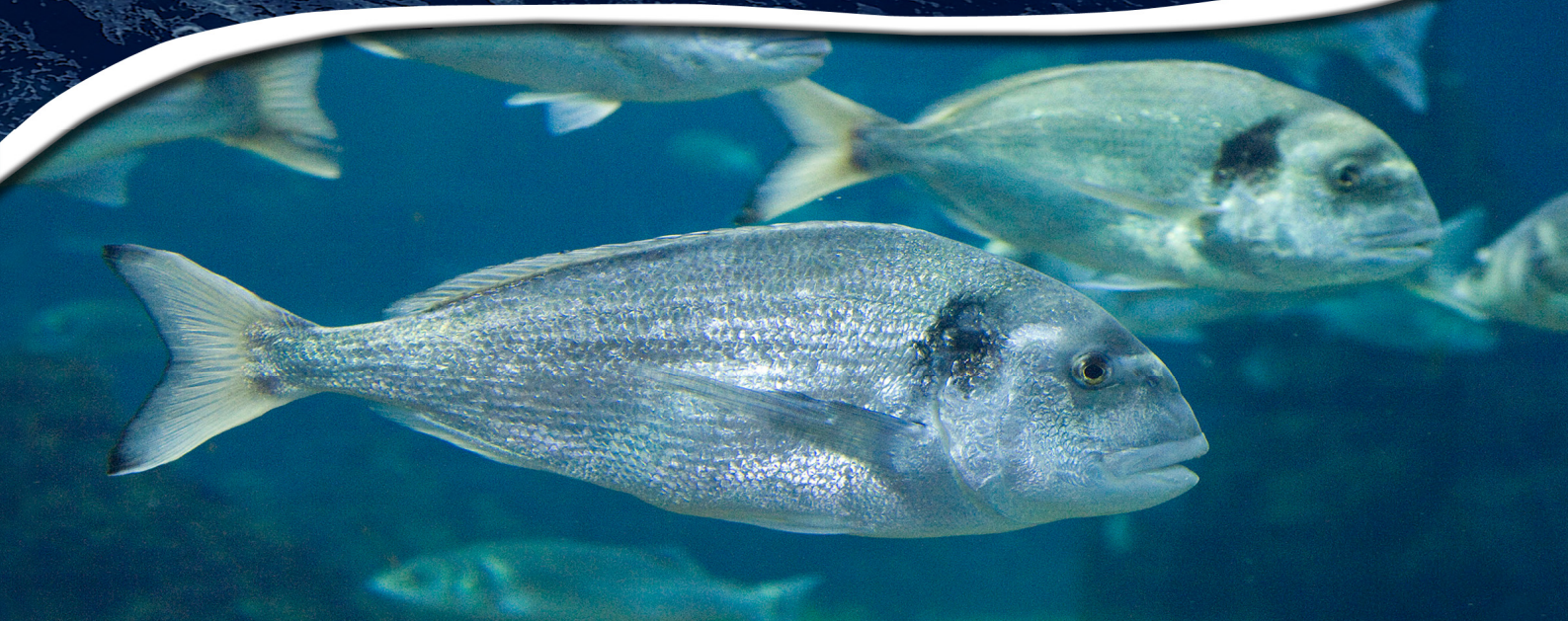
VNIVERSITAT DE VALÈNCIA
Facultat de Ciències Biològiques
INSTITUT CAVANILLES DE BIODIVERSITAT I BIOLOGIA EVOLUTIVA

Studies on metazoan parasites of two marine fish species of interest for aquaculture: *Seriola dumerili* and *Sparus aurata*

TESI DOCTORAL PER:
AIGÜES REPULLÉS ALBELDA

CO-DIRECTORS:
FRANCISCO E. MONTERO ROYO
JUAN A. RAGA ESTEVE

VALÈNCIA, DESEMBRE DEL 2012



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PROGRAMA DE DOCTORAT 119A

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D. JUAN ANTONIO RAGA ESTEVE, Catedràtic del Departament de Zoologia de la Facultat de Ciències Biològiques de la Universitat de València

CERTIFIQUEN que Dª Aigües Repullés Albelda ha realitzat sota la nostra direcció i amb el major aprofitament el treball d'investigació recollit a aquesta memoria y titulat: "Studies on metazoan parasites of two marine fish species of interest for aquaculture: *Seriola dumerili* and *Sparus aurata*", per tal d'optar al grau de Doctora en Ciències Biològiques.

I per a que així conste, en compliment de la llei vigent, expedim el present certificat a Paterna el 19 de desembre del 2012.

Signat: Francisco E. Montero

Signat: Juan A. Raga

A Joan i Mari

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Summary

Aquaculture has experienced an important expansion during the last years as a response to human population growth and overexploitation of fishery stocks. Establishing viable and profitable cultures involves the management of the entire life-cycle of fish. Therefore, understanding the behaviour, habitat, nutritional requirements, reproductive biology, and larval and juvenile physiology of each fish species is crucial in fish production systems. On the other hand, captivity conditions and fish stress facilitate pathogen transmission and thus, disease control becomes also a crucial matter in aquaculture.

This PhD study compiles various studies on metazoan parasites of two fish species of interest for aquaculture, the greater amberjack (*Seriola dumerili*) and the gilthead seabream (*Sparus aurata*). Among these parasites, and out of myxozoans, it is worth highlighting the importance of monogeneans and blood flukes since they cause the most severe parasitoses in cultures. In the Mediterranean, two heteraxinid monogeneans (*Allencotyla mcintoshi* and *Zeuxapta seriolae*) and two aporocotylid trematodes (*Paradeontacylix balearicus* and *P. ibericus*) have been related to severe infections of greater amberjack. Concerning gilthead seabream, the microcotylid monogenean *Sparicotyle chrysophrii* is the species causing the most important economic losses in this region although infections by *Furnestinia echeneis* have also been sporadically related to fish losses (Antonelli *et al.*, 2010a). Biological features and effects of *Cardicola aurata*, the blood fluke species infecting gilthead seabream, have been recently analysed and thus, this species is not included in the current PhD study.

This PhD study aims to improve the knowledge on the metazoan parasite diversity of the greater amberjack off western Mediterranean as well as to provide new taxonomical and biological data on the most hazardous parasitic species infecting greater amberjack and gilthead seabream in cultures, blood flukes and monogeneans, in order to prevent and manage infections.

To address these aims, the following objectives have been targeted:

I.- To review the metazoan parasite fauna of *S. dumerili* in the western Mediterranean Sea based on an exhaustive sampling in different localities of this region;

II.- To explore the seasonal occurrence of metazoan parasites infecting wild *S. dumerili* off Majorca focusing specially in seasonal patterns of those parasite species pathogenic in culture conditions;

III.- To determine the identity of the Mediterranean blood flukes (*Paradeontacylix* spp.) from *S. dumerili* (off Balearic Islands and Iberian Peninsula), as well as their phylogenetic relationship with the known species of the genus;

IV.- To analyse the seasonal population dynamics of *Z. seriolae* (Monogenea, Heteraxinidae) parasitising wild *S. dumerili* off Majorca and to provide comparative data for populations of juvenile fish from other localities in western Mediterranean;

V.- To study the oncomiracidial development, survival and swimming behaviour of the microcotylid monogenean *Sparicotyle chrysophrii* in order to improve disease control; and

VI.- To describe the morphological post-larval changes of *S. chrysophrii* during the development, to compare the growth of this parasite with that of other microcotylids and heteraxinids and to optimize the treatment design using the chronological data obtained.

The parasite fauna of *Seriola dumerili* in the western Mediterranean Sea is higher in species richness than previously thought. A total of 44921 metazoan parasites belonging to 26 species have been recovered from the four Mediterranean localities sampled: Majorca, Alicante, Corsica and Sardinia.Two of these species are new for science, 1 is a new host and locality record, 8 are new host records of Mediterranean parasites, 6 are new locality records and 9 of them had already been reported. According to their prevalence, a half of these species are accidental (13). The rest of them are mostly common (9) and a few of them are rare (2). The majority of species recovered in the in current study are heteroxenous and greater amberjack is the definitive host of most of them, except for the 2 widespread and unspecific brain metacercariae and the 2 metacestodes. Concerning specificity, half of the species are strict specialists or carangid fish specialists and, therefore, phylogenetic relatedness seems to be crucial for parasite-community composition in this region.

Recurrent seasonal infection patterns were detected in 5 out of the 9 common species described in *S. dumerili* off Majorca: *Bucephalus gorgon*, *Hemiurus communis*, *P. balearicus Stephanostomum ditrematis*, and *Z. seriolae*. Prevalences apparently increased from autumn to winter in *B. gorgon* and *H. communis*, and from spring to summer in *P. balearicus* (eggs), *S. ditrematis* and *Z. seriolae*. In the current study, most of the potentially harmful species were found in the gills and high infection levels of these species coincided in the same seasons, during spring and summer.

Among the parasites recovered from *S. dumerili* off the western Mediterranean Sea, blood flukes and monogeneans are the most harmful species in culture conditions as they have already been involved in outbreaks in this region. Metacercariae of heterophyids and strigeids, didymozoids,

acanthocolpids, caligids and gnathiids, belong to taxa including species causing pathologies although they have not been associated with *S. dumerili* outbreaks and they would only be considered as potential risks. The rest of species were considered harmless or less hazardous (other trematodes, cestodes, nematodes and other copepods) as they cause mild effects or are low prevalent and abundant in this region.

Two new species of *Paradeontacylix* from *S. dumerili* are described: *P. balearicus* from the Balearic Islands, and *P. ibericus* from the Iberian Peninsula. Each species can be distinguished morphologically from the other species of the genus by its size and shape. Phylogenetic trees show that *Paradeontacylix* spp. split into two well supported clades, separating (*P. ibericus*+*P. kampachi*) from (*P. godfreyi*+(*P. balearicus*+*P. grandispinus*)). *P. odhneri* sequence was not available for the analyses although in cladistic tree this species diverged earlier. The lowest sequence divergences are found between *P. grandispinus* and *P. balearicus* (0.2%, 2.5%, 6.3% for 28S, ITS2 and COI respectively) and, to a slightly smaller extent, between *P. kampachi* and *P. ibericus* (0.2%, 4.7%, 7%). *P. godfreyi* show the highest percentage of sequence divergence among all species, with values as high as 12.5% (ITS2) and 16.0% (COI). Clustering of Japanese and Mediterranean species together highlights their morphological and molecular similarity over the geographical separation. These findings suggest that both groups of blood fluke species or their ancestors existed before both fish populations (Japanese and Mediterranean) were separated.

Z. seriolae infection levels observed in fish off Majorca from 2005 to 2007 are the highest recorded in wild *S. dumerili* to date, and similar or higher than those reported in epizootics of this parasite in fish cultures. A recurrent and seasonal infection pattern is observed for this species in the populations of *S. dumerili* off Majorca, with substantially higher parasite loads during the warm season (April to June). This is consistent in spite of the significant trend of decrease in abundance from 2005 to 2007, and significantly correlated with the increases in mean and maximum seawater temperatures. However, geographical variation of the distribution and abundance of *Z. seriolae* on *S. dumerili* in the Mediterranean is also detected and requires further exploration.

Despite the high infection levels recorded in wild fish from Majorca, the Fulton's condition factor is not significantly affected and fish external appearance is healthy. However, three lines of evidence indicate a possible parasite-induced mortality of juvenile *S. dumerili*: (*i*) the significant negative association between abundance of *Z. seriolae* and fish length; (*ii*) the association between the increases in parasite abundance during the warm weather months and the sharp increases in monogenean aggregation levels and; (*iii*) the strong negative correlation between the levels of aggregation of *Z. seriolae* and mean fish total length which indicates that heavily infected individuals are rapidly removed from the population.

The chronology of oncomiracidial and post-larval development of *S. chrysophrii*, monogenean parasite of *S. aurata*, is established. Eggs hatch from 5 to 10 days after their deposition *in vitro* at 20ºC and hatching success is high (87,3 %). Most of the hatchings occur during darkness periods (> 75%), especially during the first and second nights after the first hatching. Nocturnal hatchings could be related to parasite-host coordination as gilthead seabreams group for foraging during dusk and rest during night. This behaviour increases the fish density and decreases the gill activity probably easing *S. chrysophrii* transmission. Oncomiracidial survival time does not usually exceed 24h although larvae can extraordinarily live more than 2 days (52 h) at 20ºC. After emerging, most of the oncomiracidia (93%) swim vertically, although after 12 h this percentage descends to 15%. Thus, the period to actively find a new host is short and does not usually exceed the 12 h.

New morphological data on the development of the first post-larval stages of *S. chrysophrii* are recorded. The most relevant are: (*i*) clamps do not replace lateral hooklets as, after the development of the first pairs of clamps, they are only laterally displaced and fall off with surrounding tissue; (*ii*) pharynx develops in a medial position and this could affect its functionality; (*iii*) the hook size does not change from the development of the first pairs of clamps to the hook loss and, therefore, post-larvae of different sizes attach with hooks of the same size; and (*iv*) the four granular dark spots associated to the digestive tract, observed in all the specimens at early developmental stages (without clamps), could be food waste but also yolk.

Three periods with different growth rates are distinguished in the development of *S. chrysophrii*. The first period (slow) finish with the terminal lappet loss, after the development of 5 pairs of clamps. The second period (fast) endures until specimen maturation. And the last one (slow) is observed from maturation to the development of the rest of clamps (from 36 to 70 pairs). Common timings were observed for the terminal lappet loss and the attainment of maturity for all the microcotylids and heteraxinids compared (i.e. *Microcotyle spinicirrus*, *Microcotyle donavini*, *Microcotyle gotoi*, *Microcotyle sebastis*, *Microcotyle hiatulae*, *Polylabroides multispinosus*, *Bivagina tai*, *Heteraxinoides xanthophilis*, *Heteraxine heterocerca* y *Z. seriolae*). The terminal lappet usually falls off when 10-20% of the clamps have been developed and attainment of maturity occurs when 60-70% of them have done it.

Pathological effects of *S. chrysophrii* change during its development: damages are firstly caused by the piercing action of hooks (the first 21 days after the infection) and thereafter (from the 7th day after the infection) by the compressive action of clamps, through a transition period of combined action. Gradual clamp addition during growth extends gill damage and decreases the

respiratory surface. Moreover, as parasites grow, blood ingestion increases and anaemia is more likely to occur.

In order to counteract *S. chrysophrii* infections in *S. aurata* two treatment applications, one against worms on gills and the other against oncomiracidia emerging from the surviving eggs (separated by 15 to 20 days), would allow for long periods without monogenean infection in fish cultures. Further treatments against worms would not be required before 50 days: 36 days (worm maturation) + 10 days (maximum egg incubation time) + 3 days (maximum oncomiracidial survival). Moreover, a periodic monitorization of the parasite development would also be quite effective for epizootics prevention.

Resumen

0.1. Introducción general

La acuicultura ha experimentado un importante aumento durante los últimos 50 años, resultado del incremento global de población y la creciente demanda de proteínas de alta calidad. En los últimos 40 años, la proporción de reservas pesqueras moderadamente explotadas ha descendido progresivamente hasta estabilizarse en el 15% y en la actualidad, la mayoría de los *stocks* pesqueros están sobreexplotados y (FAO, 2010). Más de la mitad de los productos acuáticos provienen de granjas, siendo los peces de agua dulce los animales más cultivados seguidos por los moluscos, los crustáceos, los peces diádromos y los peces marinos (FAO, 2010). España es el país con mayor producción acuícola de la Unión Europea seguido por Francia y el Reino Unido. Sin embargo, durante los últimos 10 años, el crecimiento del sector acuícola se ha ralentizado en estos tres países (FAO, 2010; APROMAR, 2011).

Las nuevas especies para la acuicultura se seleccionan de acuerdo con su potencial económico y biológico. Un alto valor de mercado, un rápido crecimiento, una fácil adaptación a las condiciones de cautividad o un ciclo de vida sencillo (y fácilmente controlable en cultivo) son características indispensables para la selección de una especie nueva. Posteriormente, el control de los patógenos potenciales que afectan a las especies seleccionadas se convierte también en un elemento crucial para el mantenimiento de la producción (Ogawa, 2005).

La presente tesis reúne diferentes estudios sobre la fauna parasitaria de dos especies de peces marinos de gran interés en acuicultura: la serviola, *Seriola dumerili*, y la dorada, *Sparus aurata*. Ambas especies presentan condiciones óptimas para su cultivo, aunque sólo la dorada se produce de manera intensiva en la región mediterránea. Habitualmente, la domesticación de nuevas especies (como la serviola en el Mediterráneo) requiere una primera etapa de investigación y cultivo experimental en la que el estudio de los patógenos que las afectan es de vital importancia. Posteriormente, cuando el cultivo está más establecido en una determinada región (como en el caso de la dorada) el objetivo principal del estudio es eliminar o paliar los efectos de las especies patógenas que provocan las mayores pérdidas económicas.

0.1.1. Especies hospedadoras

La serviola es una especie de distribución circumglobal que se encuentra en aguas tropicales o templadas entre 45°N-28°S y 180°W-180°E. A pesar de ser la especie del género *Seriola* predominante en el mar Mediterráneo (Smith-Vaniz, 1986), existen otras especies como *S. carpenteri*, *S. rivoliana* y *S. fasciata* que también han sido citadas esporádicamente en esta región (Deidun *et al.*, 2011; Froese y Pauly, 2011). Los individuos de esta especie cambian de hábitat

durante el crecimiento, de epibentónico a pelágico (Smith-Vaniz, 1986; Froese y Pauly, 2011). Los juveniles forman bancos y se distribuyen en aguas costeras o bien permanecen asociados a objetos flotantes en mar abierto (Massutí *et al.*, 1999; Riera *et al.*, 1999). Cuando los especímenes crecen, se vuelven migratorios, los grupos se reducen y algunos individuos viven en solitario (Dempster, 2005).

La dorada presenta una distribución principalmente mediterránea aunque también se ha citado en las islas Británicas, islas Canarias, el estrecho de Gibraltar y el mar Negro (Bauchot y Hureau, 1986). Se trata de una especie epibentónica litoral que habita fondos de posidonia y arenosos. Los individuos de esta especie son sedentarios y viven en solitario o formando pequeños grupos.

0.1.2. Enfermedades de *S. dumerili* y *S. aurata*

Las patologías que afectan a la serviola de modo más significativo en condiciones de cultivo son causadas por bacterias de los géneros *Vibrio* y *Streptococcus*, así como por la especie *Photobacterium damselae* (Alcaide *et al.*, 2000; Leong y Colorni, 2002). Por otra parte, el ciliado inespecífico *Cryptocaryon irritans* (Ciliophora), afecta principalmente a las serviolas cultivadas en tanques (Rigos *et al.*, 2001; Leong y Colorni, 2002; De la Gándara *et al.*, 2004). En el caso de la dorada, el patógeno bacteriano que provoca los efectos más graves en cultivo es también la bacteria *Photobacterium damselae* (Leong y Colorni, 2002). Asimismo, el protista dinoflagelado *Amyloodinium ocellatum* también ha sido relacionado con infecciones graves.

Los parásitos metazoos de la serviola y la dorada (ver revisión en Gibson *et al.*, 2005) han sido ampliamente estudiados debido a la importancia de estas especies para la acuicultura y a su consumo tradicional. Entre estos parásitos es importante destacar aquellos que causan mayores pérdidas en condiciones de cultivo: los mixozoos, los monogeneos y los trematodos aporocotílidos (ver detalle en los siguientes apartados). En el caso de la serviola se han citado: el mixozoo *Myxobolus* sp. (Myxozoa; Grau *et al.*, 1999; Ogawa, 2005); los monogeneos, *Neobenedenia melleni*, *Neobenedenia girellae*, *Benedenia seriolae* (Monopisthocotylea, Capsalidae) y *Allencoyla mcintoshi* y *Zeuxapta seriolae* (Polyopisthocotylea Heteraxinidae) (Montero *et al.*, 2001a; Hutson *et al.*, 2007b; Hirayama *et al.*, 2009); y los trematodos aporocotílidos *Paradeontacylix balearicus*, *P. ibericus*, *P. grandispinus* y *P. kampachi* (Crespo *et al.*, 1994; Ogawa y Fukudome, 1994; Repullés *et al.*, 2008).

En el caso de la dorada, el mixozoo *Enteromyxum leei* ha sido frecuentemente relacionado con graves mortandades en condiciones de cultivo (Paperna, 1982; Diamant, 1992; Padrós *et al.*, 2001b; Ogawa, 2005). Por otra parte, entre los helmintos, *Sparicotyle chrysophrii* (Monogenea, Microcotylidae) es la especie que causa las mayores pérdidas materiales y económicas en la

actualidad (Sanz, 1992; Sitjà-Bobadilla *et al.*, 2009). Esta especie está ampliamente distribuida en el Mediterráneo, donde provoca epizootias recurrentes en condiciones de cultivo (ver capítulos 7 y 8). Recientemente, la especie *Furnestinia echeneis* (Monogenea, Diplectanidae), también ha sido relacionada con mortandades en granjas (Antonelli *et al.*, 2010a). En cambio, el trematodo aporocotílido que infecta la dorada, *Cardicola aurata*, no ha sido asociado con infecciones graves (Padrós *et al.*, 2001a; Holzer *et al.*, 2008).

0.1.3. Parásitos de interés

En esta tesis se desarrollan diferentes estudios sobre las especies de monogeneos y aporocotílidos que parasitan a la serviola y la dorada en el Mediterráneo, exceptuando a la especie *C. aurata* cuya morfología y efectos han sido estudiados previamente (Padrós *et al.*, 2001; Holzer *et al.*, 2008). Seguidamente, se describen algunas de las características biológicas de estos grupos de parásitos.

Los monogeneos poliopistocotíleos son parásitos externos que habitan en las branquias y la piel de sus hospedadores, habitualmente peces, tanto marinos como de agua dulce (Hayward, 2005; Whittington y Chisholm, 2008). En general, estos parásitos presentan ciclos de vida directos y especificidad estricta (Hayward, 2005). Los monogeneos poliopistocotíleos que infectan a *S. dumerili* en el Mediterráneo son *Allencotyla mcintoshi* y *Zeuxapta seriolae*; mientras que los que infectan *Sparus aurata* son *Atrispinum salpae*, *Bivagina pagrosomi* y *Sparicotyle chrysophrii*. Las especies estudiadas en esta tesis, *Z. seriolae* y *S. chrysophrii*, pertenecen a la superfamilia Microcotyloidea.

La familia Aporocotylidae está constituida por trematodos que parasitan el sistema circulatorio de los peces (Smith, 2002). Los aporocotílidos marinos son especialistas estrictos de hospedador y más del 70% de ellos parasita una sola especie de pez (Smith, 2002). Al igual que el resto de trematodos, presentan un ciclo vital heteroxeno, con un estado reproductivo adulto que infecta un vertebrado y un estado larvario de reproducción asexual que infecta un invertebrado, generalmente un molusco o un poliqueto (Smith, 1972; 1997a). El género *Paradeontacylix* incluye un total de 7 especies: *P balearicus*, *P. ibericus*, *P. godfreyi*, *P. kampachi*, *P. grandispinus*, *P. sanguinicoloides* y *P. odhneri*. Entre ellas, sólo las dos primeras han sido registradas en aguas mediterráneas.

0.1.4. Este estudio

El presente estudio intenta proporcionar información útil para el manejo de la serviola y la dorada en condiciones de cultivo dando respuesta a las siguientes cuestiones generales: (*i*) ¿Qué

especies constituyen la comunidad parasitaria de la serviola en el Mediterráneo occidental?; (*ii*) ¿Existen variaciones temporales en la comunidad parasitaria de la serviola en Mallorca? (*iii*) ¿Cuáles son las especies potencialmente patógenas para la serviola? (*iv*) ¿Cuál es la cronología del desarrollo del monogeneo *Sparicotyle chrysophrii* parásito de la dorada? (*v*) ¿Cómo podríamos optimizar el diseño de tratamientos contra *S. chrysophrii* utilizando los conocimientos adquiridos sobre su desarrollo?

0.2. Justificación y objetivos

La presente tesis tiene dos propósitos generales: (*i*) mejorar el conocimiento sobre la diversidad parasitaria de *Seriola dumerili* en el Mediterráneo occidental y (*ii*) proporcionar nuevos datos taxonómicos y biológicos sobre las especies parásitas más peligrosas en condiciones de cautividad, que infectan a la serviola y la dorada, aporocotílidos y monogeneos, con el fin de facilitar su control.

Los objetivos específicos a desarrollar son:

I.- Revisar la parasitofauna de metazoos de *S. dumerili* en el Mediterráneo occidental mediante un muestreo exhaustivo realizado en diferentes localidades de esta región.

II.- Explorar la estacionalidad de los parásitos metazoos que infectan a *S. dumerili* en Mallorca, dedicando especial atención a aquellas especies especialmente patogénicas en condiciones de cultivo.

III.- Determinar la identidad de los parásitos sanguíneos (*Paradeontacylix* spp.) de *S. dumerili* en el Mediterráneo, así como su relación filogenética con el resto de especies del mismo género.

IV.- Analizar la dinámica estacional del monogeneo heteraxínido *Zeuxapta seriolae*, parásito de *S. dumerili* en Mallorca, y obtener muestras comparativas de otras localidades.

V.- Estudiar el desarrollo, la supervivencia y el comportamiento natatorio del oncomiracidio de *Sparicotyle chrysophrii* (Monogenea, Microcotylidae) con el fin de mejorar las estrategias de control sobre este parásito.

VI.- Describir los cambios morfológicos de las post-larvas de *S. chrysophrii* durante el desarrollo, comparar el crecimiento de esta especie con el de otras especies de microcotílidos y heteraxínidos y optimizar el diseño de tratamientos utilizando los datos cronológicos obtenidos.

0.3. Materiales y métodos generales

En este resumen los materiales y métodos se describen separadamente en cada una de las secciones correspondientes. A continuación se resumen los estudios realizados estructurados por capítulos.

0.4. Parasitofauna de *Seriola dumerili*

0.4.1. Introducción

El primer listado de parásitos de *S. dumerili* fue incluido en una guía práctica para la pesca deportiva en el océano Atlántico y Puerto Rico compilada por Williams y Bunkley-Williams (1996). Estos autores citaron un total de 47 especies parásitas, la mayor parte de ellas procedentes del Atlántico. La primera revisión sobre los parásitos de la serviola en el mediterráneo occidental fue publicada por Grau *et al.* (1999) e incluyó 14 especies obtenidas de Mallorca, Tarragona y el golfo de Valencia. Posteriormente, Montero (2001) y Montero *et al.* (2001b) describieron 18 especies parásitas en las serviolas de Murcia. Aunque las serviolas son peces migratorios y las localidades muestreadas en ambos estudios son relativamente cercanas, se constataron importantes diferencias en la fauna parasitaria encontrada en cada una de ellas.

El objetivo de este estudio fue describir y revisar la parasitofauna de la serviola en el Mediterráneo occidental así como explorar las diferencias entre localidades. Adicionalmente, el muestreo periódico sirvió para obtener datos sobre la dinámica estacional de los parásitos de serviola en Mallorca.

0.4.2. Materiales y métodos

Para el desarrollo del estudio se analizaron un total de 225 ejemplares de *S. dumerili* obtenidos entre 2005 y 2007 en diferentes localidades del Mediterráneo occidental. La muestra principal, que incluía un total de 165 peces juveniles procedentes de Mallorca, fue obtenida mediante 11 muestreos bimensuales de 15 especímenes cada uno. También se obtuvieron 4 muestras comparativas, de 15 peces cada una, procedentes de otras 3 localidades: 2 de Alicante (España), 1 de Córcega (Francia) y 1 de Cerdeña (Italia). Todos los peces fueron medidos y pesados y 4 peces de cada submuestra se analizaron en fresco mientras que el resto se congelaron para su análisis posterior.

Primeramente, se examinó la superficie externa de los peces incluyendo la piel, las aletas la cavidad oral y la cavidad opercular, con el fin de detectar ectoparásitos. Posteriormente, se

analizaron separadamente las branquias, los diferentes órganos del sistema digestivo y excretor y el sistema circulatorio incluyendo corazón, vena caudal, seno venoso y cono arterial así como las arterias branquiales. Por último, también se analizaron el cerebro y los ojos así como la musculatura circundante a las cinturas.

Para el análisis morfológico se fijó una muestra representativa de especímenes adultos en alcohol caliente al 70%. Posteriormente, los ejemplares se tiñeron con acetocarmín férrico (Georgiev *et al*., 1986), se deshidrataron y se montaron en preparaciones permanentes con bálsamo de Canadá. La mayor parte de los nematodos se examinó en fresco mientras que los especímenes más grandes se transparentaron en lactofenol y se montaron en preparaciones temporales. Para obtener los datos morfométricos se midieron diez especímenes adultos de cada una de las especies con la ayuda de un tubo de dibujo. Se calculó la prevalencia, abundancia e intensidad parasitaria para cada muestra y las especies se clasificaron en tres grupos según su prevalencia media: especies comunes (>30%), especies infrecuentes (entre 10 y 30%) y especies accidentales (<10%).

0.4.3. Resultados

El muestreo realizado en las cuatro localidades mediterráneas permitió obtener un total de 44921 parásitos metazoos pertenecientes a 26 especies distintas. Cinco de estas especies eran ectoparásitos de dos filos diferentes (un platelminto y cuatro artrópodos) y 21 eran endoparásitos de dos filos diferentes (17 platelmintos y 4 nematodos). También se describió el micropredador *Skogsbergia costai*, encontrado en dos de los muestreos rutinarios realizados en Mallorca y Alicante (ver tablas 4.1 y 4.2 en el capítulo correspondiente).

Entre los parásitos procedentes de Mallorca se encontraron 9 especies comunes (*Bucephalus gorgon*, *Caligus aesopus*, *Gnathia vorax*, *Hemiurus communis*, *Neometanematobothrioides periorbitalis*, *Paradeontacylix balearicus*, *Stephanostomum ditrematis*, *Tormopsolus orientalis* y *Zeuxapta seriolae*), 2 especies infrecuentes (*Prosorhynchus facilis* y *Hysterothylacium seriolae*) y 13 especies accidentales. (*Aphanurus* sp., *Caligus* sp., *Camallanus* sp., *Capillaria* sp., *Cardiocephaloides* sp., *Cucullanus* sp., *Floriceps saccatus* sp., *Galactosomum* sp., *Stephanostomum euzeti*, *Stephanostomum filiforme*, *Stephanostomum petimba*, *Parabrachiella seriolae* y la larva tetrafilídea).

En cuanto a la especificidad (tabla 4.1), del total de especies descritas diez eran generalistas, 1 era especialista de la familia Carangidae, 3 eran especialistas del género *Seriola* y 5 eran especialistas de *Seriola dumerili*. Las siete especies restantes no pudieron ser clasificadas a nivel de especie y su especificidad no pudo ser determinada.

0.4.4. Discusión

Parasitofauna de *Seriola dumerili*

Diecisiete nuevos registros fueron añadidos al conjunto de especies previamente citadas en *S. dumerili* del Mediterráneo occidental. Entre ellas se describieron 2 nuevas especies para la ciencia, 1 primer hallazgo en *S. dumerili* del Mediterráneo, 8 primeros hallazgos en *S. dumerili* de especies ya descritas en el Mediterráneo y 6 primeros hallazgos en el Mediterráneo. Este incremento en el número de especies registradas se debió principalmente al aumento del tamaño muestral, la creación de nuevas especies y el análisis de algunos órganos habitualmente ignorados.

Las diferencias más destacables entre los estudios realizados hasta la fecha en la región mediterránea se encontraron entre los representantes del phylum Nematoda. En el presente estudio, la riqueza de nematodos fue relativamente alta (N= 4) mientras que en los anteriores solamente se había citado una especie de nematodo, *Philometra* sp., aquí ausente. Otras diferencias significativas fueron la ausencia de *Wedlia bipartita*, previamente citada en Mallorca (Grau *et al.*, 1999), y *Tergestia laticollis* previamente encontrada en Murcia (Montero *et al.*, 2001b). Por último, cabe destacar la presencia de *Gnathia vorax* y *Hemiurus communis* en todas las localidades estudiadas y en los estudios previos realizados en Mallorca (Grau *et al.*, 1999) que contrasta con su ausencia en los estudios previos realizados en Murcia (Montero *et al.*, 2001b). Gran parte las diferencias podrían explicarse por la accidentalidad de algunas de estas especies. Sin embargo, diferencias más consistentes como la presencia de *Hysterothylacium seriolae*, frecuentemente encontrado en el presente estudio, y su ausencia en las publicaciones anteriores, podría estar relacionada con los factores ambientales, la disponibilidad de presas, etc. Adicionalmente, en el caso de *Gnathia vorax* y *Hemiurus communis* el cultivo de los peces también podría ser el causante de la pérdida de estos parásitos.

La mayoría de las especies registradas (N= 21) presenta un ciclo de vida heteroxeno en el que la serviola seria el hospedador definitivo. Por otra parte, este pez también seria hospedador intermediario de 4 especies accidentales: las 2 especies de trematodos en estado de metacercaria (*Cardiocephaloides* sp. y *Galactosomum* sp.) y los 2 metacestodos (*Floriceps saccatus* y la larva tetrafilídea). Los hospedadores definitivos de las metacercarias encontradas son aves, principalmente gaviotas (Pearson y Prévot, 1971; Gibson *et al.*, 2005) mientras que los hospedadores definitivos de los metacestodos son peces elasmobranquios (Bates, 1990).

Aproximadamente la mitad de las especies identificadas a nivel específico eran especialistas estrictas o especialistas de carángidos. Por tanto, las relaciones filogenéticas parecían tener un papel bastante importante en la composición de la comunidad parásita en esta región. Entre estas especies

destacarían las del género *Paradeontacylix* puesto que todas, a excepción de *P. odhneri*, son especialistas de diferentes especies del género *Seriola* (Repullés-Albelda *et al.*, 2008).

Dinámica estacional

El muestreo periódico reveló que 5 de las 9 especies comunes descritas en *S. dumerili* de Mallorca mostraban aparentes patrones estacionales de intensidad o prevalencia: *B. gorgon*, *H. communis*, *P. balearicus*, *S. ditrematis* y *Z. seriolae*. Las prevalencias e intensidades de *B. gorgon* y *H. communis* aumentaban de otoño a invierno mientras que las de *S. ditrematis, P. balearicus* (huevos), y *Z. seriolae* aumentaban de primavera a verano. De acuerdo con su importancia para la acuicultura, la dinámica estacional del monogeneo *Z. seriolae* se analizó separadamente en el capítulo 6.

En referencia a los trematodos sanguíneos, la prevalencia de los huevos de *P. balearicus* aumentaba recurrentemente de febrero a junio coincidiendo con el patrón descrito previamente para *Paradeontacylix* sp. de Mallorca (Grau, 1992) y en líneas generales con las dinámicas de infección de *P. ibericus* (Montero *et al.*, 2009), *P. kampachi* y *P. grandispinus* (Ogawa *et al.*, 1993). Por tanto, la mayor parte de los huevos eclosionarían al inicio del verano mientras que los adultos madurarían y pondrían sus huevos durante los meses anteriores.

En cuanto a los trematodos del sistema digestivo, *H. communis* mostró un patrón estacional similar al descrito en estudios previos (Meskal, 1967; in Gibson & Bray, 1986; Gibson, 1981) en el que el número de especímenes aumentaría de otoño a invierno y disminuiría gradualmente hasta la primavera. La dinámica estacional del resto de trematodos del digestivo registrados en el presente trabajo (bucefálidos y acantocólpidos) ha sido poco estudiada (Wolfgang 1955; Chubb, 1979; Taskinen *et al.*, 1991; Holmes & Bartoli, 1993). Sin embargo, existen estudios previos que relacionan el aumento estacional en el número de metacercarias en los hospedadores intermediarios con el aumento de adultos en los meses posteriores (Taskinen y Valtonen, 1995; Quinteiro *et al.* 1993; Wang *et al.*, 2001). Por tanto, un mayor conocimiento de las fluctuaciones estacionales de estos parásitos en sus hospedadores intermediarios podría contribuir a entender mejor las dinámicas de los adultos en hospedadores definitivos.

En el caso de los didimozoidos, el desconocimiento es aún mayor, sus ciclos de vida se han estudiado muy poco y sus hospedadores intermediaros son desconocidos en la mayoría de los casos (Pozdnyakov & Gibson, 2008). Hasta la fecha no se ha descrito ningún tipo de dinámica estacional en adultos pertenecientes a la subfamilia Nemathobothriinae y en el presente estudio, los especímenes de *N. periorbitalis* fueron observados durante todo el año sin aparente estacionalidad.

Los estudios previos sobre *Caligus* spp. muestran un aumento de la prevalencia y la abundancia de estos parásitos en primavera, asociado al aumento de la temperatura que causa el

incremento en la velocidad de desarrollo (Boxshall, 1974; Hogans y Trudeau, 1989). En el presente estudio, *C. aesopus* mostró prevalencias y abundancias similares en todos los muestreos. Sin embargo se observó una aparente estacionalidad en el período de reproducción puesto que la mayor parte de hembras grávidas fue encontrada de febrero a abril. La reproducción de otras especies de *Caligus* en el noroeste del océano Atlántico (i.e. *C. curtus* y *C. elongatus*) ocurre al final de la primavera o el inicio del verano (Hogans & Trudeau, 1989). Sin embargo, en el Mediterráneo parece adelantarse ligeramente. Este hecho se podría explicar por las diferencias en el periodo anual de incremento de temperaturas entre el mar Mediterráneo y el océano Atlántico.

Los ciclos de vida y las dinámicas poblacionales de los estados larvarios de los gnátidos han sido poco estudiados en el medio salvaje (Naylor, 1972; Fernández *et al.*, 1989; Smith *et al.*, 2003; Tanaka, 2007). Smit *et al.* (2003) definió diferentes tipos de ciclos vitales para las distintas especies de gnátidos: un ciclo de vida anual, uno bianual y otro con varias generaciones al año. Estos autores consideraron que el ciclo vital de *G. africana* presentaba varias generaciones al año, de acuerdo con el patrón de incremento y descenso recurrente de la abundancia y la prevalencia durante el periodo de estudio (Smit *et al.*, 2003). Posteriormente, Hadfield *et al.* (2009) describió este mismo patrón para *G. pilosus*. *G. vorax* también mostró este tipo de oscilaciones y por tanto, podría incluirse en el mismo grupo. Sin embargo, existen otras razones que podrían explicar este tipo de dinámica y sería necesario un estudio específico, en el que las diferentes etapas larvarias se considerasen separadamente, para poder obtener unos resultados más concluyentes.

Efectos potenciales de las diferentes especies parásitas en cultivo

Hasta la fecha, *Seriola lalandi* es la única especie de *Seriola* sobre la que se ha analizado su parasitofauna desde el punto de vista de los riesgos que representan las diferentes especies para el cultivo de su hospedador. De acuerdo con los efectos patológicos descritos en estudios previos, las especies del presente estudio se clasificaron en tres categorías: (*i*) especies dañinas para la serviola en el Mediterráneo que causan infecciones severas especialmente en cultivo, monogeneos y aporocotílidos; (*ii*) especies potencialmente peligrosas puesto que son taxonómicamente cercanas a otras con conocidos efectos patológicos, didimozoidos, acantocólpidos, trematodos en estado de metacercaria, calígidos e isópodos; y (*iii*) especies inofensivas para la serviola en el Mediterráneo occidental por sus efectos leves (bucefálidos y hemiúridos) o su baja prevalencia y/o abundancia (cestodos y nematodos).

En el presente estudio, tanto el monogeneo *Z. seriolae* (Ogawa y Yokoyama, 1998; Grau *et al.*, 2003; Montero *et al.*, 2004; Diggles y Hutson, 2005; Lia *et al.*, 2007; ver referencias en capítulo 6) como los aporocotílidos sanguíneos del género *Paradeontacylix* (Crespo *et al.* 1992; Crespo *et al.*,

1994; Ogawa y Fukudome, 1994) se han relacionado con epizootias, principalmente en condiciones de cultivo. Los efectos de los monogeneos están principalmente relacionados con la anemia o la pérdida de superficie branquial respiratoria (Whittington y Chisholm, 2008) mientras que los efectos de los trematodos sanguíneos están relacionados con la rotura del epitelio branquial por la eclosión de los huevos y la salida de los miracidios. Entre las dos especies de parásitos sanguíneos descritas en el Mediterráneo, *P. balearicus* es la única relacionada con mortandades (Crespo *et al.* 1992; Crespo *et al.*, 1994) y por tanto, parece ser la más patogénica. La mayor distribución de sus especímenes y huevos podría ser una de las causas que explicarían su mayor virulencia.

Los estudios sobre los efectos provocados por los acantocólpidos son escasos puesto que son especies poco dañinas en su fase adulta (Hutson et al., 2007b). Sin embargo, Grau (1992) mostró, mediante un estudio histológico, la destrucción de células epiteliales y hemorragias en los ciegos intestinales y el intestino de *S. dumerili* provocadas por diferentes estos parásitos. A pesar de la gravedad de las lesiones, no se observó ningún episodio de mortandad asociado. Por tanto, parece que los acantocólpidos no suponen una grave amenaza para la población de peces de Mallorca en el medio salvaje y tampoco en cultivo puesto que su captación se produce a través de la dieta y ésta podría modificarse fácilmente en cautividad.

En cuanto a los didimozoidos, sus efectos dependen mayoritariamente de su ubicación en el tejido del hospedador. En el presente trabajo no se observó ningún tipo de patología severa asociada a *Neometanematobothrioides periorbitalis.* Asimismo, se observaron efectos leves durante el estudio sobre la patogeneidad de una especie parásita de *Scomber australicus* perteneciente a la misma subfamilia que *N. periorbitalis* y con distribución similar (Perera, 1992). Por tanto, es bastante probable que *N. periorbitalis* no represente un alto riesgo para la salud de *S. dumerili* en el mar Balear, aunque esté presente en elevada intensidad y prevalencia.

Finalmente, los efectos de los trematodos en estado de metacercaria merecen una mención especial puesto que por su localización, afectan al sistema nervioso de los peces y pueden cambiar su comportamiento (Osset *et al.* 2005). Sin embargo, su baja incidencia en el presente estudio y la ausencia de citas en *Seriola* indican que estas especies no representan un riesgo importante para *Seriola dumerili* en el Mediterráneo occidental.

En cuanto a los calígidos, la especie *C. spinosus* (considerada sinónima de *C. aesopus* por muchos autores) ha sido relacionada con la anemia de *S. quinqueradiata* en cultivo (Egusa, 1983). Sin embargo, los efectos de esta especie sobre *S. lalandi* se consideraron leves (Hutson *et al.*, 2007b). Esta diferencia podría explicarse por los distintos niveles de infección registrados en ambas localidades. En el presente estudio, no se observaron efectos relevantes relacionados con la presencia de *C. aesopus* y su incidencia fue baja. Por tanto, el riesgo potencial que representa es

aparentemente leve. Sin embargo, al tratarse de un parásito monoxeno y de fácil transmisión, su prevalencia debería ser controlada periódicamente en condiciones de cultivo.

El caso de *Gnathia vorax* (isópodo) es similar al de *C. aesopus* con el agravante de que se trata de una especie poco específica que está ampliamente distribuida en el Mediterráneo (Naylor, 1972; Cals, 1978; Holdish & Harrison, 1980; Grau *et al.*, 1999). Se sabe que esta especie provoca hemorragias y anemia en condiciones de cautividad (Marino *et al.*, 2004) aunque no se ha sido relacionada con efectos patológicos graves en el medio salvaje. Por tanto, esta especie no representa un grave riesgo para la serviola aunque su presencia debería ser controlada en condiciones de cultivo.

0.5. Especiación de las especies de *Paradeontacylix* (Aporocotylidae) de *Seriola dumerili.* dos especies nuevas del género *Paradeontacylix* en el Mediterráneo

0.5.1. Introducción

La serviola (*S. dumerili*) ha sido cultivada experimentalmente en diferentes países alrededor del Mediterráneo. Sin embargo, su cultivo intensivo está parcialmente limitado por la existencia de parásitos patógenos que provocan graves mortandades entre los juveniles. Entre éstos destacan los trematodos sanguíneos del género *Paradeontacylix* (Trematoda Aporocotylidae) que se han relacionado con importantes pérdidas registradas tanto en Japón (océano Pacífico) como en Mallorca (mar Mediterráneo) (Crespo *et al.*, 1994; Ogawa y Fukudome, 1994). En el Mediterráneo, los episodios de aporocotilidosis se han registrado en dos localidades, Murcia y Mallorca, aunque solamente se han producido mortandades significativas en esta última (Crespo *et al.*, 1994; Montero, 2001). Las especies encontradas en el Mediterráneo presentan importantes similitudes morfológicas con las descritas en Japón. De hecho, la especie de Murcia ha sido previamente identificada como *P. kampachi* (García y Díaz, 1995; Montero *et al.*, 2003) mientras que la de Mallorca se ha sido descrita como "especie parecida a" *P. grandispinus* (Montero *et al.*, 1999). Determinar la identidad específica de las especies mediterráneas podría contribuir a comprender mejor su distinta virulencia.

0.5.2. Materiales y métodos

En el presente estudio se analizaron un total de 90 peces. Setenta y cinco de ellos fueron obtenidos mediante 5 muestreos bimensuales en Mallorca (de abril del 2005 a abril del 2006) mientras que los otros 15 se obtuvieron mediante un único muestreo en Alicante (febrero del

2006). El análisis de los peces así como la fijación y el montaje de los parásitos se realizaron tal y como se describe en el protocolo de la sección 0.4.2 del presente resumen.

Con el fin de comparar los individuos obtenidos con otras especies congenéricas se adquirieron 10 ejemplares de *P. grandispinus* y 4 de *P. kampachi* procedentes de colecciones privadas y 10 especímenes de *Paradeontacylix* sp. procedentes de Murcia. La información morfométrica, resumida en 14 caracteres, se analizó mediante análisis discriminante y análisis de componentes principales utilizando SPSS® v. 12.0 (SPSS, Inc., Norusis, 2002).

El cladograma incluyó todas las especies de *Paradeontacylix* descritas hasta el momento y se realizó mediante el criterio de máxima parsimonia. *Aporocotyle spinosicanalis* Williams, 1958 fue utilizado como grupo externo. Se analizaron un total de 11 caracteres morfológicos (8 binarios y 3 multiestado). Para el análisis filogenético molecular, se extrajo el ADN de 2 individuos de la península ibérica y 2 de las islas Baleares. Por otra parte, también se obtuvo el ADN de 2 individuos de *P. grandispinus* y de 1 individuo de *P. kampachi*, procedentes de *S. dumerili* de Japón, y 2 individuos de *P. godfreyi* procedentes de *S. lalandi* de Australia. Posteriormente, se amplificaron las secuencias de las regiones 28S e ITS2 del gen ribosomal así como la secuencia del COI del gen mitocondrial. Finalmente, los alineamientos resultantes se combinaron en una base de datos. La estimación del árbol se realizó mediante análisis bayesiano y siguiendo el criterio de optimización de máxima parsimonia. Se utilizaron dos programas informáticos: MrBayes v 3.0 y PAUP v. 4.0b10 (Swofford, 2002).

0.5.3. Resultados

Los especímenes de *Paradeontacylix* sp. procedentes de las islas Baleares y la península ibérica eran marcadamente diferentes entre ellos pero muy parecidos a *P. grandispinus* (*Pg*) y *P. kampachi* (*Pk*) de Japón, respectivamente. Sin embargo, presentaban ciertas peculiaridades morfológicas y moleculares que nos permitieron proponer dos nuevas especies: *P. balearicus* (*Pb*) y *P. ibericus* (*Pi*).

P. balearicus se diferencia del resto de especies del género por la siguiente combinación de caracteres: longitud corta (es la especie más corta), número de testículos inferior a 26, distribución de testículos en una sola fila, ovario con forma de corazón, útero post-ovárico, poro genital femenino ligeramente dextral, espinas tegumentarias de 8-12 por fila y espinas posteriores largas. La especie más parecida a *P. balearicus* es *P. grandispinus*, previamente descrita en *S. dumerili* de Japón, aunque ambas difieren en su tamaño corporal (midiendo una la mitad que la otra). También se observan diferencias en el tamaño de las espinas tegumentarias posteriores que son relativamente más largas en *P. balearicus*.

P. ibericus se distingue del resto de especies del género por la combinación de: longitud total (entre 2480 a 5700), testículos distribuidos en dos filas, ovarios en forma de riñón, útero post-ovárico, espinas tegumentarias marginales distribuidas en 678 filas (577-746) y presencia de entre 8 y 18 espinas tegumentarias posteriores cortas. La morfología de *P. ibericus* es muy parecida a la de *P. kampachi*, descrito en *S. dumerili* de Japón. *P. ibericus* puede distinguirse de *P. kampachi* por su menor longitud corporal (2480 a 5700 en *P. ibericus* y de 4680 a 8100 en *P. kampachi*) y la mayor longitud relativa de sus espinas tegumentarias posteriores. Otras características diferenciales de la nueva especie son su ovario estrecho y el mayor número de filas de espinas tegumentarias.

Las 4 especies analizadas (*Pk*, *Pg*, *Pi* y *Pb*) se separaron claramente en el análisis discriminante y en el análisis de componentes principales. Sin embargo, el análisis de componentes principales comunes mostró resultados menos concluyentes y un mayor solapamiento entre especies. Las diferencias morfológicas entre *P. grandispinus* y *P. balearicus* no resultaron estadísticamente significativas.

El único árbol filogenético, resultante del análisis cladístico mostró dos clados separados (*Pk* + *Pi*) y (((*Pg/Pb*) + *P. sanguinicoloides*) + *P. godfreyi*). *P. odhneri* se situó en posición basal, en una rama separada. El nivel de soporte de la rama (*Pk* + *Pi*) fue bastante alto y por tanto estas dos especies parecían estar claramente relacionadas. Los niveles de soporte de la rama que relacionaba (*Pg/Pb*) y *P. sanguinicoloides* fueron cercanos al 70% por lo que aparentemente estas tres especies estarían relacionadas. El análisis filogenético basado en datos moleculares mostró dos clados claramente separados y con un buen soporte: (*P. ibericus* + *P. kampachi*) y (*P. godfreyi* + (*P. balearicus* + *P. grandispinus*)). El porcentaje de diferencias entre secuencias aumentaba del 28S al ITS2 y al COI. De este modo, se observó una variación del 0,9% entre las especies de *Paradeontacylix* para el 28S y una mejor discriminación en el ITS (2,5-12,5%) y el COI (6,3–16,0%). El menor porcentage de diferencias se encontró entre *P. grandispinus* y *P. balearicus* mientras que el mayor se observó entre *P. godfreyi* y el resto de especies, con valores máximos de 12,5% (ITS2) y 16,0% (COI).

0.5.4. Discusión

Los niveles de variación interespecífica de las secuencias de trematodos son típicamente ≤ 1% para el ITS y superan el 2% para el mtDNA (ver revisión de Blouin (2002)). Por tanto, molecularmente las especies de *Paradeontacylix* del Mediterráneo serían consideradas diferentes de las japonesas. Sin embargo, se observó una clara identidad de secuencias entre *P. ibericus/P. kampachi* y *P. balearicus/P. grandispinus* en todas las regiones analizadas. Nolan y Cribb (2006a y 2006b) consideraron que las divergencias del 0,3% (una sola base) eran suficientes para la separación entre especies de aporocotílidos de los géneros *Phthinomita* y *Cardicola*. De acuerdo con

los trabajos realizados por estos autores, se concluyó que las diferencias del 4,7% entre *P. ibericus/P. kampachi* y del 2,5% entre *P. balearicus/P. grandispinus* justificarían la creación de nuevas especies para los especímenes Mediterráneos.

Las islas Baleares están separadas de la península ibérica por una distancia de aproximadamente 300 km con una profundidad máxima de 1900 m. El presente estudio reveló que, a pesar de parasitar el mismo hospedador, *P. balearicus* y *P. ibericus*, permanecen aisladas en el Mediterráneo. Sin embargo, *P. grandispinus* y *P. kampachi* se encuentran en infecciones mixtas en Japón (Ogawa y Egusa, 1986). Las especies de *Paradeontacylix* del Mediterráneo parecen infectar principalmente a peces juveniles, que viven en zonas costeras y son bastante sedentarios (Froese & Pauly, 2011). Los resultados del presente estudio sugirieron que las poblaciones de juveniles de *S. dumerili* permanecían aisladas en las dos localidades a pesar de la escasa distancia geográfica entre ellas.

La información filogenética obtenida permitió la diferenciación de dos clados separados de *P. odhneri*: uno que unía *P. kampachi* y *P. ibericus* y otro que unía *P. grandispinus*, *P. balearicus*, *P. sanguinicoloides* y *P. godfreyi*. *P. sanguinicoloides*, *P. godfreyi*, *P. grandispinus* y *P. balearicus* muestran diversos caracteres que los diferencian del grupo formado por *P. kampachi/P ibericus*. Curiosamente, *P. sanguinicoloides* y *P. godfreyi* parasitan *S. lalandi*, mientras que *P. grandispinus* y *P. balearicus* parasitan *S. dumerili*. Es posible que la especiación de los ancestros de estos dos últimos grupos de parásitos ocurriese después de la especiación de sus hospedadores (*S. lalandi* y *S. dumerili)*.

En relación a las especies de *Paradeontacylix* de *S. dumerili*, los árboles filogenéticos mostraron la asociación de *P. kampachi/P. ibericus* en un clado diferente al de *P. grandispinus/P. balearicus*. Una explicación posible para esta paridad morfológica y molecular entre las especies japonesas y los mediterráneas sería que los ancestros de *P. kampachi/P. ibericus* y *P. grandispinus/P. balearicus* hubiesen existido antes de la separación de sus dos poblaciones de hospedadores.

0.6. Dinámica estacional de la población de *Zeuxapta seriolae* parásita de *Seriola dumerili* (Carangidae) en el mediterráneo occidental

0.6.1. Introducción

Uno de los principales grupos causantes de infecciones masivas en *Seriola* spp. de cultivo son los monogeneos heteraxínidos. Su presencia se ha registrado en cultivos de diferentes localidades: Australia (Ernst *et al.*, 2002; Tubbs *et al.*, 2005; Hutson *et al.*, 2007b), Nueva Zelanda (Diggles y Hutson, 2005), Japón (Ogawa, 1996; Ogawa y Yokoyama, 1998; Hutson, 2007b), Italia

(Montero *et al.*, 2004), Malta (Nielsen *et al.*, 2003) y España (Montero *et al.*, 2001a,b; 2004; Grau *et al.*, 2003). Estos parásitos también se han detectado en peces salvajes de Nueva Zelanda (Sharp *et al.*, 2003), Australia (Rohde, 1978, 1981; Hutson *et al.*, 2007a) y el Mediterráneo (en Turquía, Genç *et al.*, 2007; e Italia, Lia *et al.*, 2007). Sin embargo, no existen estudios completos sobre la dinámica poblacional de los monogeneos heteraxínidos que infectan a las diferentes especies de *Seriola* y la información disponible a este respecto es incompleta o está basada en datos puntuales. El conocimiento de la dinámica poblacional y los parámetros de infección de estos monogeneos en el medio salvaje podría contribuir a disminuir los riesgos de epizootias en cautividad.

0.6.2. Materiales y métodos

El muestreo del presente estudio coincide con el descrito en el capítulo 4 con la excepción de que en este caso se obtuvieron dos muestras adicionales, de 10 peces cada una, para conseguir datos sobre la abundancia parasitaria en hospedadores de tamaño mayor ("peces grandes": 43.5 - 48.5 TL) y menor ("peces pequeños": 26.5 - 29.5 TL) que los del estudio general. Por tanto, el total de peces analizados fue de 245. Los ejemplares fueron medidos y pesados y se calculó el factor de condición de Fulton para cada pez. Posteriormente, se extrajeron las branquias, se separaron los 4 arcos branquiales y se examinaron con la ayuda de la lupa. La prevalencia, la abundancia y la intensidad fueron calculadas según Bush *et al.* (1997). Por último, los datos de abundancia parasitaria, longitud total del pez y factor de condición se transformaron logarítmicamente antes de la aplicación de los análisis estadísticos.

Durante el cálculo de resultados se determinó una correlación significativa entre el tamaño del pez y la abundancia parasitaria o el factor de condición. El efecto del tamaño se corrigió en los análisis posteriores, utilizando los residuos de la regresión entre estos parámetros como variables dependientes. Los efectos estacionales y anuales sobre la abundancia parasitaria se analizaron mediante un ANOVA factorial. Por último se calculó el coeficiente de correlación de Spearman entre la abundancia parasitaria y la temperatura máxima, mínima y media del agua en los alrededores de Mallorca. Los análisis estadísticos se desarrollaron utilizando el programa Statistica v. 8.0 (StatSoft, Inc., Tulsa, OK, USA).

0.6.3. Resultados

Todos los peces examinados en el presente estudio presentaron ejemplares de *Zeuxapta seriolae* en las branquias. La prevalencia en la muestra principal, obtenida de Mallorca, fue del 90,3% y la intensidad media de entre 1 y 1182 parásitos con un promedio ± desviación estándar de 173,36 ± 261,13. Se detectaron diferencias significativas de tamaño entre las submuestras

obtenidas, siendo generalmente más grandes los peces de octubre y febrero del 2005 que los muestreados en abril. Los análisis también revelaron una diferencia significativa en el factor de condición entre las submuestras mensuales y entre los peces de diferente tamaño. Sin embargo, después de realizar la corrección por tamaño, ninguna de las diferencias de factor de condición entre submuestras fue significativa. La apariencia externa de todos los peces era saludable aunque aquellos altamente parasitados presentaban palidez en branquias e hígado y una mayor secreción mucosa en la branquia.

Los mayores niveles de infección en Mallorca fueron registrados durante el periodo cálido (entre abril y junio) en los tres años de estudio. Se encontraron diferencias significativas en la abundancia parasitaria de las diferentes submuestras que revelaron una clara dinámica estacional de infección por *Z. seriolae*. Los niveles de infección aumentaron significativamente durante el periodo cálido (entre abril y junio; rango 1-1182 parásitos; 283,30 ± 287,24 en promedio por pez) en contraposición con los bajos niveles registrados durante el periodo frío (entre octubre y febrero; rango 1-21 parásitos; 4,45 ± 4,94 en promedio por pez). La abundancia de *Z. seriolae* mostró una correlación significativa con las temperaturas promedio y máxima mensual. Por otra parte, el incremento en los niveles de infección coincidió con mayores niveles de agregación parasitaria en las muestras examinadas durante el periodo cálido. Finalmente, los niveles de agregación de *Z. seriolae* también se correlacionaron fuertemente con el tamaño medio del pez.

En líneas generales, se detectó una tendencia de descenso en la abundancia de *Z. seriolae* entre 2005 y 2007 y el ANOVA reveló efectos significativos tanto de la estación como del año sobre la abundancia parasitaria. También se detectaron diferencias significativas de abundancia parasitaria entre las muestras de peces con diferentes tamaños.

La comparación de las muestras de las distintas localidades, incluyendo Mallorca, mostró diferencias altamente significativas entre ellas. Los peces obtenidos durante el periodo cálido en Mallorca y Córcega presentaron el mayor nivel de infección en comparación con los obtenidos en Mallorca y Alicante durante el periodo frío o en Cerdeña en julio del 2006.

0.6.4. Discusión

En este estudio se obtuvieron las mayores intensidades y abundancias de *Z. seriolae* registradas hasta la fecha, tanto en cultivo como en medio salvaje. Aunque *Z. seriolae* es una especie con distribución circumglobal, las infecciones masivas causadas por ésta y otras especies de monogeneos heteraxínidos se han citado principalmente en cultivos localizados en el mar Mediterráneo (Grau *et al.*, 2003; Repullés *et al.*, 2005) o en otras regiones con clima templado: Australia, Nueva Zelanda, Chile y Japón (Ogawa y Yokoyama, 1998; Wilson *et al.*, 2001; Sharp *et*

al., 2003; Hutson, 2007; Hutson *et al.*, 2007a; Whittington y Chisholm, 2008). Este hecho podría explicarse porque es en estas regiones donde el cultivo ha sido más intensivo, aunque también podría existir algún tipo de causa ambiental.

Los niveles de infección observados en la muestra principal de Mallorca fueron similares o superiores a los registrados en epizootias de *Z. seriolae* en especímenes de *S. dumerili* (Montero *et al.*, 2004) o *S. lalandi* (ver Mansell *et al.*, 2005) de tamaños similares. Por otra parte, los valores de abundancia media registrados durante abril y junio del 2005 también fueron similares o mayores que los obtenidos en el único caso de mortandades en el medio salvaje registrado hasta la fecha (Lia *et al.*, 2007).

Uno de los resultados más relevantes del presente estudio poblacional fue el patrón estacional recurrente de *Z. seriolae* en las poblaciones de *S. dumerili* de Mallorca. Este patrón era consistente y estaba significativamente correlacionado con la temperatura media o máxima del agua a pesar de la variación anual. Por tanto, se sugirieron mayores tasas de transmisión del parásito a mayores temperaturas. Los datos obtenidos en las tres localidades del Mediterráneo occidental se correspondieron con el patrón estacional de abundancia de *Z. seriolae* registrado en Mallorca. La única excepción fue la muestra de Cerdeña, obtenida en julio del 2006, que presentó una abundancia ampliamente inferior a la registrada en agosto del mismo año en Córcega. Por tanto, la variación de la distribución y abundancia de *Z. seriolae* en *S. dumerili* del Mediterráneo requeriría un estudio más amplio.

Aunque las mortandades en poblaciones de peces salvajes son casi imposibles de detectar (ver excepción en Lia *et al.*, 2007), tres evidencias podrían indicar una mortandad inducida por el parásito en las poblaciones de juveniles de *S. dumerili* en Mallorca. (*I*) Existe una asociación negativa entre la distribución de abundancias de *Z. seriolae* y la longitud del pez que contrasta con el patrón típico de asociación positiva descrito para las relaciones pez-monogeneo (Frankland, 1954; Paperna *et al.*, 1984; Buchmann, 1989; Rohde *et al.*, 1995; Grutter, 1998). Este patrón de abundancias según el tamaño del pez sugiere que la mayor parte de la población de *Z. seriolae* se mantiene en las poblaciones de peces juveniles (0+). (*II*) Por otra parte, el aumento de abundancia parasitaria observado durante los meses cálidos va unido a un mayor nivel de agregación, al contrario de lo que ocurre cuando bajan las temperaturas. (*III*) La fuerte correlación negativa entre los niveles de agregación de *Z. seriolae* y la longitud media del pez indicaría que aquellos peces altamente infectados son eliminados rápidamente de la población de hospedadores. Finalmente, la mortandad debida al efecto del sistema inmunitario del hospedador y la estacionalidad en la transmisión del parásito, también podrían influir en el patrón de abundancias observado.

0.7. Desarrollo, supervivencia y comportamiento natatorio del oncomiracidio de *Sparicotyle chrysophrii* (Van Beneden et Hesse, 1863)

0.7.1. Introducción

El monogeneo microcotílido *Sparicotyle chrysophrii* es uno de los parásitos metazoos más patógenos de la dorada en el Mediterráneo (Sanz, 1992; Álvarez-Pellitero, 2004; Athanassopoulou *et al.*, 2005; Sitjà-Bobadilla *et al.*, 2009). Se trata de una especie monoxena y su transmisión se realiza mediante oncomiracidios y huevos (Whittington *et al.*, 2000a), siendo estos últimos los más resistentes a los agentes antihelmínticos (Sitjà-Bobadilla *et al.*, 2006). En este trabajo se estudió el desarrollo, la supervivencia y la natación de los oncomiracidios de *S. chrysophrii* con el fin de obtener información biológica para diseñar tratamientos más efectivos contra este patógeno.

0.7.2. Materiales y métodos

Para el desarrollo del presente experimento se utilizaron un total de 450 huevos de *S. chrysophrii* puestos por diferentes adultos grávidos, procedentes de doradas cultivadas en Valencia (España). Estos huevos se mezclaron y se distribuyeron en grupos de entre 15 y 25 individuos. Todos los ejemplares se mantuvieron a 20ºC y fueron expuestos a periodos de 12 horas de luz y oscuridad. De acuerdo con los objetivos propuestos se realizaron dos estudios diferentes: uno sobre el desarrollo del oncomiracidio en el interior del huevo y el otro sobre su supervivencia y natación.

El estudio sobre el desarrollo del oncomiracidio incluyó 4 réplicas: 2 preliminares, en las que los huevos se observaron cada 24 h y 2 definitivas, en las que los huevos se observaron cada 8 h. A partir del quinto día, los huevos se revisaron cada 4 h en todas las réplicas, hasta que el primero de ellos eclosionó. Los cambios morfológicos observados tanto en el oncomiracidio como en el vitelo fueron registrados y se determinó el éxito de eclosión. Posteriormente, se analizaron las diferencias entre el número de huevos eclosionados en el total de periodos con iluminación y con oscuridad (test de Mann-Whitney), así como las diferencias entre el número de huevos eclosionados en cada uno de los periodos lumínicos de los diferentes días (test de Kruskal-Wallis).

Para el estudio sobre la supervivencia y la natación de los oncomiracidios se utilizaron un total de 155 larvas. Los individuos se separaron en diferentes pocillos y se observaron cada 2 horas. El tiempo de supervivencia y el periodo de natación fueron registrados y se analizó su correlación con el tiempo de incubación. Posteriormente, se analizaron las diferencias entre el tiempo de supervivencia y de natación de los oncomiracidios emergidos durante los periodos de luz y los de oscuridad. Finalmente, se calculó la velocidad de natación de los oncomiracidios y se establecieron diferentes categorías para describir el comportamiento de éstos cada dos horas.

0.7.3. Resultados

Durante los primeros muestreos, los huevos recién puestos estaban llenos de vitelo y los embriones no podían diferenciarse. Entre las 8 y las 56 h después de la puesta (horas *post-deposition*, hpd), el vitelo se había desplazado lateralmente y los embriones podían distinguirse en el centro del huevo. Entre las 48 y las 64 hpd el vitelo estaba distribuido en dos masas longitudinales, en consonancia con la forma del oncomiracidio. Los escleritos del háptor empezaron a distinguirse entre las 72 y las 88 horas, primero los *hamuli*, después los ganchos posteriores y finalmente los laterales. Entre las 88 y las 100 hpd se pudieron observar las manchas oculares, primero como acumulaciones de pigmento aisladas y después progresivamente más agregadas. Los ganchos laterales fueron visibles a partir de las 120 hpd (5 días), momento en el cual la mayor parte de los huevos ya estaban operculados.

El primer oncomiracidio nació a las 124 hpd (5 días) y el éxito de eclosión osciló entre el 68 y el 93%. La mayor parte de las eclosiones se produjo durante los periodos de oscuridad (75%), siendo la primera y la segunda noche las que presentaron un número significativamente mayor de eclosiones. El tiempo de supervivencia máximo fue de 52 horas post-eclosión*/hatching* (hph), aunque el 54% de los oncomiracidios había muerto después de 12 hph y solamente el 13% de ellos permanecían vivos después de 24 hph. El tiempo de supervivencia de los especímenes nacidos en periodos de oscuridad resultó significativamente mayor que el de aquellos nacidos con iluminación. En cuanto a la natación, el periodo máximo fue de 46 hph. La mayor parte de los oncomiracidios (93%) fueron capaces de nadar verticalmente al nacer, aunque después de 6 hph solamente el 53% de ellos era capaz de hacerlo y a las 16 hph el porcentaje había disminuido hasta el 11%. Su velocidad media de natación fue de 3,97 mm/s y no se encontraron diferencias significativas entre los periodos de natación de los individuos nacidos en oscuridad y los nacidos con iluminación.

0.7.4. Discusión

Los principales eventos del desarrollo de los oncomiracidios de *S. chrysophrii* siguieron una secuencia parecida a la descrita para otras especies de monogeneos. Las primeras estructuras visibles fueron los primordios de los ganchos, después las manchas oculares y, finalmente, los cilios y los opérculos, perceptibles poco tiempo antes de la eclosión de los huevos (Bondad-Reantaso *et al.*, 1995). El tiempo de incubación, la duración del periodo de eclosión y el elevado éxito de eclosión de *S. chrysophrii* coincidió con el descrito para otras especies de monogeneos (Kearn, 1986; Gannicott y Tinsley, 1998a; Whittington *et al.*, 2000a; Tubbs *et al.*, 2005).

La eclosión de los huevos de los monogeneos se ha vinculado a diferentes estímulos químicos, mecánicos, físicos, etc. (Kearn, 1986; Gannicott y Tinsley, 1997; Whittington *et al.*,

2000a). En concreto, el predominio de eclosión nocturna exhibido por *S. chrysophrii*, ha sido descrito para *Discocotyle sagittata* (ver Gannicott y Tinsley, 1997) y *Entobdella hippoglossi* (ver Kearn, 1974). Sin embargo, en el caso de *Entobdella soleae* (Kearn, 1973), este ritmo de eclosión aparentemente relacionado con las condiciones ambientales, resultó ser de tipo circadiano. Los datos obtenidos en el presente estudio, no permitieron descartar la hipótesis del ritmo endógeno para la eclosión de los huevos de *S. chrysophrii*. En cuanto a la supervivencia, la mayoría de los oncomiracidios conocidos no exceden las 48 hph de tiempo de vida máximo a temperaturas parecidas a las del presente estudio (Whittington *et al.*, 2000a). Los datos obtenidos para *S. chrysophrii* coincidieron con estos resultados aunque, realmente, sólo el 10% de los individuos superaron las 24 hph de vida.

Los patrones de natación exhibidos por los oncomiracidios de *S. chrysophrii* también fueron muy parecidos a los descritos en estudios previos (Kearn, 1980; Gannicott y Tinsley, 1998b; Whittington *et al.*, 2000a): (*i*) un primer periodo de natación vertical que podría contribuir a la búsqueda del hospedador en la columna de agua (Kearn, 1980) y (*ii*) un segundo periodo cerca del sustrato, posiblemente para minimizar la pérdida energética. Gannicott y Tinsley (1998b) postularon que la habilidad para nadar verticalmente estaba mucho más relacionada con el potencial infectivo de los oncomiracidios que con el tiempo de supervivencia. En el caso de *S. chrysophrii*, su tiempo de supervivencia le permitiría infectar al hospedador hasta 24 horas después de la eclosión del huevo. Sin embargo, los oncomiracidios buscarían activamente a su nuevo hospedador, y por tanto serían especialmente infecciosos, durante un periodo de entre 6 y 12 horas, en el que nadarían verticalmente. En condiciones de cultivo, este periodo infectivo se prolongaría artificialmente puesto que los huevos eclosionarían más lejos del fondo y los oncomiracidios permanecerían un mayor tiempo en la columna de agua.

A priori, las diferentes velocidades de natación entre el parásito y su hospedador supondrían una gran barrera para la transmisión del parásito a nuevos individuos (Kearn, 1986). No obstante, la coordinación entre la reproducción del parásito y los hábitos de su hospedador parece ser una estrategia muy útil para soslayar esta limitación (Kearn, 1986). En el caso de *S. chrysophrii*, el predominio de eclosiones durante los periodos de oscuridad podría estar relacionado con los hábitos de la dorada, que forman grupos de forrajeo cerca del fondo al anochecer (Watt-Pringle, 2009) y descansan durante la noche (Bégout y Lagardère, 1995). De este modo, la densidad de hospedadores aumentaría y su actividad branquial disminuiría, evitándose el lavado de parásitos y facilitándose su transmisión y establecimiento.

0.8. Desarrollo post-larvario del *monogeneo microcotílido Sparicotyle chrysophrii* (Van Beneden et Hesse, 1863): comparación con otras especies de Microcotylidae y Heteraxinidae

0.8.1. Introducción

El monogeneo poliopistocotíleo *Sparicotyle chrysophrii* causa graves epizootias en condiciones de cultivo. Estos episodios, son registrados principalmente durante la primavera y el verano (Faisal e Imam, 1990; Reversat *et al.*, 1992; Sitjà-Bobadilla *et al.*, 2009) y pueden causar importantes pérdidas económicas. Los efectos patológicos más relevantes causados por esta especie son la pérdida de sangre, debida a las heridas producidas por el anclaje y a la alimentación del parásito, y la disminución de superficie respiratoria, provocada por el aumento progresivo del tejido dañado y la ocupación branquial (Whittington y Chisholm, 2008). Los cambios en los elementos de anclaje durante el desarrollo y la tasa de crecimiento del parásito podrían modificar la gravedad de los efectos causados. Por tanto, conocer el desarrollo post-larvario de *S. chrysophrii* podría contribuir a optimizar el diseño de tratamientos y el control sobre esta especie.

0.8.2. Materiales y métodos

Para analizar el desarrollo post-larvario de *S. chrysophrii* se utilizaron un total de 76 doradas infectadas, procedentes de una piscifactoría localizada en Murcia (España). Estos peces se desparasitaron y se distribuyeron por grupos de 19 individuos en 4 tanques de 250 l mantenidos a 20ºC. La infección experimental se inició mediante la introducción de 250 huevos de *S. chrysophrii* en cada uno de los tanques. A partir de este momento, los peces fueron sacrificados y analizados en grupos de cuatro. El primer muestreo se realizó a los 3 días después de la infección (dpi, días post infección), posteriormente se muestrearon cada 2 días hasta los 21 dpi y, finalmente, cada 5 días hasta los 36 dpi, momento en el que se observaron especímenes de segunda generación enganchados a la branquia. El grupo de peces sobrante (N= 16) se sacrificó a los 51 dpi.

Los parásitos se recogieron y clasificaron según el arco que ocupaban y su estado de desarrollo. Se registraron longitud total, longitud del háptor, anchura de la pinza más grande y número total de pinzas para cada espécimen. Finalmente, se calculó la tasa de crecimiento del parásito durante su desarrollo y se comparó con las obtenidas para otras especies de microcotílidos y heteraxínidos. En este análisis comparativo se incluyeron un total de 10 especies: *Microcotyle spinicirrus*, *Microcotyle donavini*, *Microcotyle gotoi*, *Microcotyle sebastis*, *Microcotyle hiatulae*, *Polylabroides multispinosus*, *Bivagina tai*, *Heteraxinoides xanthophilis*, *Heteraxine heterocerca* y *Zeuxapta seriolae*.

0.8.3 Resultados

La prevalencia durante el experimento fue del 78% y la intensidad media de 12 ± 13 con una abundancia máxima de 46. El número total de parásitos obtenido fue 582, 36 de los cuales eran adultos. En referencia al número de parásitos por arco branquial, no se encontraron diferencias significativas.

Entre los cambios morfológicos observados durante el primer periodo del desarrollo post-larvario de *S. chrysophrii* destacaron el tamaño progresivamente mayor de los ganchos hasta la aparición del primer par de pinzas, el cese posterior del crecimiento, la posición progresivamente anterior de la faringe y la uniformidad en el aspecto y la disposición de las manchas oscuras asociadas al digestivo. Todos los especímenes encontrados durante el primer muestreo presentaban un háptor formado por los ganchos posteriores, los *hamuli* y los pequeños ganchos laterales. El primer par de pinzas se observó entre los 7 y los 15 dpi. Los ganchos laterales se mantenían durante algún tiempo después de la aparición de las primeras pinzas. Los mayores especímenes con lengüeta terminal tenían entre 5 y 6 pares de pinzas y se encontraron entre los 15 y 21 días después de la infección. Los individuos sin lengüeta terminal se registraron desde el día 15 después de la infección hasta el final del experimento. Los especímenes con el máximo número de pinzas se encontraron en el último muestreo, a los 51 dpi.

En las primeras fases del desarrollo (individuos sin pinzas) se observaron 4 masas compactas de aspecto granular y color marrón o amarillento asociadas a los ciegos digestivos que mantenían su forma hasta la aparición de los primeros pares de pinzas. El atrio genital se observó completamente desarrollado a los 19 dpi, el primordio de los testículos entre los 19 y los 21 dpi y el germario entre los 21 y los 26 dpi. Los primeros huevos intrauterinos se detectaron a los 26 dpi y las larvas de segunda generación fueron encontradas por primera vez a partir de los 36 dpi a 20ºC.

La curva de crecimiento de *S. chrysophrii* presentaba un aspecto sigmoide con 3 periodos de crecimiento diferentes (lento-rápido-lento). El mismo patrón se observó para la tasa de adición de pinzas (nº pares de pinzas/día): 0-1 durante el primer periodo, 2-3 durante el segundo y 1-2 durante el tercero. La longitud del háptor crecía de manera lineal con la longitud total del cuerpo aportando, aproximadamente, 2/7 de su crecimiento total.

La relación entre la longitud del cuerpo y el número de pinzas fue exponencial para todas las especies comparadas. Las diferentes especies se agruparon en 4 conjuntos según la pendiente de la línea de regresión entre el número de pinzas y la transformación logarítmica de la longitud total para cada una de ellas: (*i*) entre 0,0217 y 0,0229 (*S. chrysophrii*, *P. multispinosus* y *B. tai*), (*ii*) entre 0,0280 y 0,0311 (*M. sebastis*, *M. spinicirrus* y *M. hiatulae*), (*iii*) entre 0,0405 y 0,0439 (*H. xanthophilis* y

M. donavini) y (*iv*) entre 0,0517 y 0,0523 (*H. heterocerca* y *M. gotoi*). La especie *Z. seriolae*, con una pendiente de 0,0324 y se incluiría en el segundo grupo.

0.8.4. Discusión

En referencia a los cambios morfológicos del háptor durante el desarrollo de *S. chrysophrii*, uno de los datos más relevantes fue que no se observó el reemplazamiento de los ganchos laterales por parte de las pinzas, al contrario de lo que se había descrito en estudios previos (Euzet, 1958; Thoney, 1986a; Thoney y Munroe, 1987; Thoney, 1988; Roubal y Diggles, 1993). Estos ganchos eran desplazados lateralmente hasta que caían con parte del tejido circundante. Por otra parte, los ganchos posteriores y los *hamuli* no variaban su tamaño después de la aparición del primer par de pinzas y por tanto, ganchos de iguales dimensiones eran utilizados para la sujeción de especímenes de distinto tamaño, con mayor o menor contribución de las pinzas.

Por otra parte se sugirió una nueva composición para las manchas asociadas al digestivo de los primeros estados larvarios de *S. chrysophrii*. Estas manchas habían sido previamente interpretadas como residuos de sangre dentro del primordio del digestivo sacular (Euzet, 1958). Sin embargo, si se tratase de productos residuales de la digestión, su morfología, color y distribución debería mostrar un aspecto variable, puesto que la ingestión es discontinua en los monogeneos y los restos de comida se excretan (Kearn, 2004; Whittington y Chisholm, 2008). Estas manchas mantenían su forma y tamaño durante los primeros estados de desarrollo del parásito y presentaban un aspecto parecido al de las vitelógenas de los adultos o a los pequeños gránulos de vitelo encontrados de forma dispersa en el cuerpo de algunos oncomiracidios. Considerando todos estos datos, se sugirió que el vitelo también podría ser uno de los componentes de estas manchas.

Los periodos con distintas tasas de crecimiento descritas para *S. chrysophrii* se correspondían, aparentemente, con diferentes etapas de su desarrollo. La primera etapa, de crecimiento lento (*i*), coincidiría con el periodo de desarrollo de las primeras pinzas (elemento crítico para una buena sujeción de los individuos de mayor tamaño) y terminaría aproximadamente con la caída de la lengüeta terminal. A partir de este momento se iniciaría una etapa de crecimiento rápido (*ii*) hasta llegar a la madurez, después de la cual se produciría un nuevo periodo de crecimiento lento (*iii*) que finalizaría con la aparición de los primeros individuos de segunda generación.

Se observó un aparente patrón común para la pérdida de la lengüeta terminal y la maduración de las diferentes especies de microcotílidos analizadas. La lengüeta terminal se perdía cuando se habían desarrollado entre un 10 y un 20% de las pinzas, mientras que la madurez (entendida como el momento de aparición del primer huevo) se producía cuando se habían

desarrollado entre un 60 y un 75% del total de pinzas. Este patrón coincidía con el descrito previamente para *M. hiatulae*, *P. multispinosus*, y *B. tai* por Roubal y Diggles (1993).

Los cambios morfológicos producidos en el háptor de *S. chrysophrii* varían sus efectos sobre el tejido branquial. Durante el desarrollo de este parásito se diferenciaron tres periodos en los que la lesión predominante la causaban los ganchos, las pinzas o la acción combinada de ambos. Los especímenes con ganchos fueron encontrados en los primeros 21 dpi. Este sería un periodo en el que los daños estarían causados por la perforación del gancho sobre el tejido branquial. Los parásitos con pinzas se encontraron a partir del séptimo día después de la infección. Por tanto, durante 14 días las lesiones causadas por los ganchos se combinarían con las resultantes de la acción compresiva de las pinzas. Finalmente, la adición gradual de pinzas extendería el daño sobre el tejido branquial y disminuiría la superficie de intercambio gaseoso (Montero *et al.*, 2004; Buchmann y Bresciani, 2006; Whittington y Chisholm, 2008; Sitjà-Bobadilla y Álvarez-Pellitero, 2009).

Desde un punto de vista aplicado, los resultados del presente estudio, junto con los obtenidos en el estudio sobre el desarrollo larvario de *S. chrysophrii*, permitirían establecer un calendario óptimo de tratamientos para mejorar su efectividad. El primer paso sería suministrar un tratamiento que eliminase la mayor parte de especímenes adultos (aunque los huevos continuarían permaneciendo en la instalación). Después, las nuevas aplicaciones deberían realizarse a partir de los 50 días (36 días de maduración + 10 días de tiempo máximo de incubación + 3 días de tiempo máximo de supervivencia del oncomiracidio). Con el fin de mantener la instalación libre de parásitos durante más tiempo y controlar el número de juveniles se podrían aplicar tratamientos menos agresivos, de 15 a 20 días después de haber administrado la primera dosis. Entre dos y tres tratamientos (adulto-larva) al año serían suficientes para mantener la infección controlada en cultivos localizados en el área del mediterráneo occidental. Adicionalmente, un seguimiento del desarrollo parasitario, sería una medida preventiva muy recomendable.

0.9. CONCLUSIONES

1. La parasitofauna de *Seriola dumerili* en el Mediterráneo occidental es más rica en especies de lo que se pensaba. Diecisiete de las veintiséis especies obtenidas en las cuatro localidades muestreadas son nuevos registros: dos nuevas especies para la ciencia (*Paradeontacylix balearicus* and *P. ibericus*), un nuevo hallazgo en serviola del Mediterráneo (*Parabrachiella seriolae*), ocho primeras citas en *S. dumerili* (*Aphanurus* sp., *Capillaria* sp., *Camallanus* sp., *Cardiocephaloides* sp., *Cucullanus* sp., *Floriceps saccatus*, *Galactosomum lacteum*, *Lecithocladium* sp.) y seis nuevos hallazgos en el Mediterráneo (*Caligus aesopus*, *Caligus* sp., *Neometanematobothrioides periorbitalis*,

Prosorhynchus facilis, *Hysterothylacium seriolae*, Tetraphyllidean larva); y nueve son especies previamente citadas en *S. dumerili* del Mediterráneo occidental. De acuerdo con su prevalencia, la mayoría de las especies de Mallorca son accidentales (13). El resto de especies son principalmente comunes (9) y algunas de ellas son infrecuentes (2). Las especies comunes se encuentran en todas las localidades estudiadas.

2. La comunidad parasitaria de *S. dumerili* en el Mediterráneo occidental está mayoritariamente formada por parásitos heteroxenos, principalmente trematodos, adquiridos tróficamente. La serviola es el hospedador definitivo de muchos de ellos puesto que se encuentra en un nivel alto de la cadena alimenticia. La serviola también es hospedador intermediario de dos trematodos en estado de metacercaria y dos metacestodos accidentales. Muchas de las especies encontradas son especialistas estrictos o especialistas de peces carángidos. Por tanto, las relaciones filogenéticas parecen tener un papel bastante relevante en la composición de esta comunidad.

3. Cinco de las nueve especies comunes en *S. dumerili* de Mallorca presentan un patrón estacional de infección: *Bucephalus gorgon*, *Hemiurus communis*, *Paradeontacylix. balearicus* (huevos), *Stephanostomum. ditrematis* y *Zeuxapta seriolae*. Las prevalencias aumentan aparentemente de otoño a invierno en *B. gorgon* y en *H. communis*, y de primavera a verano en *P. balearicus* (huevos), *S. ditrematis* y *Z. seriolae*.

4. Entre los parásitos encontrados en Mallorca, los más peligrosos en condiciones de cultivo son los monogeneos y los aporocotílidos puesto que han sido previamente relacionados con mortandades masivas de *S. dumerili* en el Mediterráneo. Las especies de trematodos en estado de metacercaria (strigeidos y heterófidos), didimozoidos, acantocólpidos, calígidos y gnátidos se consideran potencialmente dañinos. Aunque no han sido relacionadas con infecciones importantes de *S. dumerili*, son taxonómicamente cercanas a otras especies causantes de significativos efectos patológicos, El resto de especies se consideran poco peligrosas por sus escasos efectos (bucefálidos y hemiúridos) o por su baja prevalencia (cestodos, nematodos).

5. Se describen dos nuevas especies de *Paradeontacylix* parásitas de *S. dumerili: P. balearicus* procedente de las islas Baleares y *P. ibericus* procedente de la península ibérica. Cada una de estas especies se distingue morfológicamente del resto por su tamaño y forma. Los análisis discriminantes separan estas dos especies de las que infectan a *S. dumerili* en Japón (*P. grandispinus* y *P. kampachi*). Sin embargo, las diferencias morfológicas entre *P. grandispinus* y *P. balearicus* no son estadísticamente significativas.

6. El cladograma del género *Paradeontacylix* coincide con el árbol filogenético basado en datos moleculares. Las especies de *Paradeontacylix* se distribuyen en dos clados separados y con un buen soporte: (*P. ibericus*+*P. kampachi*) y (*P. godfreyi*+(*P. balearicus*+*P. grandispinus*)). Las menores divergencias de secuencias se encuentran entre *P. grandispinus* y *P. balearicus* (0.2%/2.5%/6.3%) mientras que entre *P. kampachi* y *P. ibericus* la divergencia es ligeramente mayor (0.2%/4.7%/7%). *P. godfreyi* muestra los mayores porcentajes de divergencia con el resto de especies con valores de hasta un 12.5% en el ITS2 y un 16.0% en el COI.

7. La unión filogenética entre las especies japonesas y mediterráneas subraya la importancia de su similitud morfológica y molecular por encima de la separación geográfica entre ellas. Estos resultados sugieren que ambos grupos de especies de aporocotílidos o sus ancestros existían antes de la separación de las poblaciones de sus peces hospedadores.

8. Los niveles de infección de *Z. seriolae* observados en *S. dumerili* de Mallorca entre 2005 y 2007 son los mayores registrados hasta la fecha en individuos salvajes y similares o superiores a los registrados en epizootias de este parásito en condiciones de cultivo. Se observa un patrón estacional recurrente de infección por *Z. seriolae* en las poblaciones de *S. dumerili* de Mallorca, que presentan mayores cargas parasitarias durante las estaciones cálidas (de abril a junio). Este patrón es consistente, a pesar de la tendencia significativa de descenso en la abundancia a lo largo de los diferentes años de muestreo, y está significativamente correlacionado con el aumento del promedio y el máximo de temperatura mensual.

9. En líneas generales, los datos parasitológicos obtenidos de los muestreos adicionales en las diferentes localidades del Mediterráneo occidental (Alicante y Córcega) muestran el mismo patrón estacional de abundancias con la excepción de la población de peces de Cerdeña. Por tanto, la variación geográfica en la distribución y abundancia de *Z. seriolae* requiere una mayor exploración.

10. La infección por *Z. seriolae* no afecta significativamente a la apariencia externa de los peces o su factor de condición de Fulton. Sin embargo, existen tres evidencias que sugieren una posible mortandad de los juveniles de *S. dumerili* inducida por el parasito: (*i*) la asociación negativa significativa entre la abundancia de *Z. seriolae* y la longitud del pez; (*ii*) la asociación entre el incremento de abundancia durante los meses cálidos y el aumento notable de los niveles de agregación del parásito; y (*iii*) la fuerte correlación negativa entre estos niveles de agregación y el promedio de la longitud del pez que podría indicar que los peces altamente infectados están siendo rápidamente eliminados de la población. La mortandad del parásito debido a la respuesta

inmune del hospedador y la estacionalidad en la transmisión del parásito podría también contribuir en el patrón de abundancias y agregación espacial.

11. Los huevos de *Sparicotyle chrysophrii* eclosionan entre 5 y 10 días después de la puesta a 20ºC e in vitro y su éxito de eclosión es de 87,3 %. Muchas de las eclosiones ocurren en los periodos de oscuridad (> 75%), especialmente entre la primera y la segunda noche después de la primera eclosión. Las eclosiones nocturnas podrían estar relacionadas con la coordinación entre el parásito y su hospedador puesto que la dorada forrajea en grupos al anochecer y disminuye su actividad general durante la noche. El aumento de densidad local y el descenso de actividad branquial facilitaría la transmisión del parásito. El tiempo de supervivencia de los oncomiracidios de *S. chrysophrii* no excede habitualmente las 24h aunque las larvas pueden vivir más de dos días. La mayoría de los oncomiracidios nada verticalmente al nacer aunque después de 12h solamente el 15% de ellos puede hacerlo. Por tanto, el periodo para encontrar activamente al nuevo hospedador es inferior a 12 h.

12. Se registran nuevos datos morfológicos en el desarrollo de los primeros estados post-larvarios de *S. chrysophrii*. Los más relevantes son: (*i*) el mantenimiento de los ganchos laterales después de la aparición del primer par de pinzas; (*ii*) la posición medial de la faringe, que podría disminuir su funcionalidad; (*iii*) el mantenimiento del tamaño de los ganchos a partir del desarrollo del primer par de pinzas y por tanto la sujeción de individuos de diferente tamaño mediante ganchos iguales; y, (*iv*) la morfología invariable de las 4 manchas de aspecto granular asociadas al digestivo que podrían estar formadas por sangre y vitelo.

13. En el desarrollo de *S. chrysophrii* se diferencian tres periodos con diferentes tasas de crecimiento. El primer periodo (lento) finaliza con la pérdida de la lengüeta terminal. El segundo periodo (rápido) se desarrolla a lo largo de la maduración del parásito. El último periodo (lento) se observa desde la maduración hasta el desarrollo del total de las pinzas. En relación con estos periodos, se observan unos patrones temporales comunes para la pérdida de la lengüeta terminal y la maduración en todos los microcotílidos y heteraxínidos comparados en el presente estudio (*Microcotyle spinicirrus*, *Microcotyle donavini*, *Microcotyle gotoi*, *Microcotyle sebastis*, *Microcotyle hiatulae*, *Polylabroides multispinosus*, *Bivagina tai*, *Heteraxinoides xanthophilis*, *Heteraxine heterocerca* y *Z. seriolae*). La lengüeta terminal cae, normalmente, cuando se han desarrollado entre el 10 y el 20% del total de pinzas, mientras que la maduración ocurre cuando se han desarrollado entre el 60% y el 70% de las pinzas.

14. Los efectos patológicos causados por el enganche de *S. chrysophrii* varían durante su desarrollo. Primeramente, los ganchos ejercen una acción perforante (durante aproximadamente 21 días) y posteriormente las pinzas actúan comprimiendo los filamentos branquiales (desde el séptimo día después de la infección). La adición gradual de pinzas extiende los daños y disminuye la superficie branquial de intercambio gaseoso. Asimismo, con el crecimiento del parásito la cantidad de sangre ingerida es mayor y la anemia puede ser más severa.

15. Un tratamiento con dos dosis separadas entre 15 y 20 días, una contra los adultos y juveniles y otra contra los oncomiracidios nacidos de los huevos resistentes al primer tratamiento, seria altamente eficiente y permitiría largos periodos sin infección en condiciones de cultivo. La administración de nuevos tratamientos podría postergarse con seguridad al menos hasta 50 días después: 36 días (maduración) + 10 días (tiempo máximo de incubación del huevo) + 3 días (periodo máximo de supervivencia del oncomiracidio).

CHAPTER 1.
General introduction

Aquaculture has experienced an important expansion during the last years as a response to human population growth and the subsequent increase of high quality protein requirements. Most of the fishery stocks are overexploited and the estimated proportion of underexploited or moderately exploited stocks has declined to a 15% percent in the last 40 years (FAO, 2010). Food and Agriculture Organisation (FAO; 2010) estimates that 65% of the consumed aquatic food will be provided from aquaculture in 2030. Nowadays, more than a half of the global aquatic resources come from aquaculture. Fish represent the 46% of total supply, China being the largest producing country (FAO, 2010). The most cultured aquatic animals are freshwater fishes followed by molluscs, crustaceans, diadromous fishes, marine fishes and others. Spain is the biggest aquaculture producer in Europe (providing 21% of total tonnes), followed by France and UK (FAO, 2010). However, fish aquaculture has slightly increased from 2000 onwards in the European Union (FAO, 2010; APROMAR, 2011) and these once-leading countries have suffered a production decrease during last years.

Fish species must satisfy some requirements to be good candidates for aquaculture as having a fast growth rate, high economic value, docility, resistance to stress, simple life-cycle, acceptance of artificial feeds, positive physical features, etc. (see Liao & Huang, 2000). Moreover, viable and highly profitable culture involves the management of the entire life-cycle of fish. Therefore, understanding the behaviour, habitat, nutritional requirements, reproductive biology and larval and juvenile physiology of each fish species is necessary in closed life-cycle fish production systems (Ottolenghi *et al.*, 2004). On the other hand, overcrowding, confinement and fish stress facilitate pathogen transmission in cultures (Ogawa, 1996; Nowak, 2007; Hutson *et al.*, 2007b). Consequently, disease control is also a crucial matter in aquaculture since fish pathologies, often caused by parasites, can significantly increase the production costs (Ogawa, 2005).

This thesis compiles various studies on metazoan parasites of two fish species of interest for aquaculture, the greater amberjack and the gilthead seabream. Both species present great conditions to be cultured (i.e. fast growth, high market value, good appearance and high flesh quality) although their culture in the Mediterranean region is quite disparate. Greater amberjacks are only experimentally cultured in this region, as breeding techniques have not been optimised enough and production is constricted by availability of wild juveniles (Poortenaar *et al.*, 2003; Ottolenghi *et al.*, 2004). In contrast, the culture of gilthead seabream is well-managed and intensive in this region (APROMAR, 2011).

This thesis deals with the study of parasite pathogens affecting greater amberjack and gilthead seabream in cultures and in the wild. According to the production necessities mentioned above, the research on the diseases of these species focus on: (*i*) the general study of all potential

pathogenic organisms (mostly for new Mediterranean aquaculture finfish species, as greater amberjack) and (*ii*) the study of concrete pathologies causing fish losses and their related treatments (mostly for traditionally cultured species in the area as gilthead seabream).

In this introductory section, the main features of the two host species studied are described as well as their main diseases in culture conditions. Moreover, biological features and effects of the main parasite taxa infecting these fish, monogeneans and blood flukes, are summarized.

1.1. Fish species

Amberjacks (*Seriola* spp.) are commercially important fish species with long tradition of culture, especially in Japan (Poortenaar *et al.*, 2003). Ten species have been described within this genus (Laroche *et al.* 1984; Smith-Vaniz, 1986; Poortenaar *et al.*, 2003), the Japanese amberjack (*S. quinqueradiata* (Temminck et Schlegel, 1845)), the yellowtail kingfish (*S. lalandi* (Valenciennes, 1833)) and the greater amberjack (*S. dumerili* (Risso, 1810)) being those most widely cultured and consumed. Currently, Japanese fish farmers are promoting *S. dumerili* and *S. lalandi* instead of *S. quinqueradiata* cultures due to the higher quality of their flesh and their faster growth (Poortenaar *et al.*, 2003). In Europe, *S. dumerili* has been cultured since 1980, especially in the Mediterranean region where this species has been traditionally fished and consumed (Porrello *et al.* 1993; García & Díaz, 1995; Montero *et al.*, 2001a; Poortenaar *et al.*, 2003). Many experimental cultures of this species have been developed in different countries around the Mediterranean Sea: i.e. Italy, Malta, Spain (Di Bitteto & Lazzari, 1991; Ernst *et al.*, 2002; García & Díaz, 1995; Montero *et al.*, 2001a). Nowadays, the life-cycle of greater amberjack has been closed, and the first intensive productions have been completed (see information on the 24/7/2009 PROMAN CO. project in http://www.mispeces.com).

Sparus is a monospecific genus that only includes the gilthead seabream, *S. aurata* Linnaeus, 1758, a species traditionally produced and consumed in the Mediterranean (Colloca & Cerasi, 2005-12). *S. aurata* belongs to the family Sparidae which includes 125 species from 37 different genera (Bauchot & Hureau, 1986; Froese & Pauly, 2011). Some of these sparids (*Dentex dentex*, *Diplodus puntazzo*, *Pagellus bogaraveo*, *Pagrus pagrus*, etc.) are considered promising species for aquaculture in the Mediterranean (FEAP, 1999). Nowadays, gilthead seabream is the third most produced fish species in the European Union and one of the most cultured marine fish species in Spain (APROMAR, 2011).

1.1.1. Biological features of *Seriola dumerili* (Perciformes, Carangidae)

The greater amberjack (Fig. 1.1) is the native *Seriola* species in the Mediterranean Sea, and it is also the largest species of the genus (Poortenaar *et al.*, 2003). Three other species have been reported in this region to date: *S. carpenteri* Mather, 1971, *S. rivoliana* Valenciennes, 1833 and *S. fasciata* Rüppell, 1830, the latter being recently found in stable populations (Deidun *et al.*, 2011; Froese & Pauly, 2011). This species is distributed worldwide in tropical to temperate waters (between 45°N-28°S and 180°W-180°E; Fig. 1.2). However, in some regions, its location is difficult to determine as in the African coast where many records could be mistaken with the congeneric species *S. carpenteri* (Froese & Pauly, 2011).

The habitat of greater amberjack is epibenthic and pelagic. Young specimens are found in inshore waters (less than 10 m depth) while older ones are found between 18 and 72 m depth (Smith-Vaniz, 1986). Juveniles form shoals and live usually associated with floating objects: plants or debris in oceanic and offshore waters (Massutí *et al.*, 1999; Riera *et al.*, 1999). Fish become migratory as they grow, the size of the shoals decreases and most of the specimens live alone (Dempster, 2005). Concerning diet and eating habits, during the early weeks of life greater amberjacks are planktivorous, feeding on gastropods, amphipods and decapod larvae. Thereafter, they approach to the coast where they feed on benthic invertebrates (crustacean, polychaetes) and demersal fish. Finally, they become fast-swimming predators and mainly feed on smaller pelagic fish (Pipitone & Andaloro, 1995; Froese & Pauly, 2011).

Figure 1.1. Greater amberjack (*Seriola dumerili*). Picture provided by JM Barres.

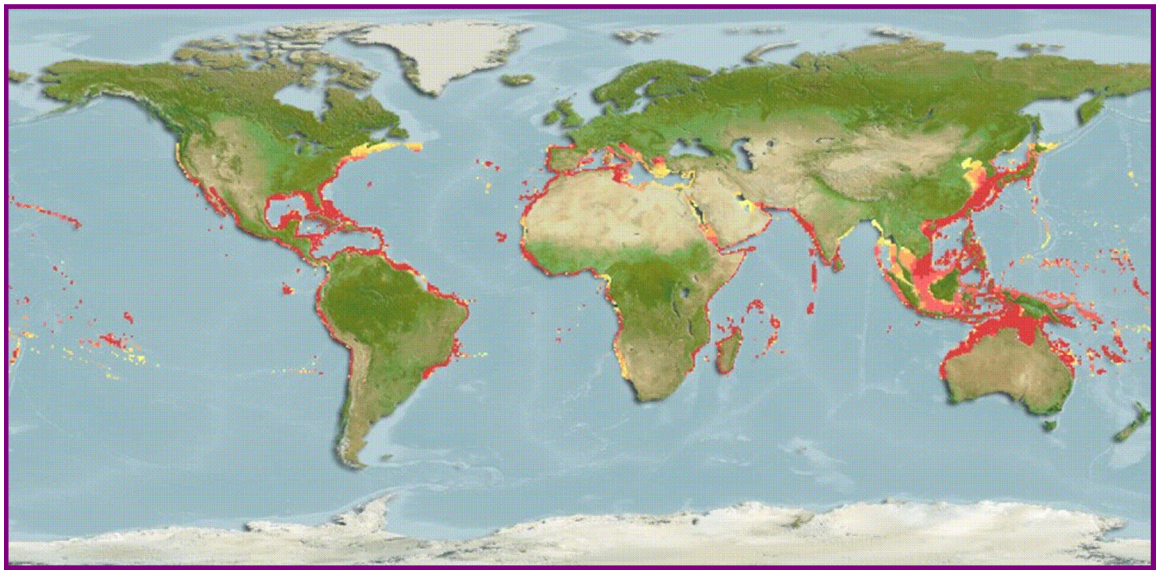


Figure 1.2. Distribution of greater amberjack (*Seriola dumerili*). Map obtained from Froese & Pauly (2011)

The reproductive patterns of greater amberjacks have been studied in the resident populations off Atlantic coasts. Greater amberjack is a dioecius species with a single spawning season extending from late spring to early summer (May to July). Spawning occurs in offshore waters and eggs and larvae are pelagic (Smith-Vaniz, 1986; Froese & Pauly, 2011). Fish grow fast, reaching sizes from 32 to 45 cm (TL) and approximately 1300 kg of weight in the first year of life (Kozul *et al.*, 2001). Individuals mature after three to five years (at about 15 kg) and life-span is around 17 years. The longest specimen captured measured 1.80 m and weighed 80.6 kg, although captures of specimens over 1.10 m long (from 25 to 40 kg) are rare (Smith-Vaniz, 1986).

1.1.2. Biological features of *Sparus aurata* (Perciformes, Sparidae)

The gilthead seabream (Fig. 1.3.) is mainly distributed in the Mediterranean and, more rarely, in the Black Sea and the eastern Atlantic (British Isles and from Gibraltar Strait to Cape Verde, including Canary Islands) (Bauchot & Hureau, 1986; Fig. 1.4).

This is an epibenthic littoral species that inhabits in *Posidonia oceanica* beds and sandy bottoms. Juveniles live in shallow depths (30 m), while adults are found deeper, at 150 m. They are sedentary fish which live alone or in small shoals. This fish species is mainly carnivorous and feeds on zooplankton, zoobenthos and nekton (mollusks, mainly mussels, crustaceans and fishes) (Bauchot & Hureau, 1986; Froese and Pauly, 2011).

Regarding reproduction, gilthead seabream is a protandric hermaphrodite species, which matures as a male during the first or second year of life and as a female after the third year. Spawning occurs from October to December and larval stages last from 43 to 50 days. Maximum

standard length registered is 70 cm, although adult specimens usually measure from 30 to 35 cm (Bauchot & Hureau, 1986; Froese and Pauly, 2011).

Figure 1.3. Gilthead seabream (*Sparus aurata*). Picture provided by JM Barres.

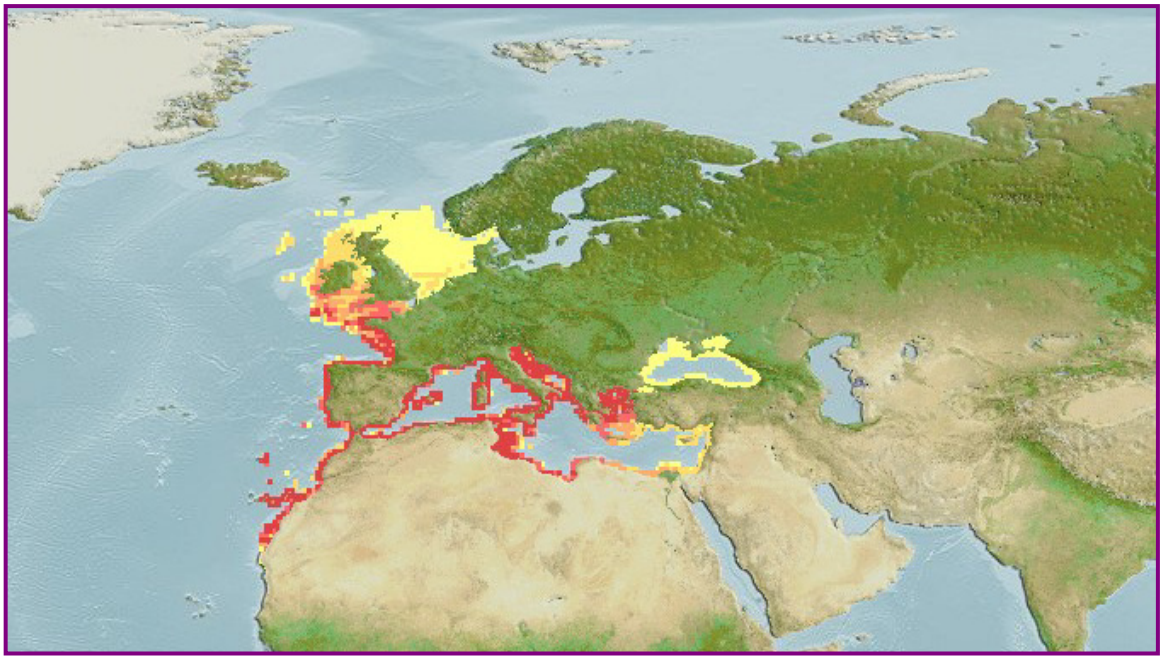


Figure 1.4. Distribution of gilthead seabream (*Sparus aurata*). Map obtained from (Froese & Pauly, 2011).

1.2. Parasites and diseases of *S. dumerili* and *S. aurata*

Several fish diseases have been associated with outbreaks or economic losses of *Seriola dumerili* and *Sparus aurata*. Out of metazoan parasites, the most important diseases of greater amberjacks in culture conditions are caused by bacterias of the genera *Vibrio* and *Streptococcus* as

well as the species *Photobacterium damselae* subsp. *piscida* McDonell et Colwell, 1986 causing pasteurellosis (Alcaide *et al.*, 2000; Leong & Colorni, 2002). The latter has also been related to outbreaks of gilthead seabream although vaccines currently available avoid important troubles in cultures. Moreover, the species *Pseudomonas anguilliseptica* Wakabayashi et Egusa, 1972 has been associated with the winter syndrome, a severe immunosuppression that affects gilthead seabream during the winter months and facilitates parasitic infections (Tort *et al.*, 1998). From the other hand, severe infections of the protist *Amyloodinium ocellatum* (Brown, 1931), affecting gilthead seabream, and epitheliocystis (probably associated with bacteria or fungus-like pathogens), affecting both fish species, have also been reported (Leong & Colorni, 2002). Finally, the ciliate *Cryptocaryon irritans* Brown, 1951, has also been associated with important losses in tank cultures of *S. dumerili* in the Mediterranean Sea (Rigos *et al.*, 2001; De la Gándara *et al.*, 2004). This parasite species is highly unspecific and has also been reported parasitising gilthead seabream (Colorni, 1985).

Metazoan parasite faunas of *S. dumerili* and *S. aurata* have been deeply studied as these fish are traditionally consumed and cultured. However, the number of parasites reported from greater amberjack is higher in accordance with its wider distribution (see lists in Gibson *et al.*, 2005 and checklist of the present PhD study). Several taxonomical papers and reviews on species parasitising greater amberjack in the Mediterranean have been published and parasite-lists have been compiled from two western localities, Murcia and Majorca (Grau *et al.*, 1999; Montero *et al.*, 2001b; Bartoli & Bray, 2004; Bartoli *et al.*, 2004; Bartoli *et al.*, 2005). Moreover, microsporidians and myxozoans have been separately studied as they cause severe pathological effects in cultures. Known species parasitising greater amberjack are: *Microsporidium seriolae* Egusa, 1982, *Myxobolus acanthogobii* Hoshina, 1952 and *Kudoa anamiensis* Egusa et Nakajima, 1978 (Ogawa, 2005). Concerning gilthead seabream, several studies have been developed, especially focused on platyhelminthes (Padrós *et al.*, 2001a; Gibson *et al.*, 2005; Holzer *et al.*, 2008). Recently, Fioravanti *et al.* (2006) has reviewed the parasite fauna of this fish in a comprehensive survey. Moreover, punctual studies on myxozoans of gilthead seabream in the Mediterranean, *Kudoa* sp. and *Enteromyxum leei* (Diamant, Lom et Dyková, 1994), have also been reported (Paperna, 1982; Diamant, 1992; Padrós *et al.*, 2001b; Ogawa, 2005).

According to their effects on fish cultures, among the metazoan parasites of greater amberjack and gilthead seabream it is worth highlighting the importance of monogeneans and blood flukes (Trematoda: Aporocotylidae) (Ogawa & Fukudome, 1994; Whittington & Chisholm, 2008). Infections of greater amberjack have been associated to four monogenean and four aporocotylid species: (*i*) the monogeneans *Neobenedenia girellae*, *Benedenia seriolae* (both Monopistocotylea, Capsalidae), *Allencotyla mcintoshi* and *Zeuxapta seriolae* (both Polyopisthocotylea, Heteraxinidae) (Montero *et al.*, 2001a; Hutson *et al.*, 2007b; Hirayama *et al.*, 2009); and (*ii*) the

aporocotylids of the genus *Paradeontacylix*: *P. balearicus*, *P. grandispinus*, *P ibericus* and *P. kampachi* (Crespo *et al.*, 1994; Ogawa & Fukudome, 1994; Repullés-Albelda *et al.*, 2008).

In the case of the gilthead seabream, *Sparicotyle chrysophrii* (Monogenea, Microcotylidae) is, currently, the metazoan parasite causing the most important material and economic losses in cultures (see Sanz, 1992; and Sitjà-Bobadilla *et al.*, 2009). This is a parasite widely distributed in the Mediterranean, where the gilthead seabreams are mainly cultured and recurrent epizootics are frequently reported (Álvarez-Pellitero, 2004). More recently, the species *Furnestinia echeneis* (Monogenea, Diplectanidae), has also been related to fish losses in culture conditions (Antonelli *et al.*, 2010a). Concerning blood flukes, *Cardicola aurata* is the only species reported in gilthead seabream to date (Holzer *et al.*, 2008). This species has not been associated with fish mortalities although severe inflammatory responses and tissue necrosis have been described (Padrós *et al.*, 2001a).

1.3. Parasites studied

This section summarizes the biological features of the two main parasite taxa investigated in this study: monogeneans (heteraxinids and microcotylids) of greater amberjack and gilthead seabream and blood flukes of greater amberjack (*Paradeontacylix* spp.). Biological features and effects of *Cardicola aurata,* the blood fluke species infecting gilthead seabream, have been recently analysed (Padrós *et al.*, 2001a; Holzer *et al.*, 2008) and, therefore, this species has not been included in the current PhD study.

1.3.1. Heteraxinids and Microcotylids (Monogenea, Polyopisthocotylea)

Polyopisthocotylea is a large group of monogeneans with about 1000 described species (Hayward, 2005; Buchmann & Bresciani, 2006). Hosts of polyopisthocotyleans are usually freshwater and marine fish although some species have also been reported in other vertebrates and even in two invertebrates (Hayward, 2005; Whittington & Chisholm, 2008). These parasites are external, monoxenous and usually strict host-specific except for a *Microcotyle* species which has been reported from teleosts of five different orders (Hayward, 2005). Polyopisthocotylean monogeneans infecting *S. dumerili* in the Mediterranean are *Allencotyla mcintoshi* (Price, 1962) and *Zeuxapta seriolae* (Meserve, 1938), while polyopisthocotyleans infecting *S. aurata* are *Atrispinum salpae* (Parona et Perugia, 1980); *Bivagina pagrosomi* (Murray, 1931), *Polylabris tubicirrus* (Paperna & Kohn, 1964) and *Sparicotyle chrysophrii*. Species herein studied (*Z. seriolae* and *S. chrysophrii*) belong to the superfamily Microcotyloidea which includes 10 families and 272 species infecting fish from 8 different orders (Hayward, 2005).

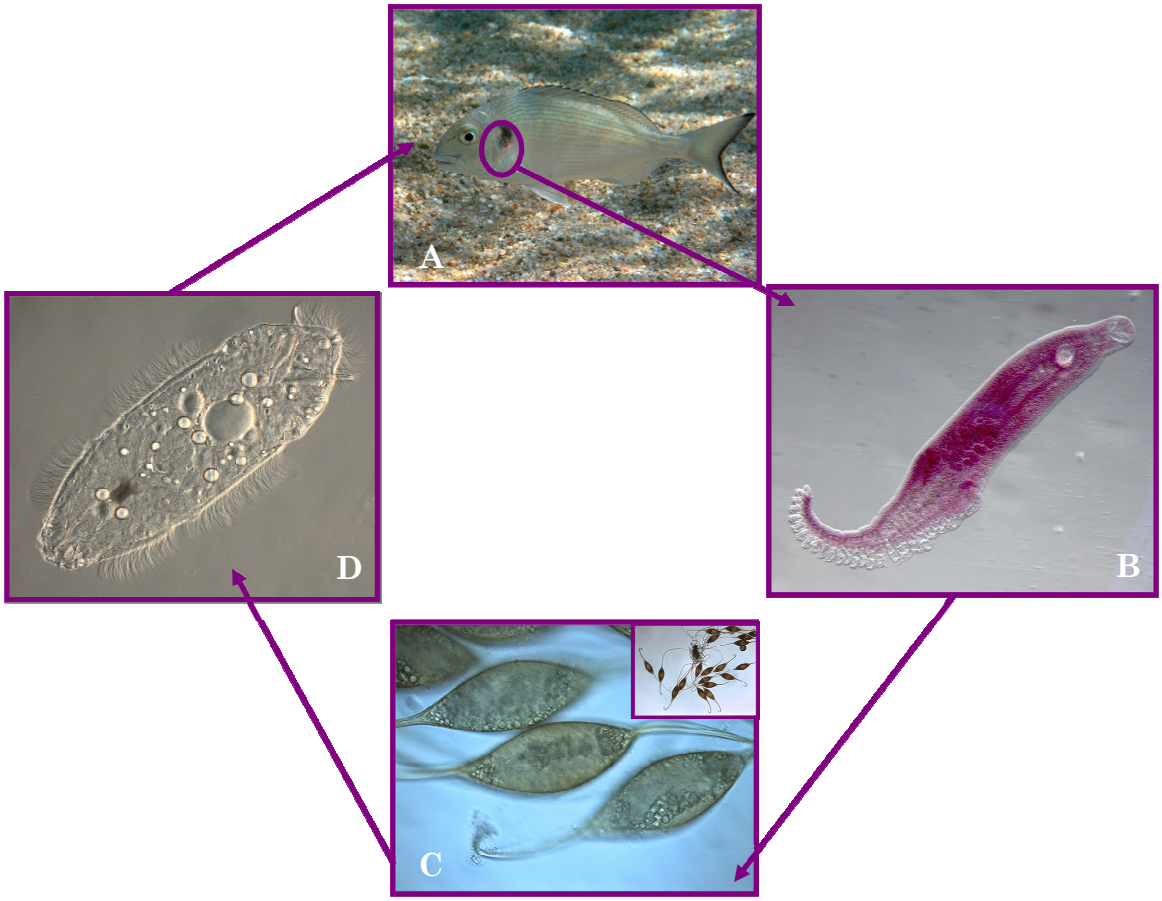


Figure 1.5. Scheme of the life-cycle of *Sparicotyle chrysophrii*. A) Host, *Sparus aurata*; B) Adult specimen; C) Eggs; D) Oncomiracidium. Picture A obtained from www. fishbase.org.

Pathologies caused by polyopishtocotyleans have often been considered mild compared to those caused by monopistocotyleans (Leong and Colorni, 2002; Whittington & Chisholm, 2008). However, the two species treated in the current study, *Z. seriolae* and *S. chrysophrii*, represent a substantial exception to this foundation as they have been frequently related to fish mortalities, especially in overcrowded culture conditions (Whittington & Chisholm, 2008; Sitjà-Bobadilla & Álvarez-Pellitero, 2009). Effects of polyopisthocotyleans are related to their attachment and their feeding habits. On one hand, hooks and clamps of these parasites cause injuries in the epithelial and branchial tissues that can be infected by opportunistic organisms, often very virulent. On the other hand, parasite blood feeding can cause anaemia and may lead to the fish death (Buchmann & Bresciani, 2006; Whittington & Chisholm, 2008). In the case of *Z. seriolae* these effects have been reported in *S. lalandi* and *S. dumerili* infections (Montero *et al.*, 2004; Mansell *et al.*, 2005). Similar effects have also been reported for *S. chrysophrii* parasitising *S. aurata*, (Sitjà-Bobadilla & Álvarez-Pellitero, 2009).

1.3.2. Blood flukes (Trematoda, Aporocotylidae): *Paradeontacylix* spp.

The family Aporocotylidae is constituted by species living in the circulatory system of fish. Their habitat and specific morphological features, as the absence of suckers, distinguish them from the rest of trematodes (Smith, 2002). In general, aporocotylids have been poorly studied as they go unnoticed in usual parasitological analyses (Bullard & Overstreet, 2002). More than 100 species, belonging to 20 different genera, have been described within this family (Smith, 1972; 1997a; 1997b; Gibson *et al.*, 2005). Aporocotylids are highly host-specific and more than 70% of them parasitise only one fish species. Moreover, when the specificity is not strict, parasites infect fish belonging to the same families. The species of *Paradeontacylix* herein studied are strict specialists of *Seriola* spp. (Smith, 1972; 1997a; 1997b).

Aporocotylids have heteroxenous life-cycles with sexually reproductive adult stages infecting vertebrates and asexually reproductive larval stages parasitising invertebrates (generally molluscs or polychaetes) (Fig. 1.6; Smith, 1972; 1997a). Hermaphroditic adults are found in the fish circulatory system, usually in heart and in gill arches, where they copulate and release the eggs, which get trapped in different capillaries of some organs along the bloodstream. Only those eggs inside the gill filaments are able to successfully hatch. Hatched miracidia tear the gill tissue and leave the fish. Then, they swim using their cilia to find the intermediary host which is a mollusc or a polychaete. These larvae, which live approximately 24 h, penetrate into the intermediate host, asexually reproduce, develop into sporocysts and/or rediae and generate the cercariae (Smith, 1972; 1997a). The asexual reproduction period lasts approximately 28 days (Smith, 1997a). The cercariae emerge through the invertebrate tissue and actively swim to find the definitive host. Aporocotylid cercariae infect definitive host by direct penetration through the eyes, gill, skin, fins or digestive system and thereafter, larvae develop into juveniles and migrate to the fish bloodstream were they become adults (Smith, 1972; 1997a). Cercariae sometimes encyst in the muscular tissue and constitute the schistosomulum before developing into juveniles (see Køie, 1982).

The blood flukes herein studied belong to *Paradeontacylix* genus which includes 7 species: *P. balearicus* Repullés-Albelda, Montero, Holzer, Ogawa, Hutson et Raga, 2008, *P. ibericus* Repullés-Albelda, Montero, Holzer, Ogawa, Hutson et Raga, 2008, *P. godfreyi* Hutson et Whittington, 2006, *P. kampachi* Ogawa et Egusa, 1986), *P. grandispinus* Ogawa et Egusa, 1986, *P. sanguinicoloides* McIntosh, 1934, and *P. odhneri* (Layman, 1930).

Pathological effects of *Paradeontacylix* spp. are directly related with the accumulation of eggs into the bloodstream as they partially obstruct the normal circulatory flux. Moreover, after egg hatching, miracidia leave the gill, tearing the filament tissue and decreasing the fish respiratory

surface. Documented pathologies of these infections are gill hyperplasia and asphyxia which may lead to the fish death (Smith, 1972; 1997a).

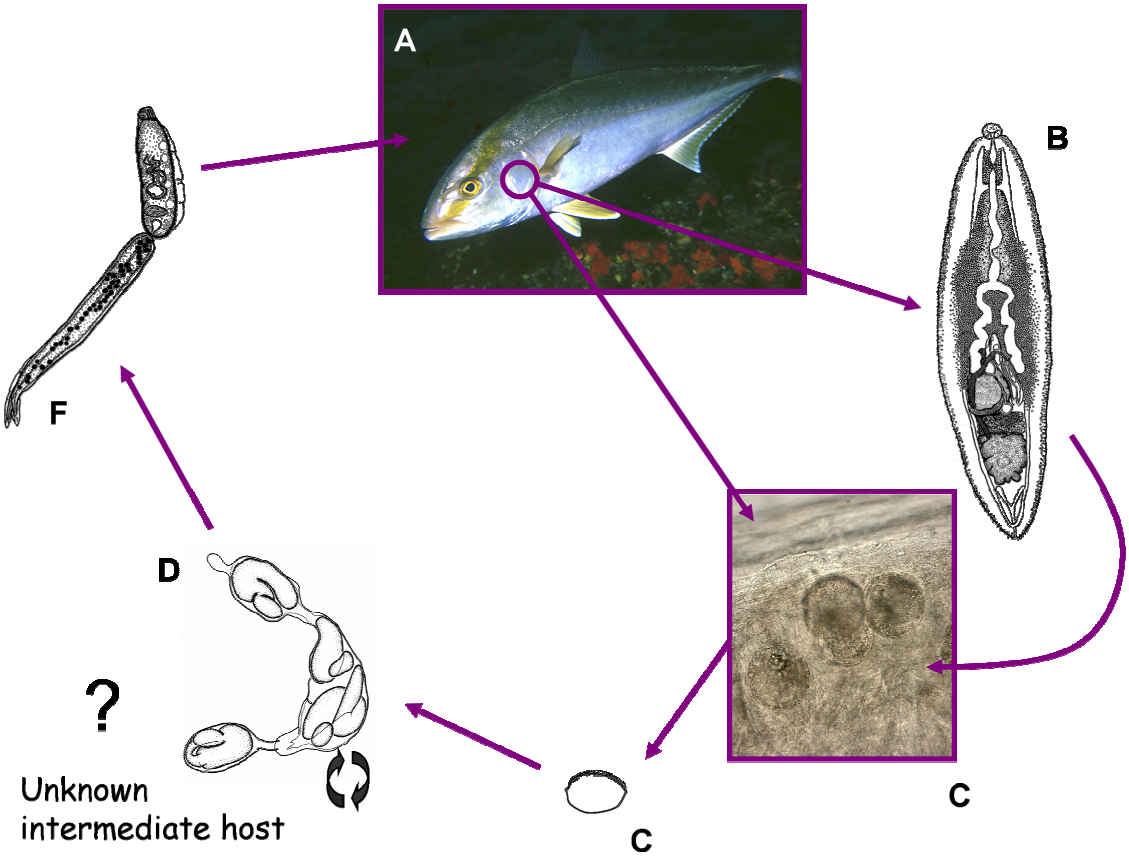


Figure 1.6. Scheme of the life-cycle of an aporocotylid species based on Smith, 1972; 1997a. Invertebrate intermediary hosts are unknown (?). A) Definitive host (greater amberjack); B & C) Adult and eggs found in the definitive host; D) Miracidium; E) Sporocysts and redia; F) Cercaria. Picture A obtained from www.fishbase.org; Pictures B, E & F obtained from Nolan & Cribb, 2004a.

1.4. This study

This study has been carried out within the framework of 4 projects, 2 of them dedicated to diseases of greater amberjack as a new candidate for aquaculture: *i)* 'Transmission, prophylaxis and treatment of *Zeuxapta seriolae* in *Seriola dumerili* cultures in the Mediterranean Sea' (funded by the Spanish Government) and, *ii)* 'Parasite pathogens in new species of Mediterranean aquaculture: an experimental approach' (funded by the European Union); and two of them dedicated to the study of the diseases of sparids of commercial interest: *iii)* 'Parasite pathogens of the sharpsnout seabream: transmission to the gilthead seabream and risks' (funded by Spanish Government) and *iv)* 'Valencian network of investigation and development of pathologies in aquaculture' (funded by Valencian Government).

The first two projects aimed to determine the biological features of the pathogenic species infecting *S. dumerili* in the Mediterranean Sea. Following this purpose, a large parasite taxonomical dataset from western Mediterranean localities has been compiled, and potentially pathogenic species have been identified. Moreover, the periodical sampling has also enabled to describe changes in the parasite community through the year. The other projects were focused on the attainment of biological data on metazoan parasites causing material and economic losses in cultures of sparids (gilthead and sharpsnout seabreams) in order to avoid crossed infections and to develop efficient treatments against them. On this purpose, the biological features of parasites shared by these two host species have been analysed.

Consequently, this research attempts to provide helpful information for the management of parasite diseases of greater amberjack and gilthead seabream in culture conditions by addressing the following questions:

(*i*) What species constitute the parasite community of greater amberjack in western Mediterranean?

(*ii*) Are there temporal variations in the parasite community off Majorca?

(*iii*) Which are the potentially pathogenic parasite species for greater amberjack?

(*iv*) What is the chronology of development of *Sparicotyle chrysophrii*, parasite of the gilthead seabream?

(*v*) How could the acquired knowledge be used to fight against this parasitic infection?

CHAPTER 2.

Aim and objectives

AIM

The aim of current study is two-fold: *i)* to improve the knowledge on the metazoan parasite diversity of the greater amberjack off western Mediterranean, and *ii)* to provide new taxonomical and biological data on the most hazardous species infecting greater amberjack and gilthead seabream in cultures (blood flukes and monogeneans) in order to prevent and manage infections.

OBJECTIVES

In order to address this aim, the following objectives have been undertaken:

I.- To review the metazoan parasite fauna of *Seriola dumerili* in the western Mediterranean Sea based on an exhaustive sampling in different localities of this region.

II.- To explore the seasonal occurrence of metazoan parasites infecting wild *S. dumerili* off Majorca focusing especially in seasonal patterns of those parasite species pathogenic in culture conditions.

III.- To determine the identity of the Mediterranean blood flukes (*Paradeontacylix* spp.) from *S. dumerili* (off Balearic Islands and Iberian Peninsula), as well as their phylogenetic relationship with the known species of the genus.

IV.- To analyse the seasonal population dynamics of *Zeuxapta seriolae* (Monogenea, Heteraxinidae) parasitising wild *S. dumerili* off Majorca and to provide comparative data for populations of juvenile fish from other localities in western Mediterranean.

V.- To study the oncomiracidial development, survival and swimming behaviour of the microcotylid monogenean *Sparicotyle chrysophrii* in order to improve disease control.

VI.- To describe the morphological post-larval changes of *S. chrysophrii* during the development, to compare the growth of this parasite with that of other microcotylids and heteraxinids and to optimize the treatment design using the chronological data obtained.

CHAPTER 3.
General materials and methods

This chapter has been structured in two sections in accordance with the two host species examined: the greater amberjack (chapters 4, 5 & 6) and the gilthead seabream (chapters 7 & 8).

3.1. Parasitological studies on greater amberjack

3.1.1. Fish samples

Two hundred and forty-five greater amberjacks, *Seriola dumerili*, from four western Mediterranean localities were obtained between April 2005 and April 2007 (see fig. 3.1 and table 3.1). The 165 specimens of the main sample, were mostly captured between Cala Figuera and Cabrera (39º8'-21'N/2º42'-3º02'E) and acquired from fish markets off Majorca (Balearic Islands, Spain) (see table 3.1 and fig. 3.1). These fish (33.0 to 42.0 cm TL) were bimonthly collected (except for the closed-fishing period from July to September) in eleven subsamples of 15 juvenile specimens, mostly young-of-the-year but also some months older individuals. Two additional samples of different fish size were also collected from this locality with the purpose of examine the parasite fauna in other fish ages and especially focusing on the monogenean *Zeuxapta seriolae*: 10 small juveniles (also referred as "smaller fish": 26.5 - 29.5 TL) in September 2005; and 10 large juveniles (also referred as "larger fish": 43.5 - 48.5 TL) in July 2006. Moreover, to improve the knowledge on the parasite fauna of the western Mediterranean fish, samples from other localities were also collected: two samples of 15 individuals each were obtained off Alicante 38º11'N/0º33'W (Iberian Peninsula); one sample of 15 fishes from Corsica 41º55'N/8º44'W and one sample of 15 fishes from Sardinia 39º12'N/9º6'W.

Table 3.1. Samplings of greater amberjacks, *Seriola dumerili*, in different localities from western Mediterranean Sea (sample size included). All fish were juveniles, from 33 to 42 cm total length, except when other lengths are indicated (*see footnote).

Locality	Samples	N of fish
Majorca		165
	• April, 2005; June, 2005; October, 2005; December, 2005	
	• February, 2006; April, 2006; June, 2006; September, 2006; November, 2006	
	• February, 2007; April, 2007	
***additional sizes**		20
	• September, 2005	
	• July, 2006	
Alicante		30
	• November, 2005	
	• February, 2006	
Corsica		15
	• August , 2005	
Sardinia		15
	• July, 2006	

* 10 small juveniles (26.5 - 29.5 TL) in September 2005; and 10 large juveniles (43.5 - 48.5 TL) in July 2006.

Figure 3.1. Map of the western Mediterranean indicating the sampling localities

All fish were first measured and weighted and parasites were collected according to a standardized protocol. Fish external surface, including skin, fins, oral and opercular cavity, was first examined for ectoparasites. Thereafter, four fish of each sample were analysed in fresh, the rest were kept frozen and parasitological dissections were carried out during the following days. Gills of fresh or thawed fish were removed and each gill arch was isolated and examined under the stereomicroscope up to 100X magnification, in order to find gill ectoparasites (i.e. monogeneans and copepods) or superficial endoparasites (didymozoids). Gill filaments were separated from the branchial arches and observed with transmitted light in order to find blood fluke eggs. The circulatory system and strongly irrigated organs, including vena caudalis, sinus venosus plus Cuvier ducts, heart, conus arteriosus, gill arch vessels, kidney, spleen and liver were scrutinized for the presence of blood flukes and eggs. Thoracic and pelvic girdles were also examined, following the suggestion of Montero *et al.* (2003) that girdles could be a target organ for some blood flukes.

All organs were examined for gross pathologies and those damaged were removed and fixed in 10% buffered-formalin. Brain, swim bladder and gonads were isolated, squashed with a small Petri dish and analysed separately under the stereomicroscope. The digestive tract was removed and stomach, caeca, and intestine were isolated, opened and washed with physiological saline. The contents were analysed and parasites were collected. Finally, in order to look for encapsulated parasites or metacercariae, visceral organs were also squashed with a Petri dish and observed under

the stereomicroscope at up to 100X magnification. Most parasites were collected, fixed and stored in ethanol 70%. Fresh helminth parasites were fixed in hot ethanol 70% for detailed morphological studies. Moreover, some specimens were fixed in ethanol 100% when required for molecular analysis.

Seasonal population dynamics of some of the parasite species recovered were explored. For this purpose, registers of Majorcan sea water temperature were kindly provided by State Ports System of the Ministry of Development, Spanish Government.

3.1.2. Morphological analyses

All parasites were identified and counted. Platyhelminthes were stained with iron acetocarmine (Georgiev *et al.*, 1986), dehydrated in ethanol series and mounted on slides with Canada balsam. Most of the nematodes and crustaceans were examined and identified in fresh but some of them were fixed and kept in ethanol 70% for posterior analyses. Large specimens were cleared with lactophenol or lactic acid, mounted on temporary slides and observed under a light microscope at 50-1000X magnification. Ten adult specimens of each species (when it was possible) were drawn and measured with the aid of a drawing tube. Moreover, digital images were obtained and analysed using Image Tools for Windows 3.00. Co. 1995-2002, UTHSCSA. Drawings were designed and provided in new species descriptions. Measurements are given in microns as the mean ± standard deviation (SD) followed by ranges in parentheses. Type-specimens of new species were deposited in the Meguro Parasitological Museum of Japan and the Natural History Museum of London, UK.

Ecological terms follow Bush *et al.* (1997). Prevalences, abundances and intensities were calculated. Species were classified into 3 categories according to their total mean prevalence: (*i*) common species >30% prevalence; (*ii*) rare species, from 10 to 30% prevalence and, (*iii*) accidental species, less than 10% prevalence. Parasite specificity was assessed after an exhaustive review of available publications including the Host-Parasite database (Gibson *et al.*, 2005)

3.1.3. Molecular analysis

Molecular data were only provided for new species. Genomic DNA was extracted from two individual worms each from the Iberian and the Balearic *Paradeontacylix* morphotypes from *S. dumerili*. Fixed specimens were transferred to 300 µl TNES urea (10 mM Tris-HCl (pH 8), 125 mM NaCl, 10 mM ethylenediaminetetra-acetic acid (EDTA), 0.5% sodium dodecyl sulphate (SDS), 4 M urea) and DNA was extracted using a phenol-chloroform protocol as described in Holzer *et al.* (2004). DNA of ribosomal and mithocondrial gene regions, i.e. 28S rDNA region, Internal

Transcribed Spacer 2 (ITS2) region and the mitochondrial Cytochrome C Oxidase 1 (COI), depending on species, were amplified using the primers listed in table 2 of chapter 5. The same procedure was carried out with one/two specimens of *P. kampachi* and *P. grandispinus* from *Seriola dumerili* off Japan (Ushine, Kagoshima Prefecture, 31°33'N/130°43'E, June 2006), and two individuals of *P. godfreyi* from *S. lalandi* off Australia (Port Augusta, South Australia, 32° 42'S/137° 46'E, August 2006).

Each 30 µl of PCR reaction volume containing 1.5 U of Thermoprime Plus DNA polymerase and 10X buffer with 1.5 mM MgCl2 (ABgene), 0.2 mM of each dNTP, 15 pmol of each primer and 50–80 ng of template DNA. Denaturation of DNA (95°C for 2 min) was followed by 35 cycles of amplification (95°C for 50 s, annealing temperature for 50 s and extension at 72°C for 50 s) and terminated by 8-min of extension hold (72°C). Annealing temperature was 56°C for the 28S reactions, 58°C for the ITS2 and 50°C for the CO1 ones. PCR products obtained were purified for sequencing using QIAquick PCR purification kit (Qiagen). PCR fragments were cycle-sequenced from both strands using Genetic Analyzer 3130xl (Applied Biosystems). Nucleotide sequences were submitted to the GenBank™ database and accession numbers are reported in chapter 5. Specific phylogenetic analyses were carried out with several software programs: PAUP* v.4.0b10 (Swofford, 2002), Clustal X (Thompson *et al.* 1997), Modeltest v.3.7 (Posada & Candall, 1998) and Mr Bayes (Ronquist and Huelsenbeck, 2003).

3.1.4. Statistical analyses

Statistical analyses for quantitative data were performed using Quantitative Parasitology program v.3.0 (QP3.0, Reiczigel & Rozsa, 2005), CPC (Phillips, 1994-7), SPSS® v. 15.0 (SPSS, Inc., 1989-2006) and Statistica v. 8.0 (StatSoft, Inc., Tulsa, OK, USA). Total mean prevalence and intensity of infection, as well as confidence intervals were calculated for each subsample in each locality. Spearman's correlations between mean monthly prevalence and/or mean monthly intensity of infection and mean monthly seawater temperature were also tested for some parasite species. Fisher's exact test was performed when required to compare prevalences of subsamples. Particular features analysed and comparisons tested are indicated in the material and methods of each chapter.

3.2. Parasitological studies on *Sparicotyle chrysophrii* infecting gilthead seabream

3.2.1. Oncomiracidial development and hatching

Sparicotyle chrysophrii eggs were obtained from gravid worms collected from cultured gilthead seabreams, *Sparus aurata*, off Valencia (Spain). Worms were isolated from the gills and eggs were collected, randomly distributed into wells and maintained at 20 °C with alternating 12 h periods of fluorescent light and darkness (LD 12:12). Eggs were examined using a stereomicroscope and a research microscope with bright field and differential interference contrast optics (DIC).

First, two preliminary replicates were performed to find the approximate timings of the main developmental events of *S. chrysophrii* and then, detailed observations at short time intervals were obtained in the two definitive replicates. Preliminary replicates were carried out with 250 eggs that were observed on a slide every 24 hours post deposition until 5 days post deposition and every 6 h until the earliest egg hatching. In the definitive replicates, 200 eggs were observed every 8 h until 5 days post deposition and every 4 h until the first egg hatching. Then, eggs and oncomiracidia were observed every 2 h. The first hatching and the number of hatchings were registered in all the replicates. Oncomiracidial morphological changes were recorded and hatching success was determined. Differences between the number of oncomiracidia emerging during light and darkness as well as differences between the numbers of hatchings recorded during each of the different light or dark periods were analysed.

To study the survival and swimming behaviour of the oncomiracidia, 155 specimens were followed up periodically. Data on survival time and swimming period were recorded for each specimen. A correlation was calculated between incubation time and survival time or swimming period. Moreover, differences in survival and swimming times of oncomiracidia emerging during light and those emerging during darkness were tested. To characterise the vitality of oncomiracidia, specimens were grouped according to 7 behaviour patterns (from 0 to 6, numbered from low to high activity): 0, dead oncomiracidia; 1, alive without cilia movement; 2, whirling or static but all or some ciliary movement; 3, slow swimming on the bottom; 4, fast swimming on the bottom; 5, swimming slowly up and down; 6, swimming fast up and down. Statistical tests were performed using SPSS® v. 15.0 (SPSS, Inc., 1989-2006).

3.2.2. Post-larval development

Seventy-six *S. chrysophrii*-infected gilthead seabreams, were collected from sea-cages of a fishfarm off Murcia, Spain and transported alive to the Central Service for the Support to

Experimental Research ("Servei Central de Suport a la Investigació i Experimental", –SCSIE–, University of Valencia). Experimental fish were arranged separately in four groups of 19 individuals/each and reared into four isolated tanks (250 l volume) at 20°C ("infected tanks"). Fish were dewormed and temporarily reared in four "parasite-free" tanks (250 l volume each). After three days, the experimental fish were returned to the four "infected tanks" and 1000 extra eggs were added to the water (250 eggs per tank). From this moment, four fish, one from each tank, were killed and analysed, first after 3 dpi (days post infection) and then every two days for 21 dpi. Thereafter, as main developmental events had occurred, fish were sampled every 5 days until 36 dpi when second generation larvae were observed attached to the gills. The remaining fish (N=16) were sampled at 51 dpi.

Parasites were collected and the developmental stage of each specimen was recorded, paying special attention to the haptor, gut and genitalia changes. Morphological traits of *S. chrysophrii* and their changes during development were analysed and the growth rate was calculated. Finally, chronology of development and morphological changes of this and other microcotylid species were compared Statistical tests were performed using SPSS® v. 15.0 (SPSS, Inc., 1989-2006).

CHAPTER 4.

Parasite fauna of *Seriola dumerili*

4.1. Introduction

Many studies on the parasite fauna of *Seriola* spp. have been published worldwide, *S. dumerili*, *S. lalandi* and *S. quinqueradiata* being the most studied species (Egusa, 1992; Hutson *et al.*, 2007a; Williams & Bunkley-Williams, 1996). Among the taxonomical studies on metazoan parasites of *S. dumerili* the great contribution of Prof. S. Yamaguti should be highlighted, as he described 14 of the 84 species reported to date (see checklist in the present chapter). The first check-list on the parasite fauna of greater amberjack (*S. dumerili*) was compiled by Williams & Bunkley-Williams (1996) within a parasite guide for fishermen of Atlantic Ocean and Puerto Rico. However, in this publication the authors reported that "*S. dumerili* has also been called *S. lalandi*" and, consequently, some of the species therein listed for *S. dumerili* could also be parasites of *S. lalandi*. Williams & Bunkley-Williams (1996) compiled 42 pathogen species: 37 metazoans, 1 protozoan and 4 bacteria. These species were mainly reported from western Atlantic Ocean (N= 21) but also from Pacific Ocean (N= 12), western Africa (N= 2), Red Sea (N= 1), and Mediterranean Sea (N= 4). After Williams & Bunkley-Williams (1996), no additional worldwide reviews of parasites of *S. dumerili* have been published although many new species have been described, especially in the Mediterranean region (e.g. Bartoli *et al.*, 2004; Bartoli & Bray, 2004).

The first parasitological survey on greater amberjack from western Mediterranean was provided by Grau *et al.* (1999) who found 14 parasite species in fish from Majorca, Tarragona and the Valencian Gulf (Balearic Sea) collected between 1995 and 1996. Thereafter, Montero (2001) and Montero *et al.*, (2001b), reported 18 parasite species from greater amberjacks off a more southern locality, Murcia (Iberian Peninsula). Interestingly, the parasite faunas reported in both studies showed important differences considering the short distance among localities and the migratory behaviour of the greater amberjack (Dempster, 2005; Froese & Pauly, 2011).

The aim of this study is to describe and review the parasite fauna of greater amberjack in the western Mediterranean, and to explore the differences among the species reported in each locality. Moreover, this study also aims at getting preliminary and descriptive records on seasonal occurrence of parasites infecting wild greater amberjacks from Balearic Islands, especially those pathogenic in culture conditions.

4.2. Materials and methods

Materials and methods of this chapter are reported in section 2.1 of the general materials and methods. Moreover metazoan parasites of *Seriola dumerili* reported to date have been compiled in a check-list which is included in the appendix of the present PhD study.

4.3. Results

A total of 44921 metazoan parasites belonging to 26 species were recovered from the four Mediterranean localities sampled: Majorca, Alicante, Corsica and Sardinia (see table 4.1). Infection parameters of the parasite species found in each locality are reported in table 4.1. Data from the main sample (Majorca) is also indicated by each monthly subsample in table 4.2. Twenty-six parasite species from three different phyla (Platyhelminthes, Nematoda and Arthropoda) were collected; five of them were ectoparasites while 21 were endoparasites. Ectoparasites belong to four different families: Heteraxinidae (Platyhelminthes, Monogenea); Caligidae and Lernaeopodidae (Arthropoda, Copepoda); and Gnathiidae (Arthropoda, Isopoda). Endoparasites belong to: seven trematode families (Acanthocolpidae, Aporocotylidae, Bucephalidae, Didymozoidae, Hemiuridae, Heterophyidae and Strigeidae); two cestode families (Lacystorhynchidae and Tetraphyllidea fam.); and four nematode families (Camallanidae, Cucullanidae, Raphidascarididae and Trichinellidae). Each family was represented by one species except for Acanthocolpidae which was represented by five species and Aporocotylidae, Bucephalidae and Caligidae which were represented by two. Additionally, the ostracod *Skoksbergia costai*, a scavenger species, was also recorded in two of the examined localities (Majorca and Alicante).

According to their prevalences, three groups of parasite species were distinguished in the sample from Majorca (see table 4.1). (*I*) Nine species were considered common (prevalence over 30%): *Bucephalus gorgon*, *Neometanematobothrioides periorbitalis* and *Zeuxapta seriolae*, with total mean prevalence over 70%; *Caligus aesopus*, *Gnathia vorax*, *Paradeontacylix balearicus* (eggs), *Stephanostomum ditrematis* and *Tormopsolus orientalis*, with prevalence between 50 - 70%; and *Hemiurus communis* with prevalence between 30 - 50% (*II*) Two species were considered rare (prevalences from 10 to 30%): *Hysterothylacium seriolae* and *Stephanostomum filiforme*; and. (*III*) Thirteen species were considered accidental (prevalences below 10%): *Aphanurus* sp., *Caligus* sp., *Camallanus* sp., *Capillaria* sp., *Cardiocephaloides* sp., *Cucullanus* sp., *Floriceps saccatus sp.*, *Galactosomum* sp., *Parabrachiella seriolae*, *Prosorhynchus facilis*, *Stephanostomum euzeti*, *Stephanostomum petimba* and the Tetraphyllidean larva.

Specificity of parasites of greater amberjack from western Mediterranean found in current study is indicated in table 4.1. Among the described species ten were generalist, infecting fish from at least two teleost families (*H. seriolae* and *S. ditrematis* were classified as generalists although they mainly infect *Seriola* spp. or Carangidae spp. respectively), one was a specialist of Carangidae, three were specialist of *Seriola*, and five were specialist of *Seriola dumerili*. Seven additional species could not be classified as they were only identified to generic level.

Species found in the present study are described below and additional information on their occurrence is provided. They have been arranged in five groups: new species for science, new host and locality records, new host records of Mediterranean species, new locality records, and species previously reported in *S. dumerili* from the Mediterranean Sea. Finally the description of the scavenger *Skogsbergia costai* has also been included.

Table 4.1. Metazoan parasites and scavenger of *Seriola dumerili* from 4 western Mediterranean localities. Mean abundance (MA ± SD) and mean intensity (MI ± SD) and prevalence (expressed in percent, with 95% confidence interval) of parasites of *Seriola dumerili* from different Mediterranean localities. Specificity: Ss, *Seriola dumerili* specialist; Gs, *Seriola* specialist; Fs, Carangidae specialist; G, generalist. Occurrence in this study: Common (C); Rare (R); Accidental (A). Number of sampled fish in parentheses.

	Occurrence	Specificity	Majorca		Alicante			Corsica		Sardinia	
			Parasite number	April '05-'07 (N=165)	Parasite number	November '05 (N=15)	February '06 (N=15)	Parasite number	August '05 (N=15)	Parasite number	April '06 (N=15)
PHYLLUM PLATYHELMINTHES											
Zeuxapta seriolae	C	Fs	25831		359			1357		156	
MA ± SD				156.55 ± 253.34		8.46 ± 7.94	15.47 ± 13.61		90.46 ± 63.34		10.40 ± 9.64
MI ± SD				172.21 ± 245.89		10.58 ± 7.47	17.84 ± 13.04		90.46 ± 63.34		13.00 ± 9.02
Prevalence				90.3 (84.6-94)		80.0 (53.4-94.3)	86.7 (60.3-97.6)		100 (77.8-100)		80.0 (53.4-94.3)
***Aphanurus* sp.**	A	-	1		-			-		-	
MA ± SD				0.01 ± 0.07		-	-		-		-
MI ± SD				1.00		-	-		-		-
Prevalence				0.6 (0-3.5)		-	-		-		-
Bucephalus gorgon	C	Ss	7638		166			125		2096	
MA ± SD				46.29 ± 126.74		51.06 ± 31.25	55.13 ± 73.171		8.33 ± 7.11		139.73 ± 107.78
MI ± SD				57.00 ± 138.54		51.06 ± 31.25	61.92 ± 76.01		11.36 ± 6.75		139.73 ±107.78
Prevalence				74.6 (67.3-80.7)		100 (77.8-100)	80.0 (53.4-94.3)		66.7 (39.7-85.8)		100 (77.8-100)
***Cardiocephaloides* sp. (metacercaria)**	A	-	25		-			-		-	
MA ± SD				0.15 ± 0.67		-	-		-		-
MI ± SD				3.00 ± 0.87		-	-		-		-
Prevalence				5.5 (2.8-10.2)		-	-		-		-
***Galactosomum* sp. (metacercaria)**	A	-	3		-			-		-	
MA ± SD				0.02 ± 0.23		-	-		-		-
MI ± SD				3.00		-	-		-		-
Prevalence				0.6 (0-3.5)		-	-		-		-
Hemiurus communis	C	G	594		6			11		7	
MA ± SD				3.60 ± 8.23		0.13 ± 0.35	0.27 ± 0.47		0.73 ± 1.28		0.47 ± 1.06
MI ± SD				6.83 ± 10.34		1.00	1.00		2.20 ± 1.30		1.75 ± 1.50
Prevalence				47.9 (40.3-55.6)		13.3 (2.4-39.7)	26.7 (9.7-53.4)		33.3 (14.2-60.3)		26.7 (9.7-53.4)
***Lecithocladium* sp.**	-	-	-		1			-		-	
MA ± SD				-		0.07 ± 0.26	-		-		-
MI ± SD				-		1.00	-		-		-
Prevalence				-		6.7 (0.35-30.2)	-		-		-
Neometanematobothrioides periorbicularis	C	Ss	299		23			54		10	
MA ± SD				1.81 ± 2.23		0.47 ± 0.64	1.07 ± 0.97		3.69 ± 2.16		0.67 ± 0.90
MI ± SD				2.49 ± 2.27		1.17 ± 0.41	1.60 ± 0.70		3.86 ± 1.99		1.43 ± 0.79
Prevalence				72.7 (65.5-79.2)		40.0 (19.1-66.8)	66.7 (39.7-85.8)		93.3 (69.8-99.7)		46.7 (22.2-70.6)
Paradeontacylix balearicus	C	Ss	15								
MA ± SD				0.09 ± 0.29		-	-		-		-
MI ± SD				1.00		-	-		-		-
Prevalence				9.1 (5.3-14.5)		-	-		-		-
Paradeontacylix ibericus	-	Ss	-		8			1		-	
MA ± SD				-		0.20 ± 0.41	0.33 ± 0.49		0.07 ± 0.26		-
MI ± SD				-		1.00	1.00		1.00		-
Prevalence				-		20.0 (5.7-46.6)	33.3 (14.2-60.3)		6.7 (0.4-30.2)		-
***Paradeontacylix* spp.** (eggs)	-	-	-		-			-		-	
Prevalence				49.5 (42.1-57.3)		86.7 (60.3-97.6)	60 (33.2-80.9)		66.7 (39.7-85.8)		53.3 (29.4-77.8)
Prosorhynchus facilis	A	G	34		-			-		-	
MA ± SD				0.21 ± 1.28		-	-		-		-
MI ± SD				3.40 ± 4.20		-	-		-		-
Prevalence				6.1 (3.2-10.8)		-	-		-		-
Stephanostomum ditrematis	C	G (Fs?)	529		15			24		79	
MA ± SD				3.21 ± 5.11		0.27 ± 0.60	0.73 ± 1.53		1.60 ± 1.73		5.27 ± 10.47
MI ± SD				5.19 ± 5..65		1.33 ± 0.58	1.83 ± 2.04		2.67 ± 1.41		7.90 ± 12.14
Prevalence				62.4 (54.6-69.7)		20.0 (5.7-46.6)	40.0 (19.1-66.8)		60.0 (33.2-80.9)		66.7 (39.7-85.8)
Stephanostomum euzeti	A	Ss	9		3			23		16	
MA ± SD				0.05 ± 0.23		-	0.20 ± 0.57		1.53 ± 2.07		1.07 ± 2.37
MI ± SD				1.00 ± 0		-	1.50 ± 0.71		3.83 ± 1.17		3.20 ± 3.35
Prevalence				4.2 (2-8.7)		-	13.3 (2.4-39.7)		40.0 (19.1-66.8)		33.3 (14.2-60.3)
Stephanostomum filiforme	R	Gs	65		-			9		19	
MA ± SD				0.40 ± 1.03		-	-		0.60±0.83		1.27 ± 2.66
MI ± SD				2.24 ± 1.41		-	-		1.50±0.55		3.17 ± 3.55
Prevalence				17.6 (12.4-24.2)		-	-		40 (19.1-66.8)		40 (19.1-66.8)

Table 4.1. (cont.) Metazoan parasites and scavenger of *Seriola dumerili* from 4 western Mediterranean localities. Mean abundance (MA ± SD) and mean intensity (MI ± SD) and prevalence (expressed in percent, %; with 95% confidence interval) of parasites of *Seriola dumerili* from different Mediterranean localities. Specificity: Ss, *Seriola dumerili* specialist; Gs, *Seriola* specialist; Fs, Carangidae specialist; G, generalist. Occurrence in this study: Common (C); Rare (R); Accidental (A). Number of sampled fish in parentheses.

	Occurrence	Specificity	Majorca		Alicante			Corsica		Sardinia	
			Parasite number	April '05-'07 (N=165)	Parasite number	November '05 (N=15)	February '06 (N=15)	Parasite number	August '05 (N=15)	Parasite number	April '06 (N=15)
Stephanostomum petimba	A	G	3		-			-		-	
MA ± SD				0.02 ± 0.13		-	-		-		-
MI ± SD				1.00		-	-		-		-
Prevalence				1.8 (0.5-5.3)		-	-		-		-
Tormopsolus orientalis	C	G	575		166			28		25	
MA ± SD				3.48 ± 5.11		1.47 ± 1.99	9.60 ± 11.84		1.87 ± 1.72		1.67 ± 3.56
MI ± SD				5.04 ± 5.48		3.14 ± 1.77	12.00 ±12.12		2.54 ± 1.51		3.13 ± 4.49
Prevalence				67.8 (60.3-74.6)		46.7 (22.2-70.6)	80.0 (53.4-94.3)		66.7 (39.7-85.8)		53.3 (29.4-77.8)
PHYLUM CESTODA											
Floriceps saccatus	A	G	3		-			-		-	
MA ± SD				0.02 ± 0.13		-	-		-		-
MI ± SD				1.00		-	-		-		-
Prevalence				1.8 (0.5-5.3)		-	-		-		-
Tetraphyllidean larva	A	-	42		-			-		-	
MA ± SD				0.25 ± 2.21		-	-		-		-
MI ± SD				4.20 ± 8.42		-	-		-		-
Prevalence				6.2 (3.2-10.8)		-	-		-		-
PHYLUM NEMATODA											
Hysterothylacium seriolae	R	G (Gs?)	84		-			2		1	
MA ± SD				0.51 ± 2.22		-	-		0.13 ± 0.35		0.07 ± 0.26
MI ± SD				3.65 ± 4.96		-	-		1.00		1.00
Prevalence				13.9 (9.3-20.2)		-	-		13.3 (2.4-39.7)		6.7 (0.35-30.2)
Camallanus sp.	A	-	1		-			-		-	
MA ± SD				0.01 ± 0.08		-	-		-		-
MI ± SD				1.00		-	-		-		-
Prevalence				0.6 (0-3.5)		-	-		-		-
***Capillaria* sp.**	A	-	2		-			-		-	
MA ± SD				0.01 ± 0.16		-	-		-		-
MI ± SD				2.00		-	-		-		-
Prevalence				0.6 (0-3.5)		-	-		-		-
Cucullanus sp.	A	-	2		-			-		-	
MA ± SD				0.01 ± 0.16		-	-		-		-
MI ± SD				2.00		-	-		-		-
Prevalence				0.6 (0-3.5)		-	-		-		-
PHYLUM ARTHROPODA											
Caligus aesopus	C	Gs	242		17			11		3	
MA ± SD				1.47 ± 1.96		0.33 ± 0.49	0.80 ± 1.01		0.73 ± 1.10		0.20 ± 0.41
MI ± SD				2.55 ± 1.97		1.00	1.33 ± 1.00		1.83 ± 0.98		1.00
Prevalence				55.7 (47.9-63.4)		33.3 (14.2-60.3)	60.0 (33.2-80.9)		53.3 (29.4-77.8)		20.0 (5.7-46.6)
Caligus sp.	A	-	1		-			-		-	
MA ± SD				0.01 ± 0.07		-	-		-		-
MI ± SD				1.00		-	-		-		-
Prevalence				0.6 (0-3.5)		-	-		-		-
Parabrachiella seriolae	A	Gs	7		1			-		-	
MA ± SD				0.04 ± 0.23		-	0.07 ± 0.26		-		-
MI ± SD				1.17 ± 0.41		-	1.00		-		-
Prevalence				3.6 (1.6-7.8)		-	6.7 (0.35-30.2)		-		-
Gnathia vorax (praniza & zuphea)	C	G	819		228			96		56	
MA ± SD				4.96 ± 14.14		0.67 ± 1.49	14.53 ± 36.92		6.43 ± 5.04		3.73 ± 8.75
MI ± SD				9.99 ± 18.91		3.33 ± 1.53	24.22 ± 46.06		9.60 ± 2.32		6.22 ± 10.79
Prevalence				50.5 (42.7-57.9)		20.0 (5.7-46.6)	60.0 (33.2-80.9)		66.7 (39.7-85.8)		60.0 (33.2-80.9)
SCAVENGER: OSTRACODA											
Skogsbergia costai	-	-	30		158			-		-	
MA ± SD				0.18 ± 1.45		-	10.60 ± 24.53		-		-
MI ± SD				7.50 ± 6.40		-	26.5 ± 34.34		-		-
Prevalence				2.4 (0.8-6.2)		-	40.0 (19.1-66.8)		-		-

Table 4.2. Metazoan parasites of *Seriola dumerili* from Majorca. Data from main fish sample (165 specimens) collected in 11 subsamples of 15 specimens each. Mean abundance (MA ± SD), mean intensity (MI ± SD) and prevalence (expressed in percent; with 95% confidence interval).

	2005					2006				2007		TOTAL
	April	June	October	December	February	April	June	September	November	February	April	
PHYLUM PLATYHELMINTHES												
MONOGENEA												
Zeuxapta seriolae												
MA ± SD	628.87 ± 262.04	455.80 ± 363.20	3.93 ± 4.13	5.60 ± 5.54	3.40 ± 3.13	247.47±134.92	273.47±137.47	5.47±6.09	3.53±3.60	5.80±7.25	88.73 ± 47.06	156.55 ± 253.34
MI ± SD	628.87 ± 262.04	455.80 ± 363.20	4.21 ± 4.14	6.46 ± 5.46	4.64 ± 2.73	247.47±134.92	273.47±137.47	7.45±5.97	4.42±3.50	6.69±7.41	88.73 ± 47.06	173.36 ± 261.13
Prevalence	100 (77.8-100)	100 (77.8-100)	100 (69.8-99.7)	86.7 (60.3-97.6)	73.3 (46.6-90.3)	100 (77.8-100)	100 (77.8-100)	73.3 (46.6-90.3)	80 (53.4-94.3)	86.7 (60.3-97.6)	100 (77.8-100)	90.3 (84.6-94)
TREMATODA												
***Aphanurus* sp.**												
MA ± SD	-	-	-	-	0.07 ± 0.26	-	-	-	-	-	-	0.01 ± 0.07
MI ± SD	-	-	-	-	1.00	-	-	-	-	-	-	1.00
Prevalence	-	-	-	-	6.7 (0.35-30.2)	-	-	-	-	-	-	0.6 (0-3.5)
Bucephalus gorgon												
MA ± SD	10.13 ± 18.37	4.13 ± 6.91	111.47 ± 260.87	18.93 ± 25.82	12.33 ± 19.14	42.07 ± 75.40	133.26 ± 171.36	11.73 ± 12.80	123.53 ± 224.14	29.13 ± 74.13	12.46 ± 22.54	46.29 ± 126.74
MI ± SD	15.20 ± 20.96	6.2 ± 7.74	128.61 ± 277.50	20.28 ± 26.24	16.81 ± 20.74	42.07 ± 75.40	166.58 ± 176.97	16.00 ± 12.42	123.53 ± 224.15	36.41 ± 81.89	17.00 ± 25.04	57.00 ± 138.54
Prevalence	66.7 (39.7-85.8)	66.7 (39.7-85.8)	86.7 (60.3-97.6)	100 (69.8-99.7)	66.7 (39.7-85.8)	66.7 (39.7-85.8)	73.3 (46.6-90.3)	80 (53.4-94.3)	100 (77.8-100)	53.3 (29.4-77.8)	66.7 (39.7-85.8)	74.6 (67.3-80.7)
***Cardiocephaloides* sp.**												
MA ± SD	0.20 ± 0.77		0.40 ± 1.06	0.53 ± 1.46	0.13 ± 0.52				0.40 ± 0.91			0.15 ± 0.67
MI ± SD	3.00		3.00	4.00 ± 1.41	2.00				2.00 ± 1.00			3.00 ± 0.87
Prevalence	6.7 (0.35-30.2)	-	13.3 (2.4-39.7)	13.3 (2.4-39.7)	6.7 (0.35-30.2)	-	-	-	20.0 (5.7-46.6)	-	-	5.5 (2.8-10.2)
***Galactosomum* sp.**												
MA ± SD		0.20 ± 0.77										0.02 ± 0.23
MI ± SD		3.00										3.00
Prevalence	-	6.7 (0.35-30.2)	-	-	-	-	-	-	-	-	-	0.6 (0-3.5)
Hemiurus communis												
MA ± SD	5.20 ± 8.13	0.73 ± 2.09	0.87 ± 2.13	8.53 ± 11.91	14.20 ± 17.25	2.00 ± 1.97	0.53 ± 0.83	0.33 ± 0.62	0.93 ± 1.67	4.00 ± 7.99	1.26 ± 1.79	3.60 ± 8.23
MI ± SD	8.67 ± 9.04	3.67 ± 3.78	2.60 ± 3.21	10.67 ± 12.48	19.36 ± 17.51	3.00 ± 1.63	1.14 ± 0.90	1.25 ± 0.50	2.33 ± 1.97	7.50 ± 8.72	1.90 ± 1.91	6.83 ± 10.34
Prevalence	60.0 (33.2-80.9)	20.0 (5.7-46.6)	26.7 (9.7-53.4)	80 (53.4-94.3)	40.0 (19.1-66.8)	66.7 (39.7-85.8)	40.0 (19.1-66.8)	26.7 (9.7-53.4)	33.3 (14.2-60.3)	73.3 (46.6-90.3)	60.0 (33.2-80.9)	47.9 (40.3-55.6)
Neometanematobothrioides periorbicularis												
MA ± SD	2.60 ± 2.87	3.86 ± 4.31	1.20 ± 1.15	1.27 ± 1.67	0.87 ± 1.68	1.67 ± 1.88	2.20 ± 1.93	1.80 ± 1.78	1.46 ± 1.50	1.13 ± 1.19	1.87 ± 1.73	1.81 ± 2.23
MI ± SD	2.60 ± 2.87	3.87 ± 4.31	1.80 ± 0.92	2.71 ± 1.38	2.60 ± 2.07	2.27 ± 1.85	2.36 ± 1.91	2.70 ± 1.49	2.00 ± 1.41	1.70 ± 1.06	2.33 ± 1.61	2.49 ± 2.27
Prevalence	100 (77.8-100)	100 (77.8-100)	66.7 (39.7-85.8)	46.7 (22.2-70.6)	33.3 (14.2-60.3)	73.3 (46.6-90.3)	100 (69.8-99.7)	66.7 (39.7-85.8)	73.3 (46.6-90.3)	66.7 (39.7-85.8)	80 (53.4-94.3)	72.7 (65.5-79.2)
Paradeontacylix balearicus												
MA ± SD	0.07 ± 0.26	-	0.07 ± 0.26	0.13 ± 0.35	0.27 ± 0.46	0.13 ± 0.35	-	-	-	0.13 ± 0.35	0.20 ± 0.41	0.09 ± 0.29
MI ± SD	1.00	-	1.00	1.00	1.00	1.00	-	-	-	1.00	1.00	1.00
Prevalence	6.7 (0.35-30.2)	-	6.7 (0.35-30.2)	13.3 (2.4-39.7)	26.7 (9.7-53.4)	13.3 (2.4-39.7)	-	-	-	13.3 (2.4-39.7)	20.0 (5.7-46.6)	9.1 (5.3-14.5)
***Paradeontacylix balearicus* (eggs)**												
Prevalence	66.7 (39.7-85.8)	100 (69.8-99.7)	13.3 (2.4-39.7)	33.3 (14.2-60.3)	53.3 (29.4-77.8)	80 (53.4-94.3)	73.3 (46.6-90.3)	13.3 (2.4-39.7)	26.7 (9.7-53.4)	33.3 (14.2-60.3)	53.3 (29.4-77.8)	49.5 (42.1-57.3)
Prosorhynchus facilis												
MA ± SD	0.33 ± 0.72	0.07 ± 0.26	-	-	0.2 ± 1.21	0.40 ± 1.12	-	-	0.07 ± 0.26	-	1.20 ± 3.89	0.21 ± 1.28
MI ± SD	1.67 ± 0.58	1.00	-	-	3.00	3.00 ± 1.41	-	-	1.00	-	9.00 ± 8.49	3.40 ± 4.20
Prevalence	20.0 (5.7-46.6)	6.7 (0.35-30.2)	-	-	6.7 (0.35-30.2)	13.3 (2.4-39.7)	-	-	6.7 (0.35-30.2)	-	13.3 (2.4-39.7)	6.1 (3.2-10.8)
Stephanostomum ditrematis												
MA ± SD	2.47 ± 5.19	3.80 ± 4.24	2.47 ± 3.56	3.33 ± 3.35	0.60 ± 0.99	2.87 ± 4.80	3.33 ± 3.08	2.47 ± 3.27	6.13 ± 7.13	1.47 ± 2.40	6.33 ± 10.16	3.21 ± 5.11
MI ± SD	6.17 ± 6.94	4.75 ± 4.25	3.70 ± 3.83	4.17 ± 3.24	1.80 ± 0.84	6.14 ± 5.52	3.85 ± 3.00	3.36 ± 3.41	11.50 ± 5.58	3.67 ± 2.50	7.92 ± 10.85	5.19 ± 5..65
Prevalence	40.0 (19.1-66.8)	80 (53.4-94.3)	73.3 (46.6-90.3)	80 (53.4-94.3)	33.3 (14.2-60.3)	46.7 (22.2-70.6)	86.7 (60.3-97.6)	73.3 (46.6-90.3)	53.3 (29.4-77.8)	40.0 (19.1-66.8)	80 (53.4-94.3)	62.4 (54.6-69.7)
Stephanostomum euzeti												
MA ± SD	0.07 ± 0.26	-	-	-	-	-	-	0.07 ± 0.26	0.13 ± 0.35	0.13 ± 0.35	0.13 ± 0.35	0.05 ± 0.23
MI ± SD	1.00	-	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00
Prevalence	6.7 (0.35-30.2)	-	-	-	-	-	-	6.7 (0.35-30.2)	6.7 (0.35-30.2)	13.3 (2.4-39.7)	13.3 (2.4-39.7)	4.2 (2-8.7)
Stephanostomum filiforme												
MA ± SD	0.53 ± 1.13	0.20 ± 0.56	0.27 ± 0.70	0.33 ± 0.72	0.13 ± 0.52	0.67 ± 1.76	0.33 ± 0.82	0.40 ± 0.91	0.73 ± 1.39	0.07 ± 0.26	0.67 ± 1.59	0.40 ± 1.03
MI ± SD	2.00 ± 1.41	1.50 ± 0.71	2.00	1.67 ± 0.58	2.00	5.00	1.67 ± 1.15	1.50 ± 1.29	2.75 ± 1.26	1.00	3.33 ± 2.08	2.24 ± 1.41
Prevalence	26.7 (9.7-53.4)	13.3 (2.4-39.7)	13.3 (2.4-39.7)	20.0 (5.7-46.6)	6.7 (0.35-30.2)	13.3 (2.4-39.7)	20.0 (5.7-46.6)	26.7 (9.7-53.4)	26.7 (9.7-53.4)	6.7 (0.35-30.2)	20.0 (5.7-46.6)	17.6 (12.4-24.2)
Stephanostomum petimba												
MA ± SD	-	-	-	0.07 ± 0.26	0.07 ± 0.26	-	-	-	0.07 ± 0.26	-	-	0.02 ± 0.13
MI ± SD	-	-	-	1.00	1.00	-	-	-	1.00	-	-	1.00
Prevalence	-	-	-	6.7 (0.35-30.2)	6.7 (0.35-30.2)	-	-	-	6.7 (0.35-30.2)	-	-	1.8 (0.5-5.3)

Table 4.2. (cont.) Metazoan parasites of *Seriola dumerili* from Majorca. Data from main fish sample (165 specimens) collected in 11 subsamples of 15 specimens. Mean abundance (MA ± SD), mean intensity (MI ± SD) and prevalence (expressed in percent; with 95% confidence interval).

	2005					2006				2007		TOTAL
	April	June	October	December	February	April	June	September	November	February	April	
Tormopsolus orientalis												
MA ± SD	2.53 ± 5.57	3.13 ± 3.48	6.73 ± 8.78	6.53 ± 6.99	1.80 ± 3.30	2.67 ± 4.67	1.47 ± 1.81	0.73 ± 0.70	3.87 ± 2.47	3.47 ± 4.13	5.4 ± 5.94	3.48 ± 5.11
MI ± SD	4.75 ± 7.09	3.62 ± 3.50	8.42 ± 9.10	8.91 ± 6.71	3.38 ± 3.96	5.71 ± 5.30	2.44 ± 1.74	1.22 ± 0.45	4.46 ± 2.07	4.73 ± 4.17	6.23 ± 5.97	5.04 ± 5.48
Prevalence	53.3 (29.4-77.8)	86.7 (60.3-97.6)	80 (53.4-94.3)	73.3 (46.6-90.3)	53.3 (29.4-77.8)	46.7 (22.2-70.6)	60.0 (33.2-80.9)	60.0 (33.2-80.9)	86.7 (60.3-97.6)	73.3 (46.6-90.3)	86.7 (60.3-97.6)	67.8 (60.3-74.6)
CESTODA												
Floriceps saccatus												
MA ± SD	-	-	-	-	0.07 ± 0.26	0.07 ± 0.26	-	-	-	0.07 ± 0.26	-	0.02 ± 0.13
MI ± SD	-	-	-	-	1.00	1.00	-	-	-	1.00	-	1.00
Prevalence	-	-	-	-	6.7 (0.35-30.2)	6.7 (0.35-30.2)	-	-	-	6.7 (0.35-30.2)	-	1.8 (0.5-5.3)
Tetraphyllidean *larva*												
MA ± SD	0.07 ± 0.26	-	0.07 ± 0.26	2.13 ± 7.23	0.13 ± 0.52	-	0.07 ± 0.26	-	0.07 ± 0.26	0.27 ± 0.59	-	0.25 ± 2.21
MI ± SD	1.00	-	1.00	16.00 ± 16.97	2.00	-	1.00	-	1.00	1.33±0.58	-	4.20 ± 8.42
Prevalence	6.7 (0.35-30.2)	-	6.7 (0.35-30.2)	13.3 (2.4-39.7)	6.7 (0.35-30.2)	-	6.7 (0.35-30.2)	-	6.7 (0.35-30.2)	20.0 (5.7-46.6)	-	6.2 (3.2-10.8)
PHYLUM NEMATODA												
Hysterothylacium seriolae												
MA ± SD	0.33 ± 0.90	2.00 ± 4.88	1.33 ± 3.41	0.27 ± 0.59	0.07 ± 0.26	-	0.20 ± 0.56	-	0.13 ± 0.52	0.07 ± 0.26	1.20 ± 3.90	0.51 ± 2.22
MI ± SD	2.50 ± 0.71	4.29 ± 6.65	5 ± 5.48	1.33 ± 0.58	1.00	-	1.5 ± 0.71	-	2.00	1.00	9.00±8.48	3.65 ± 4.96
Prevalence	13.3 (2.4-39.7)	46.7 (22.2-70.6)	26.7 (9.7-53.4)	20.0 (5.7-46.6)	6.7 (0.35-30.2)	-	13.3 (2.4-39.7)	-	6.7 (0.35-30.2)	6.7 (0.35-30.2)	13.3 (2.4-39.7)	13.9 (9.3-20.2)
Camallanus sp.												
MA ± SD	0.07 ± 0.26	-	-	-	-	-	-	-	-	-	-	0.01 ± 0.08
MI ± SD	1.00	-	-	-	-	-	-	-	-	-	-	1.00
Prevalence	6.7 (0.35-30.2)	-	-	-	-	-	-	-	-	-	-	0.6 (0-3.5)
***Capillaria* sp.**												
MA ± SD	-		-	-	0.13 ± 0.52	-	-	-	-	-	-	0.01 ± 0.16
MI ± SD	-		-	-	2.00	-	-	-	-	-	-	2.00
Prevalence	-		-	-	6.7 (0.35-30.2)	-	-	-	-	-	-	0.6 (0-3.5)
Cucullanus sp.												
MA ± SD	0.07 ± 0.26	-	-	-	0.13 ± 0.52	-	-	-	-	-	-	0.01 ± 0.16
MI ± SD	1.00	-	-	-	2.00	-	-	-	-	-	-	2.00
Prevalence	6.7 (0.35-30.2)	-	-	-	6.7 (0.35-30.2)	-	-	-	-	-	-	0.6 (0-3.5)
PHYLUM ARTHROPODA COPEPODA												
Caligus aesopus												
MA ± SD	1.00 ± 1.00	1.06 ± 1.22	1.00 ± 1.51	3.67 ± 3.73	1.07 ± 1.10	0.87 ± 0.99	1.20 ± 1.61	1.60 ± 1.64	1.00 ± 1.41	1.47 ± 1.55	2.20 ± 2.62	1.47 ± 1.96
MI ± SD	1.67 ± 0.71	2.00 ± 0.93	1.87 ± 1.64	4.23 ± 3.70	1.78 ± 0.83	1.63 ± 0.74	3.00 ± 0.76	3.00 ± 1.89	2.14 ± 1.35	2.44 ± 1.24	3.66 ± 2.45	2.55 ± 1.97
Prevalence	60.0 (33.2-80.9)	53.3 (29.4-77.8)	53.3 (29.4-77.8)	86.7 (60.3-97.6)	60.0 (33.2-80.9)	40.0 (19.1-66.8)	40.0 (19.1-66.8)	53.3 (29.4-77.8)	46.7 (22.2-70.6)	60.0 (33.2-80.9)	60.0 (33.2-80.9)	55.7 (47.9-63.4)
***Caligus* sp.**												
MA ± SD	-	-	-	-	0.07 ± 0.26	-	-	-	-	-	-	0.01 ± 0.07
MI ± SD	-	-	-	-	1.00	-	-	-	-	-	-	1.00
Prevalence	-	-	-	-	6.7 (0.35-30.2)	-	-	-	-	-	-	0.6 (0-3.5)
Parabrachiella seriolae												
MA ± SD	0.07 ± 0.26	0.07 ± 0.26	-	-	-	0.07 ± 0.26	-	-	-	-	0.27 ± 0.59	0.04 ± 0.23
MI ± SD	1.00	1.00	-	-	-	1.00	-	-	-	-	1.33 ± 0.58	1.17 ± 0.41
Prevalence	6.7 (0.35-30.2)	6.7 (0.35-30.2)	-	-	-	6.7 (0.35-30.2)	-	-	-	-	20.0 (5.7-46.6)	3.6 (1.6-7.8)
ISOPODA												
***Gnathia vorax* (praniza & zuphea)**												
MA ± SD	3.40 ± 6.07	6.67 ± 12.71	0.07 ± 0.28	0.40 ± 0.74	14.73 ± 30.73	2.33 ± 2.16	4.73 ± 5.13	0.27 ± 0.59	13.93 ± 28.93	6.27 ± 8.29	1.80 ± 2.04	4.96 ± 14.14
MI ± SD	7.28 ± 7.27	10.00 ± 14.64	1.00	1.50 ± 0.58	31.57 ± 38.91	2.92 ± 2.02	7.10 ± 4.77	1.33 ± 0.58	26.12 ± 36.19	8.55 ± 8.65	3.00 ± 1.80	9.99 ± 18.91
Prevalence	46.7 (22.2-70.6)	66.7 (39.7-85.8)	6.7 (0.35-30.2)	20.0 (5.7-46.6)	46.7 (22.2-70.6)	80 (53.4-94.3)	66.7 (39.7-85.8)	46.7 (22.2-70.6)	53.3 (29.4-77.8)	66.7 (39.7-85.8)	60.0 (33.2-80.9)	50.5 (42.7-57.9)
SCAVENGER. OSTRACODA												
Skogsbergia costai												
MA ± SD	-	-	-	-	2.00 ± 4.54	-	-	-	-	-	-	0.18 ± 1.45
MI ± SD	-	-	-	-	7.50 ± 6.40	-	-	-	-	-	-	7.50 ± 6.40
Prevalence	-	-	-	-	26.7 (9.7-53.4)	-	-	-	-	-	-	2.4 (0.8-6.2)

4.3.1. New species for science

Phylum Platyhelminthes Gegenbaur, 1859

Class Trematoda Rudolphi, 1808

Order Diplostomida Olson, Cribb, Tkach, Bray et Littlewood, 2003

Family Aporocotylidae Odhner 1912

Genus *Paradeontacylix* McIntosh, 1934

Paradeontacylix balearicus Repullés-Albelda, Montero, Holzer, Ogawa, Hutson et Raga, 2008

& ***Paradeontacylix ibericus*** Repullés-Albelda, Montero, Holzer, Ogawa, Hutson et Raga, 2008

***Localities of collection*:** *P. balearicus*, Majorca; *P ibericus*, Alicante and Corsica*

***Site*:** *P. balearicus* flukes were found in the afferent gill arteries, afferent filament arteries and heart, while *P. ibericus* specimens were found in the afferent gill arteries and heart. Eggs of *P. balearicus* were found in the afferent filament arteries as well as within the interlamellar vessels (from the afferent to the efferent side) (Fig 4.1 A & B). In contrast, eggs of *P. ibericus* were mostly found in the afferent filament arteries and, sporadically, within the afferent side of the interlamellar vessels (Fig 4.1 C).

***Description & Remarks*:**

These 2 new species are described in Repullés-Albelda *et al.* (2008) (see chapter 5).

*One specimen of *Paradeontacylix* sp. was also found in Corsica. This specimen was morphologically similar to *P. ibericus*, although a molecular study of blood flukes from this region would be required to confirm the species identity.

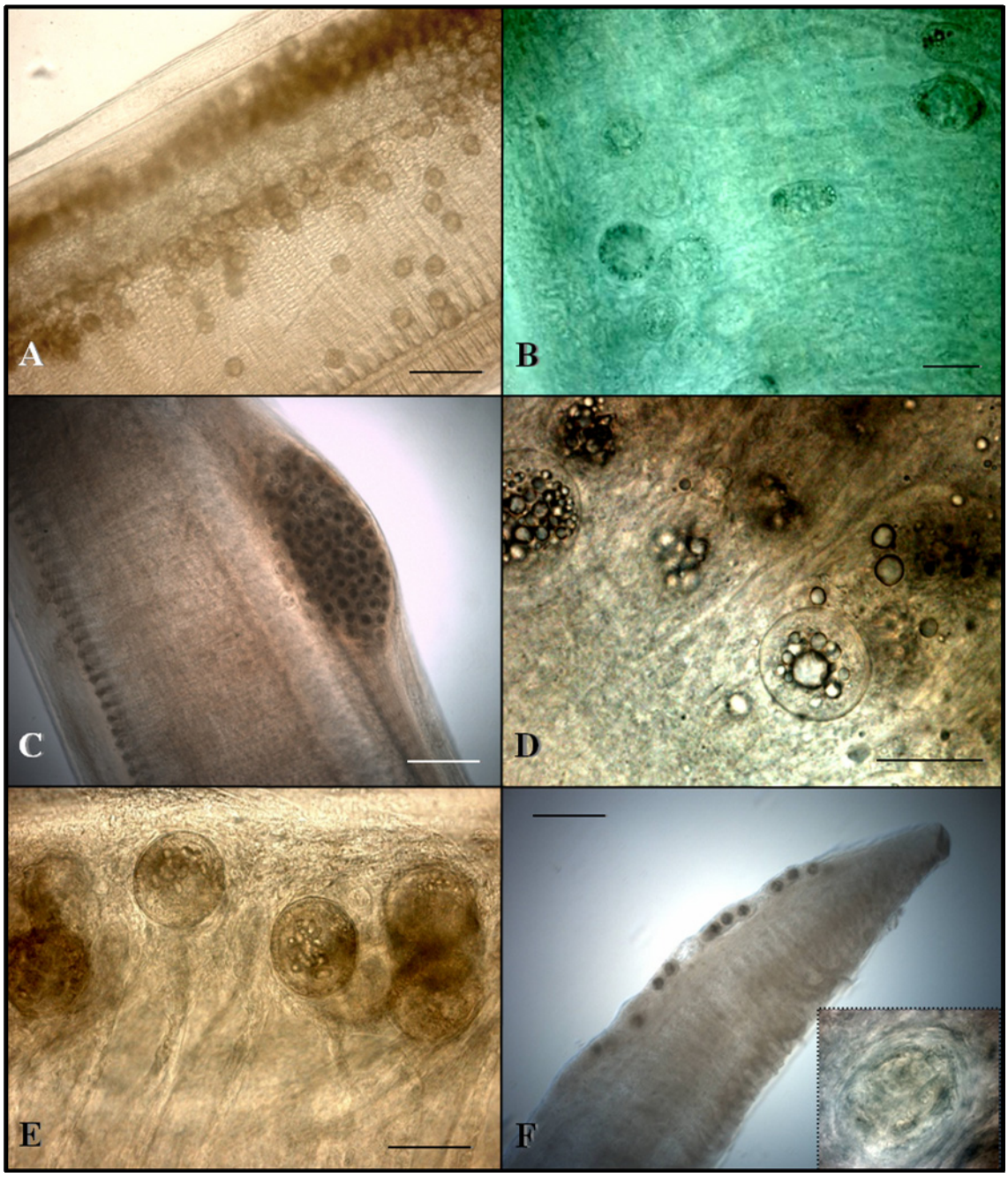


Figure 4.1. Eggs of *Paradeontacylix* spp. in *Seriola dumerili* from the Mediterranean Sea. A & B, Eggs of *P. balearicus*: A, eggs aligned along the secondary lamellae; B, detail of the eggs in the interlamellar vessels of the secondary lamellae. C) Group of eggs of *P. ibericus* in the afferent filament artery. D-F, Eggs of *P. balearicus*: February eggs (D); June eggs (E); eggs sampled in October (F), including detail of encysted egg. Scale bars represent 200 µm in A, C and F while they represent 50 µm in B, D and E.

Seasonal occurrence:

Monthly occurrence of *P. balearicus* (worms and eggs) is indicated in table 4.2 and figure 4.2. Most of the worms (all mature) were detected in February and April, when water temperatures increased. Prevalence of *P. balearicus* eggs increased gradually from October to June during the two

years of study while from June to September it appeared to decrease (July and August data were not available; fig. 4.2). The amount of eggs also apparently increased from February to June.

The developmental stage of the eggs evolved throughout the year. Most of the eggs observed during February were in an early developmental stage (Fig. 4.1 B & D). In April and June, embryos became progressively more defined (Fig. 4.1 E) until the miracidia were developed. During October and December, eggs were encysted (Fig. 4.1 F) and the miracidia appeared to be degraded. Finally, in December, some new eggs were found together with the old encysted ones.

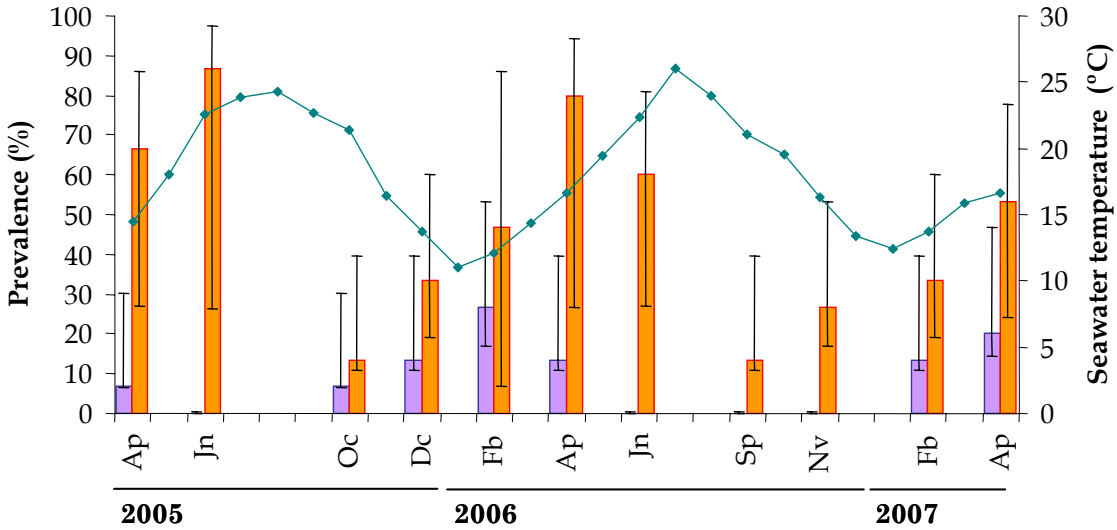


Figure 4.2. Prevalences of adults (purple) and eggs (orange) of *Paradeontacylix balearicus* in *Seriola dumerili* off Majorca from April 2005 to April 2007. Mean month seawater temperature off Majorca waters indicated by green line. Bars represent 95% confidence interval.

4.3.2. New host and locality records

Phylum Arthropoda Latreille, 1829

Class Maxillopoda Dahl, 1956: Subclass Copepoda Milne-Edwards, 1840

Order Siphonostomatoida Thorell, 1859

Family Lernaeopodidae Olsson, 1869

Genus Parabrachiella Wilson, 1915

Parabrachiella seriolae (Yamaguti et Yamasu, 1960)

***Localities of collection*:** Majorca and Alicante

***Site*:** Female specimens of *P. seriolae* were found attached to the gill filaments. Male specimens were found attached to the female posterior processes.

***Description*:**

Female

Specimens (N= 7) 10041 ± 888 (8711 - 12350) long and 1928 ± 270 (1612 - 2291) wide, with maximum width at trunk level (Fig. 4.3 A & B). Cephalothorax cylindrical 2166 ± 353 (1917 - 236) long and 1061 ± 107 (927 - 1101) wide, unflexed, anteriorly expanded to form head, covered by a rectangular dorsal shield with slightly convergent postero-lateral margins. Head constituting about 2/5 of cephalothorax length. Border between cephalothorax and trunk at second maxillae level. Trunk granulose, roughly trapezoid, 4681 ± 257 (4427 - 4963) long and 1928 ± 270 (1612 - 2291) wide, dorsoventrally flattened and with four rounded margins. Anterior neck-like region cylindrical, short and narrow, connecting with cephalothorax. Anal slit on a central prominent tubercle in posterior extremity of trunk. Dorsal posterior process cylindrical and elongated (more than 1/2 of trunk length), 3997 ± 777 (3447 - 5133) long, on each side of anal tubercle. Ventral posterior process cylindrical, 2149 ± 613 (1125 - 2433) long, often narrower than dorsal process. All females non-ovigerous.

Male

Specimens (N= 4) 1277 ± 76 (1259 - 1324) long and 369 ± 12 (360 - 378) wide (Fig. 4.3 C & D). Cephalothorax wide, unflexed, 552 ± 111.01 (474 - 631) long and 369.28 ± 12.73 (360.32 - 378.27) wide, with well-developed dorsal shield. Separation between cephalothorax and trunk slightly constricted. Trunk cylindrical, 727 ± 140 (628 - 826) long and 299 ± 4 (296 - 301) wide,

with rounded posterior extremity equipped with short uropods, 93 ± 14 (71 - 102) long and 29 ± 4 (25 - 36) wide.

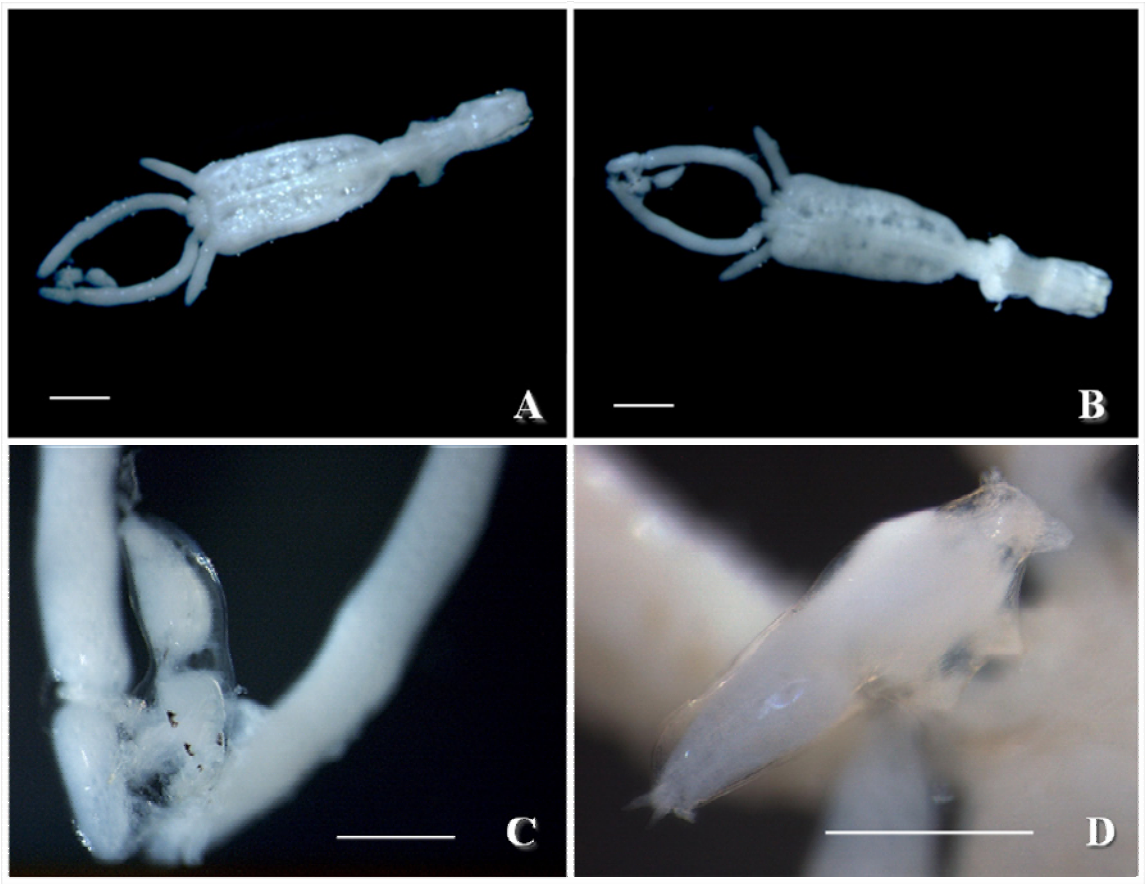


Figure 4.3. *Parabrachiella seriolae* from *Seriola dumerili* off the western Mediterranean Sea. A & B, Female: whole specimen in dorsal (A) and ventral (B) views. C & D, Male: whole specimens attached to the distal (C) or to the proximal (D) section of posterior processes. Scale bars represent 1000 µm in A and B, and 500 µm in C and D.

***Remarks*:**

P. seriolae was first described in *Seriola quinqueradiata* from Pacific Ocean (Yamaguti et Yamasu, 1960) but this species has not been reported in other *Seriola* spp. to date. Rohde (1978) and Sharp *et al.* (2003) found an unidentified *Parabrachiella* sp. parasitising *S. lalandi* and Williams & Bunkley-Williams (1996) reported *Brachiella thynni* Cuvier, 1830 and *Eobrachiella elegans* (Richiardi, 1880; as *Brachiella elegans*) in *S. dumerili*. Some of these Lernaeopodidae genera have been taxonomically controversial. In 1970, Kabata suppressed the genus *Parabrachiella* Wilson, 1915 and synonymised it with the genus *Brachiella* Cuvier, 1830. Thereafter, in 1979, the same author erected the genus *Neobrachiella* and transferred some of the *Brachiella* spp. to this new genus, including *B. seriolae*. Diagnostic features of *Neobrachiella* and the previously suppressed *Parabrachiella* were very similar, only differing in the arrangement of posterior processes and the number of terminal papillae of the maxillule (Kabata, 1979; Boxshall & Halsey, 2004). Thus, more recently,

the genus *Parabrachiella* has been reaccepted while the genus *Neobrachiella* has been suppressed and its species have been transferred to *Brachiella* or *Parabrachiella* genera (Boxshall & Halsey, 2004). *P. seriolae* is differentiated from the rest of *Parabrachiella* spp. by total body length and width, unflexed to slightly flexed cephalothorax, granulose appearance of trunk of females and presence of a pair of elongated posterior ventral processes, more than 1/2 longer than the dorsal ones (Yamaguti, 1963).

***Seasonal occurrence*:**

Monthly occurrence of females of *P. seriolae* is reported in table 4.2 (males are free-living and their occurrence has not been reported). Prevalence of infection was very low (below 20%) and intensity ranged from 1 to 2 specimens per fish. All the specimens were found in the subsamples of April or June (spring-summer) between 2005 and 2007.

4.3.3. New host records of Mediterranean species

Phylum Platyhelminthes Gegenbaur, 1859
Class Trematoda Rudolphi, 1808
Order Diplostomida Olson, Cribb, Tkach, Bray et Littlewood, 2003
Family Strigeidae Railliet, 1919
Genus Cardiocephaloides Sudarikov, 1959
Cardiocephaloides sp. (metacercaria)

***Locality of collection*:** Majorca

***Site*:** *Cardiocephaloides* specimens were found encysted within the brain in large composite cysts (up to 5 metacercariae within one cyst) of host origin.

***Description*:**

Encysted ellipsoidal metacercariae (N= 5), 1436 ± 83 (1389 - 1553) long and 957 ± 41 (929 - 989) wide (Fig. 4.4 A). Excysted metacercariae cup-shaped, with anterior end evaginable (see fig 4.3 B). Evaginated section subtriangular and short, 280.82 ± 35.51 (240 - 302) long and 338 ± 17 (318 - 351) wide, separated by transverse fold from posterior part. Oral sucker subterminal, 84 ± 3 (81 - 89) long and 83 ± 3 (81 - 87) wide. Pharynx rounded, 39 ± 2 (37 - 41) in diameter. Posterior part of body 1177 ± 67 (1124 -1261) long and 957 ± 41 (929 - 989) wide. Oesophagus short, bifurcated into two caeca. Ventral sucker medial, 205 ± 12 (185 - 217) long and 214 ± 9 (207 - 227) wide. Pseudosuckers well-developed. Holdfast organ large, located at posterior body end, 201 ± 3 (106 - 207) long and 1203 ± 117 (1099 - 1407) wide.

***Remarks*:**

Specimens were identified as *Cardiocephaloides* sp. by their infection site (the brain) and a combination of morphological features, i.e.: body shape, with an anterior evaginable section; two pseudosuckers, arranged laterally to pharynx; and large holdfast organ located at the posterior body end. The only *Cardiocephaloides* sp. reported in the Mediterranean Sea to date is *C. longicollis* (Rudolphi, 1819) (Gibson *et al.*, 2005). Interestingly, in spite that this is a quite unspecific species, it has never been reported in any carangid (Prévot & Bartoli, 1980; Gibson *et al.*, 2005). Morphological features and dimensions of the metacercariae from the current study do not completely agree with those originally described for *C. longicollis* (Prévot & Bartoli, 1980) or for any

other *Cardiocephaloides* spp. reported to date (Hunter & Vernberg, 1960; Abbott, 1968; Moravec *et al.* 1997; Timi *et al.*, 1999; Vidal-Martínez *et al.*, 2011). Unfortunately, some of these species have not been described in detail (e.g. *C. mediconiger* Dubois et Vigueras, 1949) and, therefore, they could not be compared with the specimens herein reported.

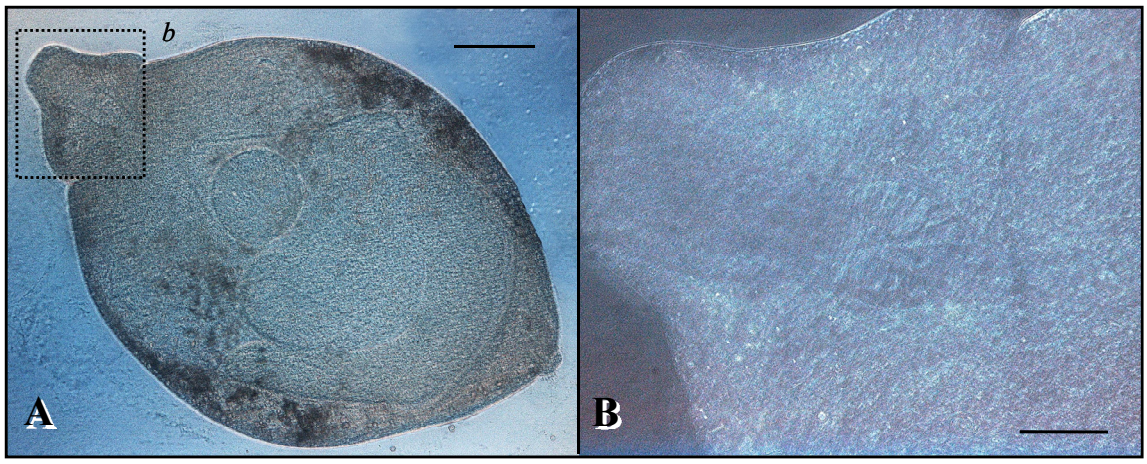


Figure 4.4. Metacercaria of *Cardiocephaloides* sp. from *Seriola dumerili* off Majorca. A, Whole specimen in ventral view; B, detail of anterior end. Scale bars represent 200 **µm** in A and 50 in B.

Based on general morphological features, these specimens mostly resemble to *C. longicollis* (Prévot & Bartoli, 1980), or to *Cardiocephaloides* sp. from *Engraulis anchoita* Hubbs et Marini, 1935 off Argentina (Timi *et al.*, 1999). However, they are abnormally large comparing with dimensions originally described for these species. From the other hand, dimensions of the specimens found in greater amberjack mostly resemble to those reported for *Cardiocephaloides* sp. (described as *Cardiocephalus* sp.), found in *Epinephelus morio* (Valenciennes, 1828) from southeastern Mexico (Moravec *et al.*, 1997), or those reported for *Cardiocephaloides* spp., parasitising *Crenimugil crenilabris* (Forsskål, 1775) and many other fish species from the eastern Indo-Pacific waters (Vidal-Martínez *et al.*, 2011). However, some differences were also observed: oral sucker and pharynx herein described were smaller than those of metacercariae in *E. morio* (Moravec *et al.*, 1997) whereas holdfast organ was longer and narrower than that reported by Moravec *et al.* (1997) and Vidal-Martínez *et al.*, (2011). The large size of the specimens found in greater amberjack could be explained by several causes and, therefore, species was identified as *C. longicollis*. However, molecular analysis would be required to reliably identify the specimens.

***Seasonal occurrence*:**

Monthly occurrence of *Cardiocephaloides* sp. is indicated in table 4.2. Prevalence and intensity of infection of this species was low in all subsamples (<15% - <5 parasites/fish). Seasonal infection patterns were not observed.

Order Plagiorchiida La Rue, 1957

Family Hemiuridae Looss, 1899

Genus *Aphanurus* Loss, 1907

***Aphanurus* sp.** (immature)

***Locality of collection*:** Majorca

***Site*:** The specimen of *Aphanurus* sp. was found in the stomach.

***Description*:**

Immature specimen (N= 1), 598 long and 175 wide. Body surface with annular plications (Fig. 4.5). Small vestige of ecsoma at posterior end. Oral sucker subterminal and subspherical, 41 long and 48 wide. Pharynx 25 long and 22 wide. Prepharynx absent. Ventral sucker subspherical, 87 long and 86 wide, at the middle of forebody. Primordial gonads diffuse. Primordium of distal genitalia 31 long, posterior to ventral sucker. Primordium of vitellarium 78 long and 41 wide, observed as a single, well-defined and ellipsoidal postovarian follicle (see arrow in fig 4.5).

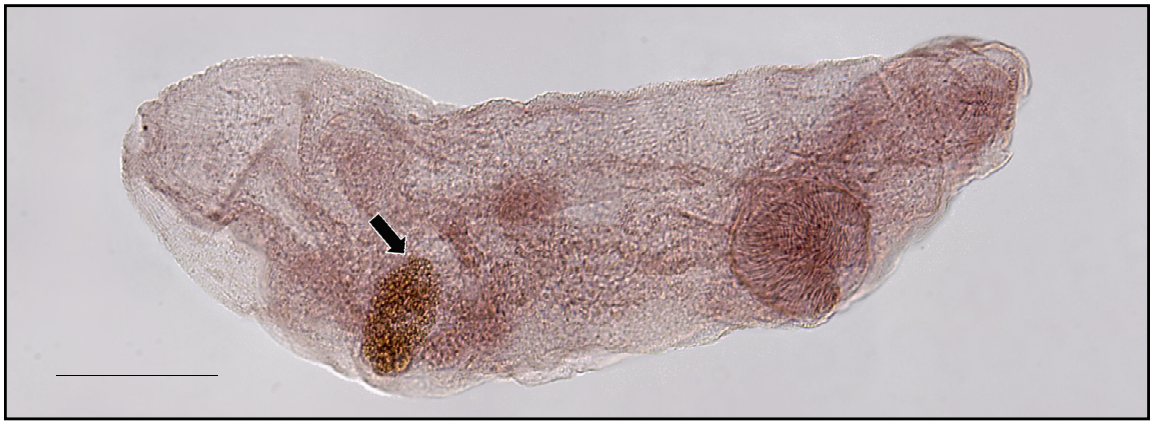


Figure 4.5. Immature specimen of *Aphanurus* sp. from *Seriola dumerili* off Majorca. Whole specimen in ventral view. Arrow points to the single ellipsoidal vitellarium. Scale bar represents 100 µm.

***Remarks*:**

Only 6 out of the 10 Hemiuridae spp. parasitising *Seriola* spp. have been reported in *S. dumerili* (see check-list in appendix). This is the first record of *Aphanurus* sp. parasitising *Seriola* spp. although species of this genus have been often reported in other carangid fish (i.e. *Trachurus* spp.) (Gibson *et al.*, 2005). The immature specimen reported here could be confused with other hemiurids with ecsoma, as *Lecithochirium* sp. or *Ectenurus* sp., previously reported in *Seriola* spp. (Gibson *et al.*, 2005). However, this was identified as *Aphanurus* sp. by its plicated body surface, its

vestigial ecsoma, and its single oval postovarian vitellarium (Gibson, 2002). Identification to species-level could not be achieved as only one immature individual was recovered.

***Seasonal occurrence*:**

The only specimen of *Aphanurus* sp. was found in February 2006 (see table 4.2).

Genus Lecithocladium Lühe, 1901

Lecithocladium sp. (immature)

***Locality of collection*:** Alicante

***Site*:** The only specimen of *Lecithocladium* sp. was found in the stomach.

***Description*:**

Immature specimen (N= 1) 1480 long and 225 wide (Fig. 4.6). Body surface smooth. Ecsoma well-developed 410 long and 127 wide. Oral sucker subterminal, funnel-shaped, 182 long and 153 wide, larger than ventral sucker. Ventral sucker 118 long and 134 wide. Pharynx elongated 112 long and 51 wide. Prepharynx absent. Primordial testes undifferentiated. Primordium of proximal feminine genitalia diffuse.

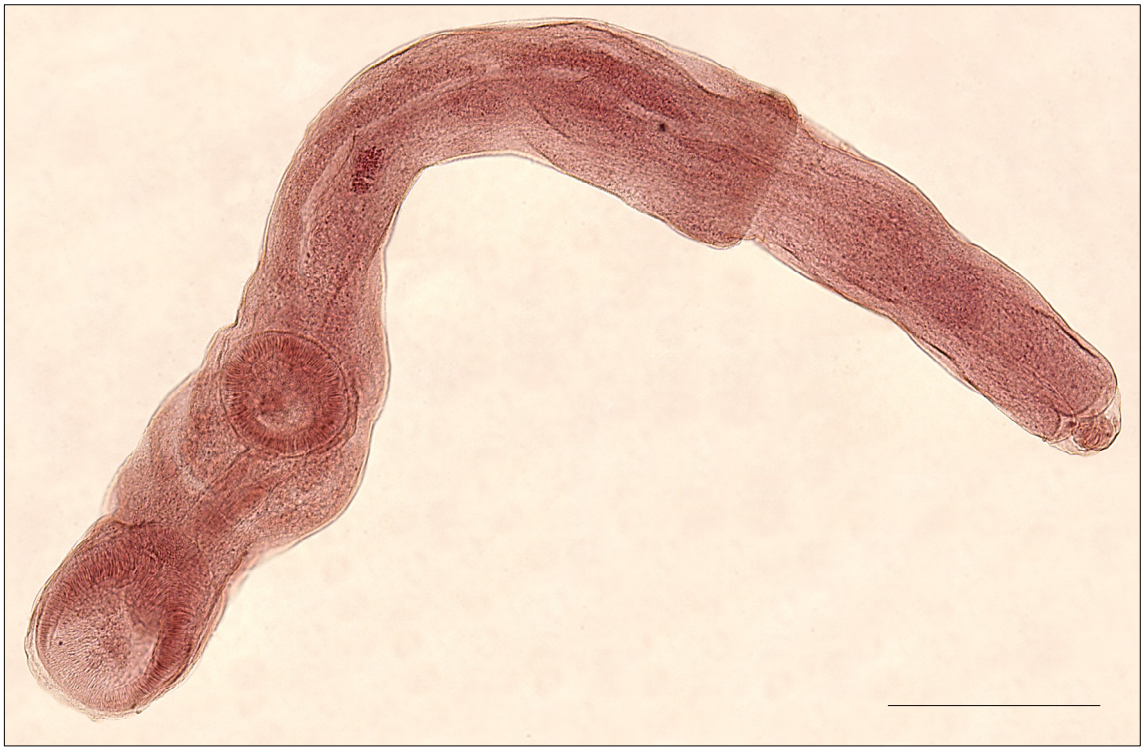


Figure 4.6. Immature specimen of *Lecithocladium* sp. from *Seriola dumerili* off Alicante. Whole specimen in ventral view. Scale bar represents 200 µm.

***Remarks*:**

This is the first record of *Lecithocladium* sp. parasitising *Seriola* sp. However, most of the species of this genus are quite unspecific and have been reported in many teleosts belonging to different families, including Carangidae (see Gibson, 2002 and Gibson *et al.*, 2005). Gibson (2002) stated that the main features used to distinguish species within the family Hemiuridae are the

presence or absence of a well-developed ecsoma and the shape, arrangement and number of vitellarium masses. According to the generic descriptions reported by this author, the specimen found in the current study was identified as *Lecithocladium* sp. by the large ecsoma, the typical cupular shape of the oral sucker and the arrangement of this sucker and the pharynx (Gibson, 2002). However, identification to species-level could not be achieved as vitellarium was indistinguishable due to the immaturity of the specimen.

Seasonal occurrence:

The only specimen of *Lecithocladium* sp. was found in the fish sample collected from Alicante in November 2005 (see table 4.1).

Family Heterophydae Odhner 1914

Genus *Galactosomum* Loss 1899

Galactosomum lacteum (Jägerskiöld, 1896) Looss, 1899 (metacercaria)

***Locality of collection*:** Majorca

***Site*:** Specimens of *Galactosomum* were found encysted in individual cysts within the brain tissue.

***Description*:**

Elongated metacercariae (N= 3) 1886 ± 148 (1779 - 2055) long and 222 ± 11 (213 - 235) wide, encapsulated in ovoid cysts. Spined tegument. Oral sucker subterminal, spherical, 226 ± 19 (212 - 240) long 230 ± 20 (210 - 250) wide, more than three times larger than ventral sucker (Fig. 4.7). Ventral sucker 67 ± 3 (65 - 71) long and 70 ± 2 (69 - 72) wide, anterosinistrally inclined, apex slightly spined and protractile. Pharynx 92 ± 6 (91 - 94) long and 114 ± 2 (112 - 116) wide. Two testes well-defined, rounded, 108 ± 15 (94 - 133) in diameter, arranged in tandem. Seminal vesicle bipartite, elongated 156 ± 22 (140 - 172) long and 68 ± 4 (65 - 71) wide. Vitellarium not observed.

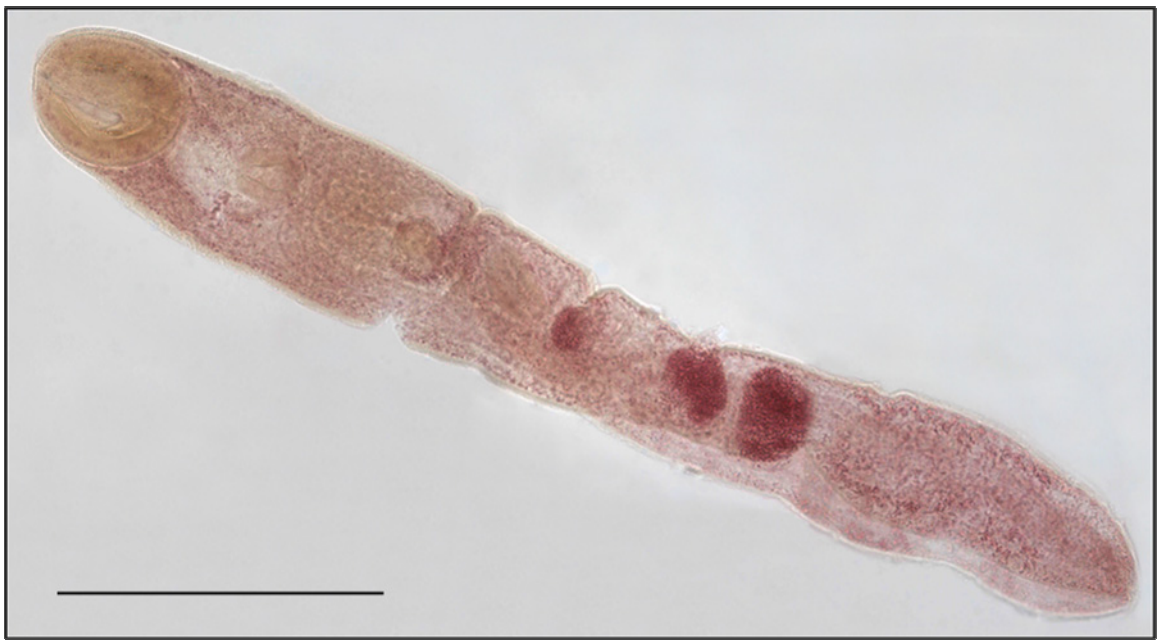


Figure 4.7. Excysted metacercaria of *Galactosomum* sp. from *Seriola dumerili* off Majorca. Whole specimen in ventral view. Scale bar represents 500 µm.

***Remarks*:**

Specimens were identified as *Galactosomum* sp. by their infection site (the brain) and the elongated spined body with parallel margins and blunt ends (Pearson, 1973). Many marine fish

species have been reported to be second intermediate hosts of *Galactosomum* spp. (Yasunaga *et al.* 1981), including *Seriola quinqueradiata* from the Japanese coast (Ogawa & Yokoyama, 1998). In the Mediterranean, most of the *Galactosomum* metacercariae have been identified as *G. lacteum* (Jägerskiöld, 1896) which infects fish from different families, i.e. Centracanthidae, Gobiidae, Labridae, Serranidae, Sparidae (Culurgioni *et al.*, 2007). The other two *Galactosomum* spp. reported in this region, *G. timondavidi* Pearson et Prévot, 1971 and *G. erinaceum* (Poirier, 1886), have only been reported from definitive hosts (gulls and marine mammals respectively; Pearson & Prévot, 1971; Buriola & Ceroni, 1995). Based on Pearson, (1973) and Culurgioni *et al.* (2007), the specimens herein reported were identified as *G. lacteum* by the asymmetrical and spined ventral sucker and the two-chambered seminal vesicle. This is the first report of *Galactosomum* sp. parasitising a carangid fish (*S. dumerili*) in the Mediterranean region.

Seasonal occurrence

Specimens of *Galactosomum* sp. were found in the same fish which was sampled in October 2005 (see table 4.2).

Class Cestoda **Rudolphi, 1808**

Order Trypanorhyncha Diesing, 1863

Family Lacisthorhynchidae Guiart, 1927

Genus *Floriceps* Cuvier, 1817

Floriceps saccatus Cuvier, 1817 (plerocercoid larva)

***Locality of collection*:** Majorca

***Site*:** *Floriceps saccatus* specimens were found encapsulated in blastocysts which were located in the visceral cavity.

***Description*:**

Plerocercoid (N= 1) with cylindrical blastocyst 18321 long (including narrow elongation of posterior end 3628 long; see fig. 4.8 A) and 5319 wide. Excysted specimen 16510 long and 3158 wide (Fig. 4.8 B); pars bulbosa 1287 wide. Scolex peduncle 5884 long, with 2 lateral bothridia, each 1668 long. Pars vaginalis 4189 long and pars bulbosa 1517 long. Four spined eversible tentacles emerging near apex of scolex (usually partially invaginated in fixed specimens) (Fig. 4.8 C). Poecilacanthous armature (Fig. 4.8 D), arranged in half-spiral rows of large spines interrupted by single lines of two-winged chainettes and adjacent small hooks.

***Remarks*:**

Based on descriptions of Campbell & Beveridge (1994), specimens were identified as *Floriceps* spp. by the poeciloacanthous armature and the arrangement of hooks. *F. saccatus* was first reported in a *Seriola* species by Bates (1990), who found this parasite in *S. lalandi* (as *S. mazatlana*). Thereafter, Montero *et al.* (2001b) reported *Floriceps* sp. in *S. dumerili* from Murcia (Iberian Peninsula). Specimens of the present study were identified as *F. saccatus* by the similar shape of hooks 1 (1') and 2 (2'), the large bulbs (>1,000) and the ratio between pars bulbosa and pars vaginalis which is lower than 1:3 (aprox. 1:2.8) (Campbell & Beveridge, 1994). Dimensions and features of these specimens coincide with those described by Montero (2001) from specimens off Murcia (identified to genus-level). Thus, it is highly probable that specimens from Montero (2001) and Montero *et al.*, (2001b) are also *F. saccatus*.

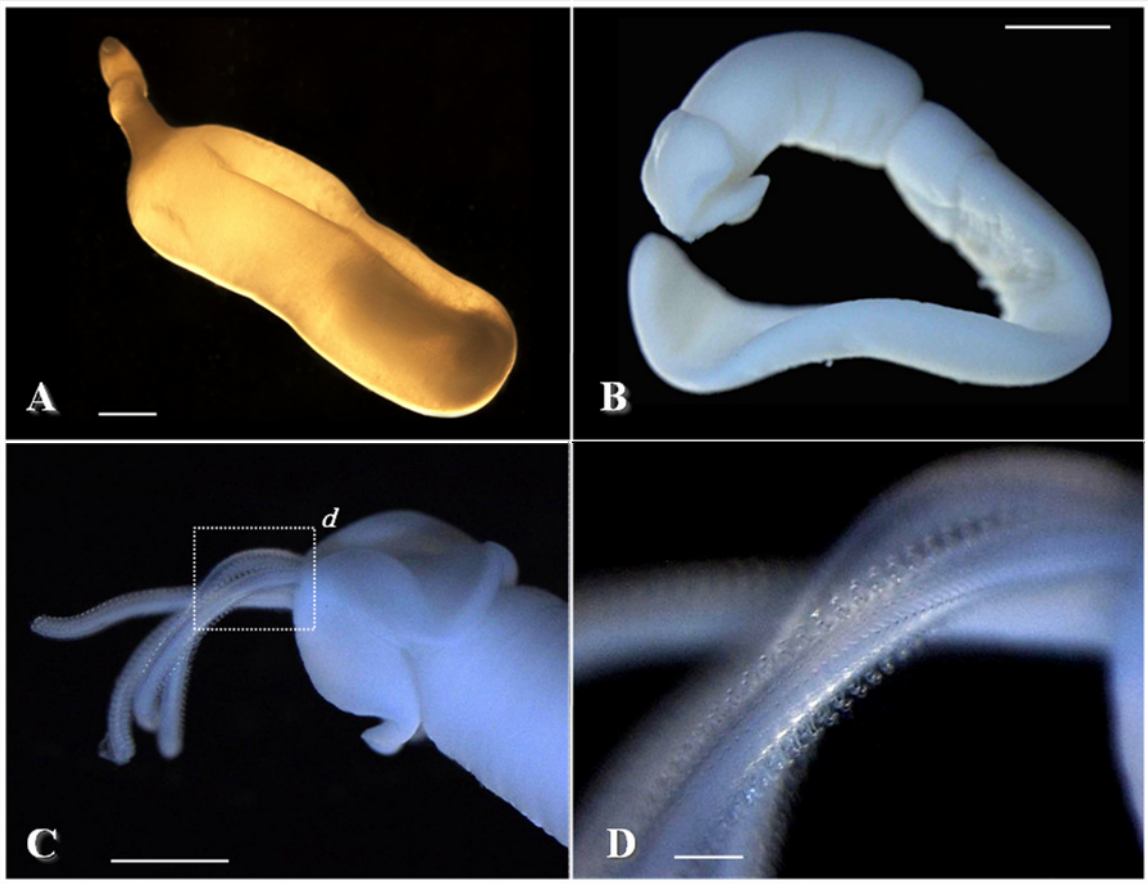


Figure 4.8. *Floriceps saccatus* from *Seriola dumerili* off Majorca. A, Blastocyst. B-D, Excysted larva: B, whole specimen; C, detail of pars botridialis at the anterior body end; D, detail of the armature of tentacles. Scale bars represent 2000 µm in A and B, 1000 in C and 100 in B.

***Seasonal occurrence*:**

The three specimens of *F. saccatus* reported here were found in different subsamples obtained in February and April of different years (see table 4.2).

Phylum Nematoda (Rudolphi, 1808) Lankester, 1877
Class Adenophorea Von Linstow, 1905
Order Enoplida Filipjev, 1929
Family Trichuridae Ransom, 1911
Genus *Capillaria* Zeder, 1800
Subgenus *Procapillaria* Moravec, 1987
***Capillaria* (*Procapillaria*) sp.**

***Locality of collection*:** Majorca

***Site*:** Specimens of *Capillaria* sp. were found in the intestine.

***Description*:**

Adult females (N=2) 11127 - 12701 long and 68 - 70 wide (Fig. 4.9 A). Oesophagus 5248 - 6131 long which represents 47 - 48 % of total body length. Muscular oesophagus 338 - 418 long and stichosome 4910 - 5713 long, with elongated stichocytes, 78 - 140 long, 37 to 43 in number (Fig. 4.9 A & B). Vulva located posterior to level of junction between oesophagus and intestine at 29 - 41. Vulvar appendage 32 long and 24 wide, present in one of the specimens, (Fig.4.9 C). Uterus with numerous elongated eggs arranged in one row. Eggs (N= 5) 65 - 72 long and 26 - 28 wide. Posterior end rounded, anus subterminal and tail 12 - 15 long.

***Remarks*:**

The only Trichuridae species reported in *Seriola* spp. to date is *Pseudocapillaria carangi* (Parukhin, 1971, 1976) while *Capillaria* spp. have never been found in any carangid fish. In the Mediterranean, three *Capillaria* spp. have been reported: *Capillaria gracilis* (Bellingham, 1840) *C. adriatica* (Nikolaeva et Naidenova, 1964) and *C. bainae* (Justine et Radjukovic, 1988). However, the latter two have been reassigned to *Pseudocapillaria* genus (Moravec. 1982; Gibbons, 2010). Based on Gibbons (2010), current study specimens could not belong to *Pseudocapillaria* genus as one of them has a vulvar appendage. Therefore, these specimens were identified as *Capillaria* (*Procapillaria*) sp. by the stichosome with one row of stichocytes and ending at oesophagus-intestine junction level, the eggs without special capsule, the vulvar appendage and their host, which is a fish species (Moravec, 1982; Moravec, 1994).

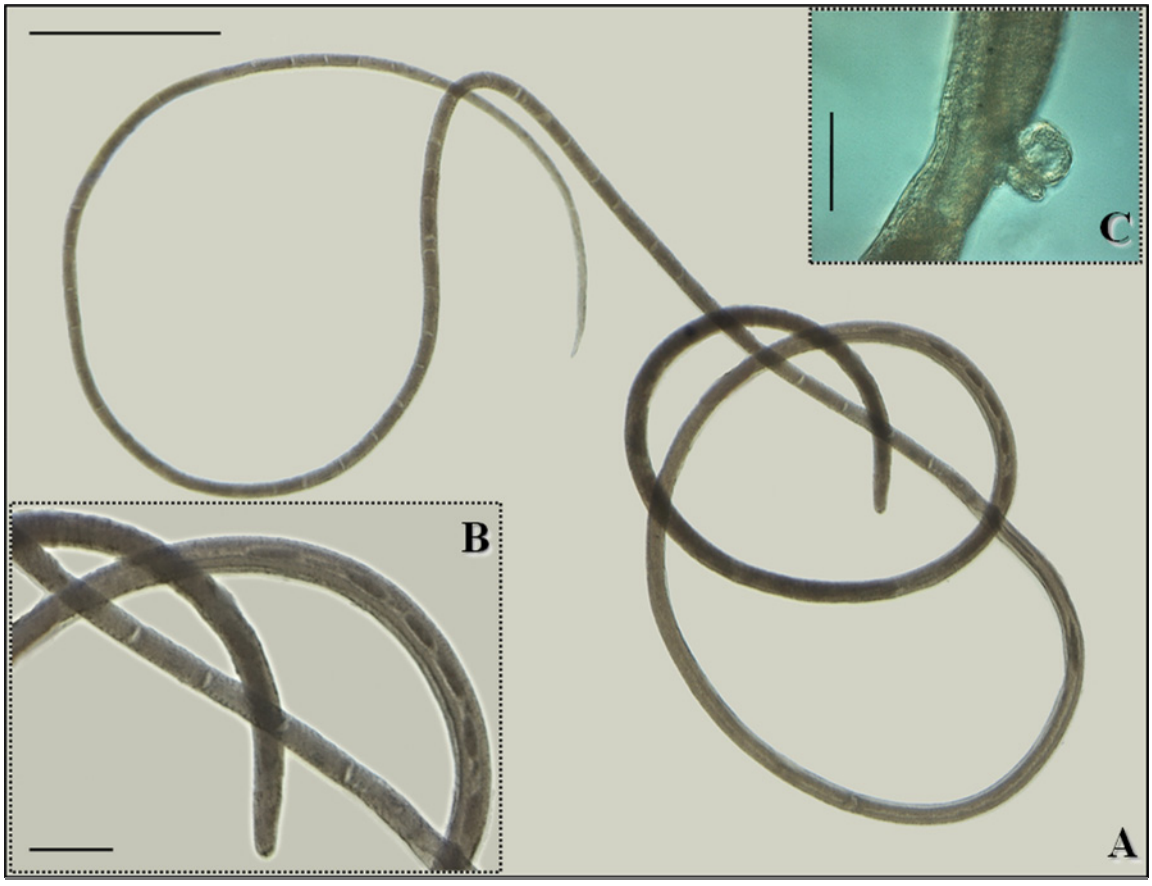


Figure 4.9. *Capillaria* sp. from *Seriola dumerili* off Majorca. A) Whole specimen; B) detail of the eggs and stichocytes; C) detail of vulvar appendage. Scale bars represent 500 µm in A, 100 in B and 50 in C.

Most dimensions and features of the specimens described here agree with those of C. *gracilis* (see Moravec, 2001), except for the slightly smaller size of the vulvar appendage of latter. Moreover, in accordance with the geographic location, the most reliable species would also be C. *gracilis* although it mainly parasitise gadiform fish (see Moravec, 2001). The diagnosis of Capillariinae is mainly based on the morphology of males (Moravec, 1982; Moravec, 1994) and species identification is very difficult when these are not available. Therefore, as only two female specimens were collected, identification to species-level could not be performed.

Seasonal occurrence:

The two specimens of *Capillaria* (*Procapillaria*) sp. were found in the same fish which was sampled in February 2006 (see table 4.2).

Class Secernentea Von Linstow 1905

Order Spirurida Chitwood, 1933

Family Camallanidae Railliet et Henry, 1915

Genus *Camallanus* Railliet et Henry, 1915

***Camallanus* sp.** (larva 4)

***Locality of collection*:** Majorca

***Site*:** The specimen of *Camallanus* sp. was found in the intestine.

***Description*:**

Larva (N= 1) 9969 long and 190 wide (Fig. 4.10 A). Buccal capsule 104 long and 91 wide, with continuous longitudinal striation (Fig. 4.10 C). Sclerotized lateral tridents 72 long, exceeding posterior border of buccal capsule. Muscular oesophagus 1809 long and glandular oesophagus 872 long (Fig. 4.10 B). Nerve ring at 227 from anterior end. Tail straight, conical, 222 long, ending in three small processes 15 long (Fig. 4.10 D).

***Remarks*:**

This is the first report of *Camallanus* sp. infecting *Seriola* spp. although *C. carangis* (Olsen 1954) has been previously reported in carangids and other fishes from Indo-Pacific waters (see review in Rigby *et al.* 1998). *Camallanus* spp. are mainly distinguished by the well-developed buccal capsule, composed of two chitinous valves longitudinally striated, the telorhabdion separating buccal cavity and oesophagus, the presence and arrangement of papillae and the ratio between muscular and glandular oesophagus (Moravec, 1994; Skryabin, 1992; Chabaud, 2009). In fact, the specimen found in *S. dumerili* differed from *C. carangis* in its relatively longer muscular oesophagus (muscular/glandular oesophagus ratio 2/1 instead of 1.4/1). According to Gibson *et al.* (2005), the only species of *Camallanus* reported in the Mediterranean region is *C. melanocephalus* (Stromberg & Crites, 1974) which is usually found in digestive tract of scombrids (Rudolphi, 1819; Sinderman, 1990). Features and dimensions of the specimen described in the current study agree with those reported by Rudolphi (1819) and Tornquist (1931) for *C. melanocephalus* except for the ratio between muscular and glandular oesophagus (2/1 in present study and 1.3/1 in *C. melanocephalus* descriptions). Further specimens would be required in order to identify this species.

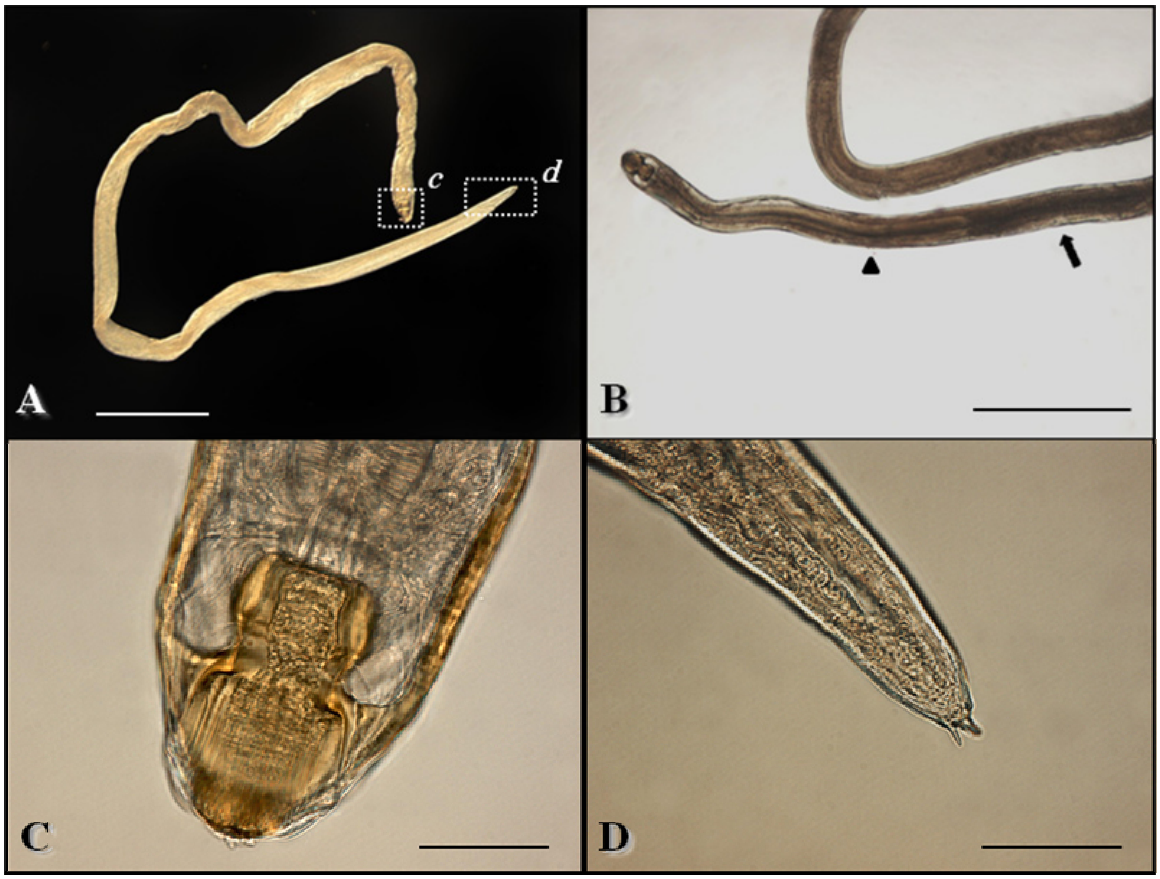


Figure 4.10. *Camallanus* sp. from *Seriola dumerili* off Majorca. A, Whole specimen; B, detail of the oesophagus, arrow head points to muscular oesophagus and arrow points to glandular oesophagus; C, detail of the buccal capsule; D, detail of the tail. Scale bars represent 1000 µm in A & B, 50 in C and 100 in D.

Seasonal occurrence:

The only specimen of *Camallanus* sp. was found in April 2005 (see table 4.2).

Order Ascaridida

Family Cucullanidae Cobbold, 1864

Genus *Cucullanus* Müeller, 1777

***Cucullanus* sp.** (larvae 4)

***Locality of collection*:** Majorca

***Site*:** specimens of *Cucullanus* sp. were found in the stomach.

***Description*:**

Degraded larvae (N= 2) 878 - 886 long and 58 - 64 wide (Fig. 4.11 A). Buccal capsule well-defined (Fig. 4.11 B), followed by oesophagus 195 - 205 long ending at the intestine. Intestinal caecum absent. Cone-tail 88 - 101 long (Fig. 4.11 C).

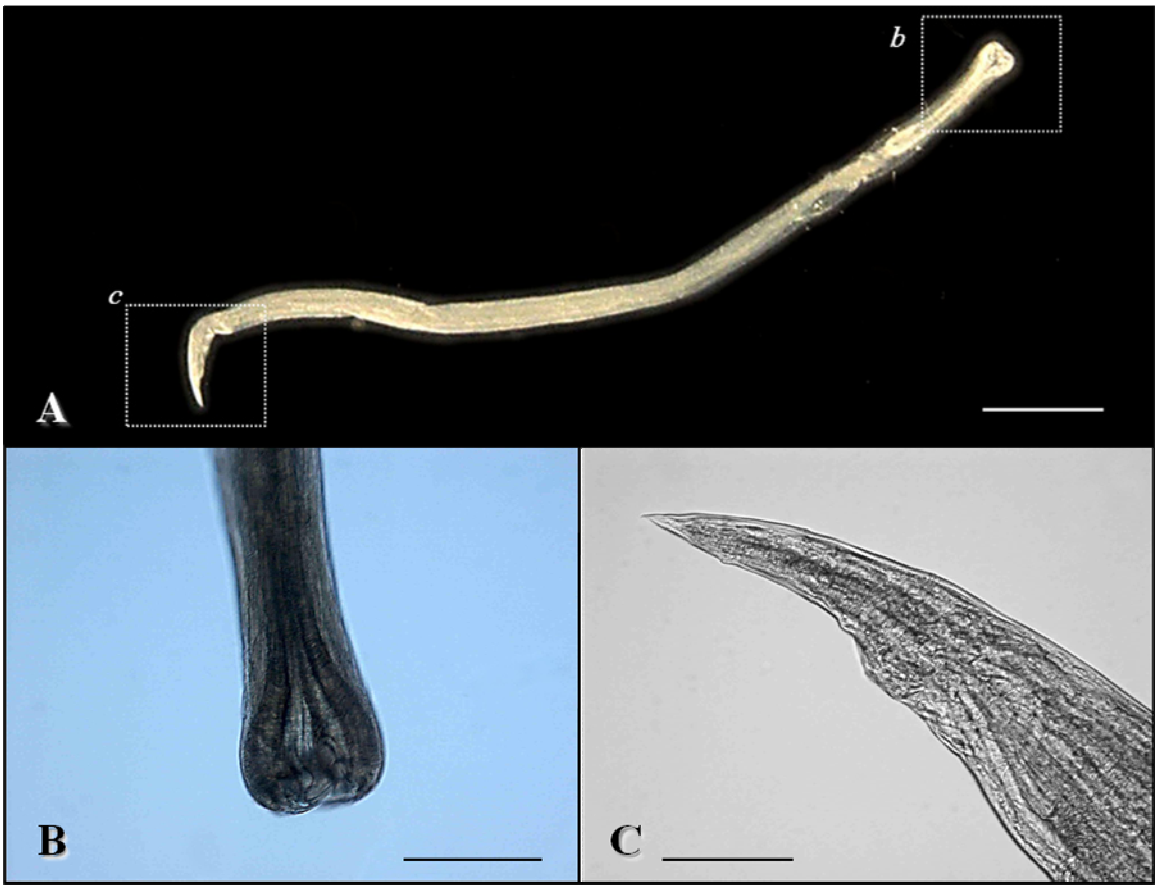


Figure 4.11. *Cucullanus* sp. from *Seriola dumerili* off Majorca. A, Whole specimen; B, detail of buccal capsule; C, detail of the tail. Scale bars represent 100 µm in A and 50 in B and C.

***Remarks*:**

Very few *Cucullanus* spp. have been reported from carangid fishes to date (Gibson *et al*., 2005), most of them in *Caranx* spp. and none of them parasitising *Seriola* spp. In the Mediterranean, *Cucullanus* spp. have never been found parasitising carangid fishes although many of them have been reported from fishes belonging to other families (see for example Campana-Rouget & Chabaud, 1956; Muñoz *et al*., 1988; Petter & Radujkovic, 1989; Campos & Carbonell, 1994). Specimens found in greater amberjack were identified as *Cucullanus* sp. by the well-defined buccal capsule, the mouth perpendicular to body axis and the absence of intestinal caecum (Petter, 1974; Moravec, 2001). Identification to species-level could not be performed as adult males are required for taxonomy within this genus and only two larvae 4 were found.

***Seasonal occurrence*:**

The two specimens of *Cucullanus* sp. were found in the same fish collected in February 2006 (see table 4.2).

4.3.4. New locality records:

Phylum Plathelminthes Gegenbaur, 1859

Class Trematoda Rudolphi, 1808

Order Azygiida Yamaguti, 1971

Family Didymozoidae Poche, 1907

Subfamily Nemathobothriinae Ishii, 1935

Genus *Neometanematobothrioides* Yamaguti, 1970

Neometanematobothrioides periorbitalis Yamaguti, 1970

***Localities of collection*:** Majorca, Alicante, Corsica and Sardinia

***Site*:** Specimens of *N. periorbitalis* were found within connective tissue of gills and in the mesentery.

***Description*:**

Specimens (N= 10) 78173 ± 46918 (23986 - 210128) long and 361 ± 187 (192-620) wide, dorsoventrally flattened, especially at the anterior body end (Fig. 4.12 A, C & D). Oral sucker 95 ± 22 (69 - 133) long and 90 ± 10 (74 - 103) wide. Pharynx absent. Ventral sucker rounded 45 ± 10 (32 - 63) in diameter. Testes 2, filiform and unbranched, obliquely arranged in the anterior half of body. Ovary filiform, overlapping with testes. Seminal receptacle present (Fig. 4.12 F). Uterus with single loop. Genital junction in Fig. 4.12 E. Vitellarium tubular, extending from uterus to posterior end. Eggs 33.02 ± 2.25 (29.54 - 37.03) long and 15.42 ± 0.93 (13.86 - 19.16) wide (Fig. 4.12 B).

***Remarks*:**

The genus *Neometanematobothrioides* includes only two species: *N. periorbitalis*, from *Seriola dumerili*, and *N. rachycentri* (Parukhin, 1969) from *Rachycentrum canadum* (Linnaeus, 1766) (Yamaguti, 1970). Based on Yamaguti (1970), current study specimens were identified as *N. periorbitalis* by the two unbranched testes obliquely arranged. Previous studies on *S. dumerili* from the Mediterranean Sea reported the presence of *Neometanematobothrioides* sp. in Murcia (Montero, 2001; Montero *et al.*, 2001b). However, in these studies the identification to species-level was not provided because, according to the authors, testes were unbranched and arranged in tandem, differing from the two species previously reported in this genus (Montero, 2001). In this case, arrangement of testes seems to be an ambiguous trait. Testes of *N. periorbitalis* are arranged in parallel but one of them begins anteriorly. As a consequence, according to the distance between the anterior end of both testes, different authors could consider their arrangement as parallel, obliquous or in tandem.

Therefore, it is highly probable that the species described by Montero (2001) is also *N. periorbitalis*. Features and dimensions of the specimens found in the current study are similar to those reported in the original description of the species (Yamaguti, 1970) and in Montero (2001) although specimens in the latter were slightly larger.

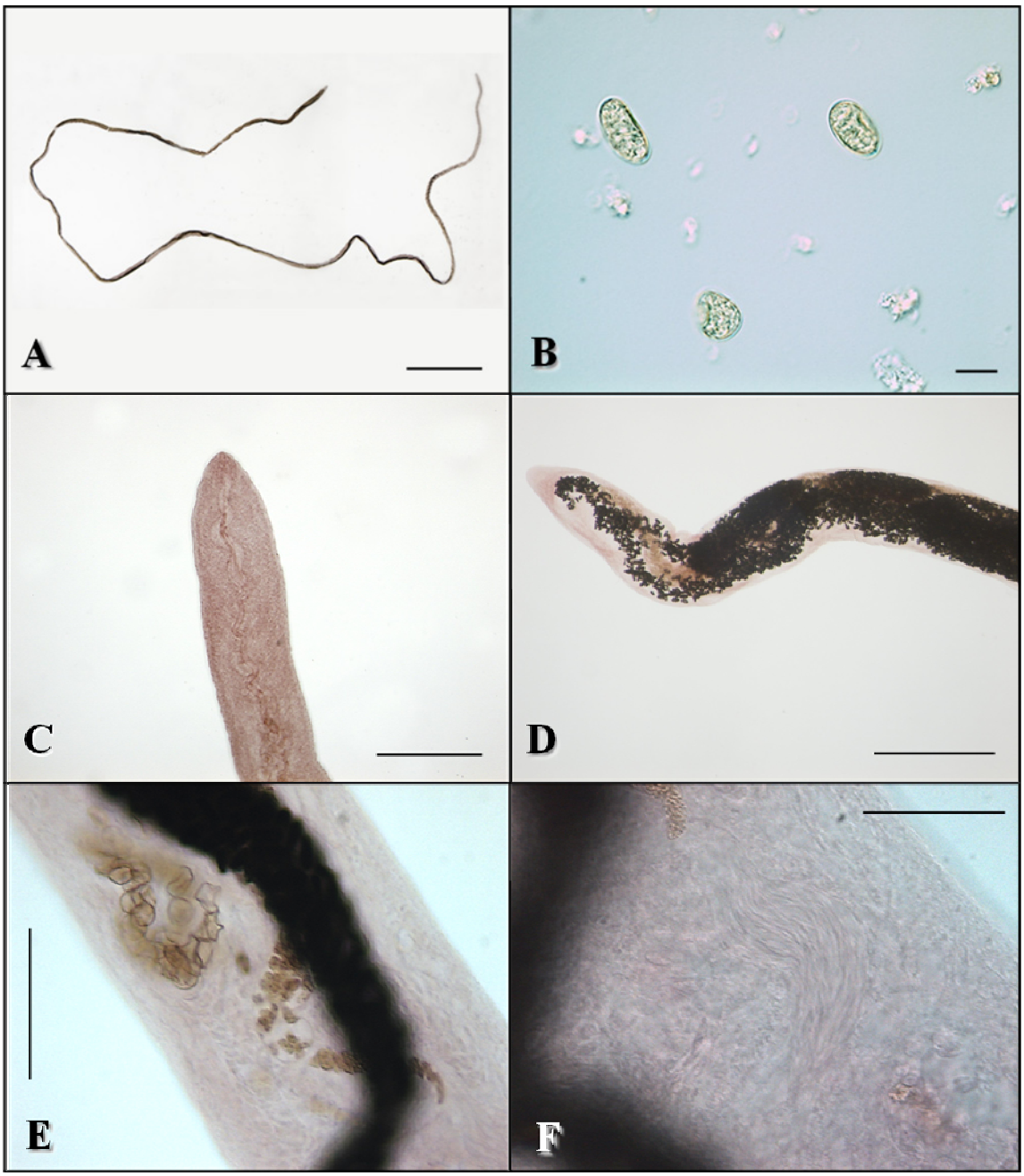


Figure 4.12. *Neometanematobothrioides periorbitalis* from *Seriola dumerili* off the western Mediterranean Sea. A, Whole specimen in ventral view; B, detail of the eggs; C, detail of anterior end; D, detail of posterior end with uterus; E, detail of genital junction; F, detail of the seminal receptacle Scale bars represent 2000 μm in A; 20 in B; 200 in C & D and 100 in E & F.

***Seasonal occurrence*:**

Monthly occurrence of *N. periorbitalis* in *S. dumerili* from Majorca is indicated in table 4.2. During the first year of study (2005), prevalence and mean intensity increased from April to June (spring-summer) while they decreased from October to February (autumn-winter). However, during the second year this apparent pattern was only observed for intensities.

Order Plagiorchiida, La Rue 1957

Family Bucephalidae Poche, 1907

Genus *Prosorhynchus* Odhner, 1905

Prosorhynchus facilis (Ozaki, 1924) Eckmannn, 1932

***Localities of collection*:** Majorca

***Site*:** Specimens of *P. facilis* were found in the digestive tract, mostly in pyloric caeca and intestine.

***Description*:**

Specimens (N= 4) 2220 ± 578 (1331 - 3039) long and 412 ± 104 (289 - 587) wide (Fig. 4.13 A). Rhynchus 181.53 ± 32.50 (141 - 204) long and 175 ± 27 (139 - 199) wide (Fig. 4.13 B). Mouth and pharynx near midbody; intestinal caecum extending forward. Testes obliquely arranged 208 ± 3 (203 - 211) long and 185 ± 7 (177 - 187) wide. Cirrus sac 198 ± 5 (195 - 205) long and 92 ± (89 - 97) wide. Ovary pretesticular 173 ± 5 (169 - 181) long and 131 ± 4 (129 - 136) wide. Vitelline follicles at midlevel, extending along the second third of total body length, never reaching to the anterior third. Vitellarium arranged in two lateral rows from 11 to 14 follicles each. Anterior section of uterus never extending over vitellarium. Eggs 24 ± 3 (21 - 31) long, 20 ± 1 (19 - 22) wide.

***Remarks*:**

Based on Overstreet & Curran (2002), specimens of this study were classified as *Prosorhynchus* sp. by the elongated seminal vesicle (at least twice as long as wide), the curved pars prostatica and the simple cone-rhynchus without anterior disc. However, these specimens were very similar to *Rhipidocotyle bartolii* Bray et Justine, 2011 which has been previously reported in Mediterranean greater amberjacks (Bartoli *et al*., 2005; Bray & Justine, 2011). *Rhipidocotyle* mainly differs from *Prosorhynchus* by the simpler rhynchus of the latter (Overstreet & Curran, 2002) but this feature is quite difficult to interpret.

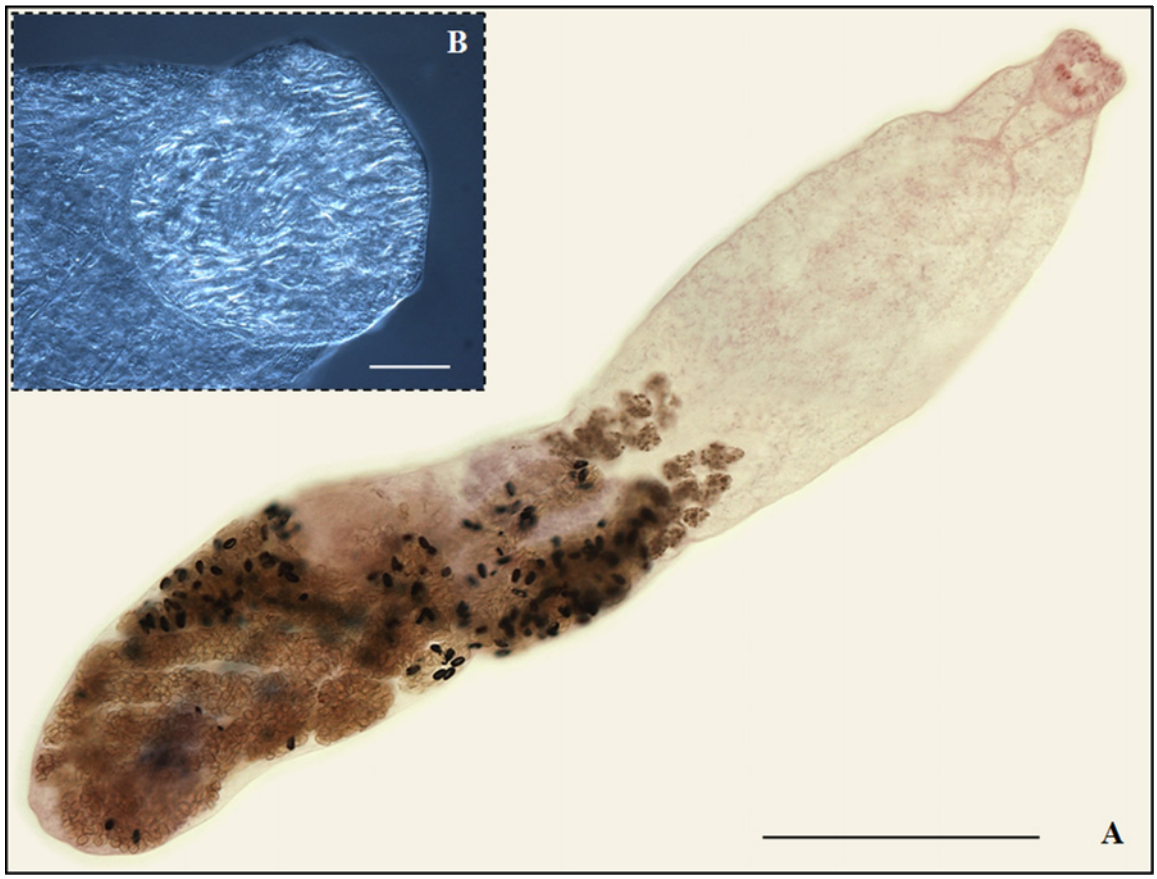


Figure 4.13. *Prosorhynchus facilis* from *Seriola dumerili* off the western Mediterranean Sea. A) Whole specimen; B) detail of rhynchus. Ventral views. Scale bar represent 500 µm in A and 50 in B.

Three *Prosorhynchus* species have been reported in *S. dumerili* to date: *P. crucibulum* Rudolphi, 1819, *P. facilis* (Ozaki, 1924), and *P. kahala* Yamaguti, 1970 (Gibson *et al.*, 2005); although none of them in the Mediterranean. Specimens found in the present study showed the diagnostic features of *P. facilis*, which is distinguished from the most similar species (*P. kahala*) by the vitellarium extending anteriorly to more than mid-body, and the eggs being larger (*P. facilis* eggs 27-34 x 19-22; *P. kahala* eggs: 14-18 x 9-12; Yamaguti, 1970). Despite the obvious similarities of the current study specimens and *P. facilis*, deeper analyses (morphological and molecular) would be required to confirm this identification. Indeed, current study specimens differ from *P. facilis* in their larger body size and their shorter cirrus sac. Moreover, all known records of *P. facilis* are from Pacific and Indian Oceans (Gibson *et al.*, 2005).

***Seasonal occurrence*:**

Monthly occurrence of specimens of *P. facilis* is indicated in table 4.2. Similar infection levels were recorded in June (summer) and in November (autumn) although prevalences and abundances were slightly higher in the 3 subsamples obtained in April (2005-2007). Seasonal infection patterns were not observed.

Class Cestoda **Rudolphi, 1808**

Order Tetraphyllidea Carus, 1863

Tetraphyllidean (plerocercoid larva)

***Localities of collection*:** Majorca

***Site*:** Tetraphyllidean larvae were found in the intestine.

***Description*:**

Tetraphyllidean plerocercoid (N= 7) with lanceolated unsegmented body, 1022 ± 241 (744 - 1172) long (Fig. 4.14). Maximum width at level of scolex, 199 ± 20 (176 - 212); maximum width at the rest of body 134 ± 14 (124 - 145). Scolex 203 ± 31 (178 - 239) long, with four bothridia. Bothridium 171 ± 10 (160 - 178) long, with 2 loculi: anterior small loculus 68 ± 11 long and posterior loculus 102 ± 9 long, separated by a tegumentary septum. Small apical accessory sucker at anterior end.

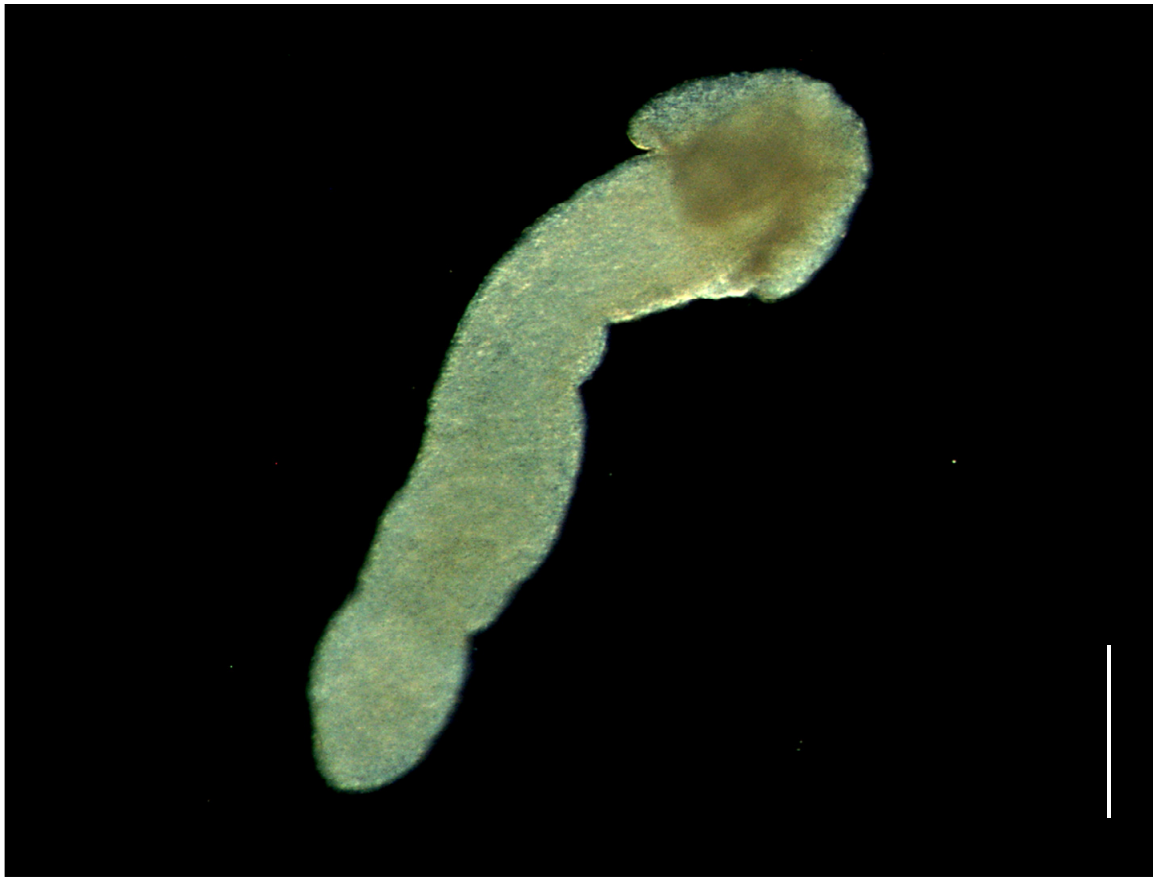


Figure 4.14. Tetraphyllidean plerocercoid from *Seriola dumerili* off the western Mediterranean Sea. Whole specimen. Scale bar represent 200 µm.

***Remarks*:**

Specimens were identified as tetraphyllidean plerocercoids as they showed the typical morphology of these larvae within the second vertebrated intermediate host: the unsegmented body and the scolex with 4 bothridia and an accessory apical sucker. This type of plerocercoids has traditionally been identified as *Scolex* spp., a complex of tetraphyllidean larval species morphologically undistinguishable (Euzet, 1994; Chervy, 2002). The morphotype "*Scolex pleuronectis*" has been reported in different regions including the Mediterranean Sea where it has been found in three carangid species: *Trachurus mediterraneus* (Steindachner, 1868), *T. picturatus* (Bowdich, 1825) and *T. trachurus* (Linnaeus, 1758) (Gibson *et al.*, 2005). In this case, molecular data would be mandatory for species identification. However, very few sequences of adult tetraphyllidean cestodes are available (see genbank database) and larvae recovered from intermediate hosts are rarely identificated.

***Seasonal occurrence*:**

Monthly occurrence of this species is indicated in table 4.2. Prevalence was very low in all subsamples (below 20%) and intensity of infection did not exceed two parasites per fish in most of them. Periodical infection patterns were not observed.

Phylum Nematoda (Rudolphi, 1808) Lankester, 1877

Class Secernentea Von Linstow 1905

Order Ascaridida Skrjabin et Schulz, 1940

Family Raphidascarididae Hartwich, 1954 (rank sensu Fagerholm, 1991)

Genus *Hysterothylacium* Ward et Magath, 1917

Hysterothylacium seriolae Yamaguti, 1941

***Localities of collection*:** Majorca, Corsica and Sardinia

***Site*:** Specimens of *H. seriolae* were found in the digestive tract, mostly in stomach and intestine.

***Description*:**

Female adult specimens (N= 2) 10760 - 12074 long and 329 - 369 wide (Fig. 4.15 A). Lips 43 long, with thin interlabia. Conical tail 216 - 260 long, with terminal spined cactus-tail at posterior end. Pharynx 1572 - 1683 long and 65 - 67 wide. Ventricular appendage 865 long, almost double the length of the intestinal caecum (461 long; ratio 1.9:1). Vulva at 2414 of anterior end (in female 12074 long). Eggs (N= 5).41 ± 2 (40 - 44). Tail 127 - 146.

Additional measures from other stages:

Male adult specimen (N= 1, out of 1 found) 8684 long and 267 wide. L4 larvae (N= 5, out of 51 found) 8289 ± 1159 (6281 - 9771) long and 252 ± 40 (193.26 - 300.83) wide (Fig. 4.15 B & C), bearing cactus-tail. L3 larvae (N= 5, out of 25 found) 5796 ± 782 (4437 - 6485) long and 60 ± 36 (136 - 197) wide, with terminal mucron at posterior end (Fig. 4.15 D & F).

***Remarks*:**

Most of the *H. seriolae* specimens found in the current study were larval stages (90%), L4 being predominant. Adult *Hysterothylacium* specimens of greater amberjack showed morphological similarities with *H. seriolae* described by Oliveira-Rodrigues *et al.* (1975). However, their morphological features differ from those reported in the original description of the species (Yamaguti, 1941). *H. seriolae* was originally described as *Contracaecum seriolae* (Yamaguti, 1941) in *Seriola quinqueradiata* from Japan (Yamaguti, 1941) and thereafter, Deardorff & Ovestreet (1980) included the species within *Hysterothylacium* genus.

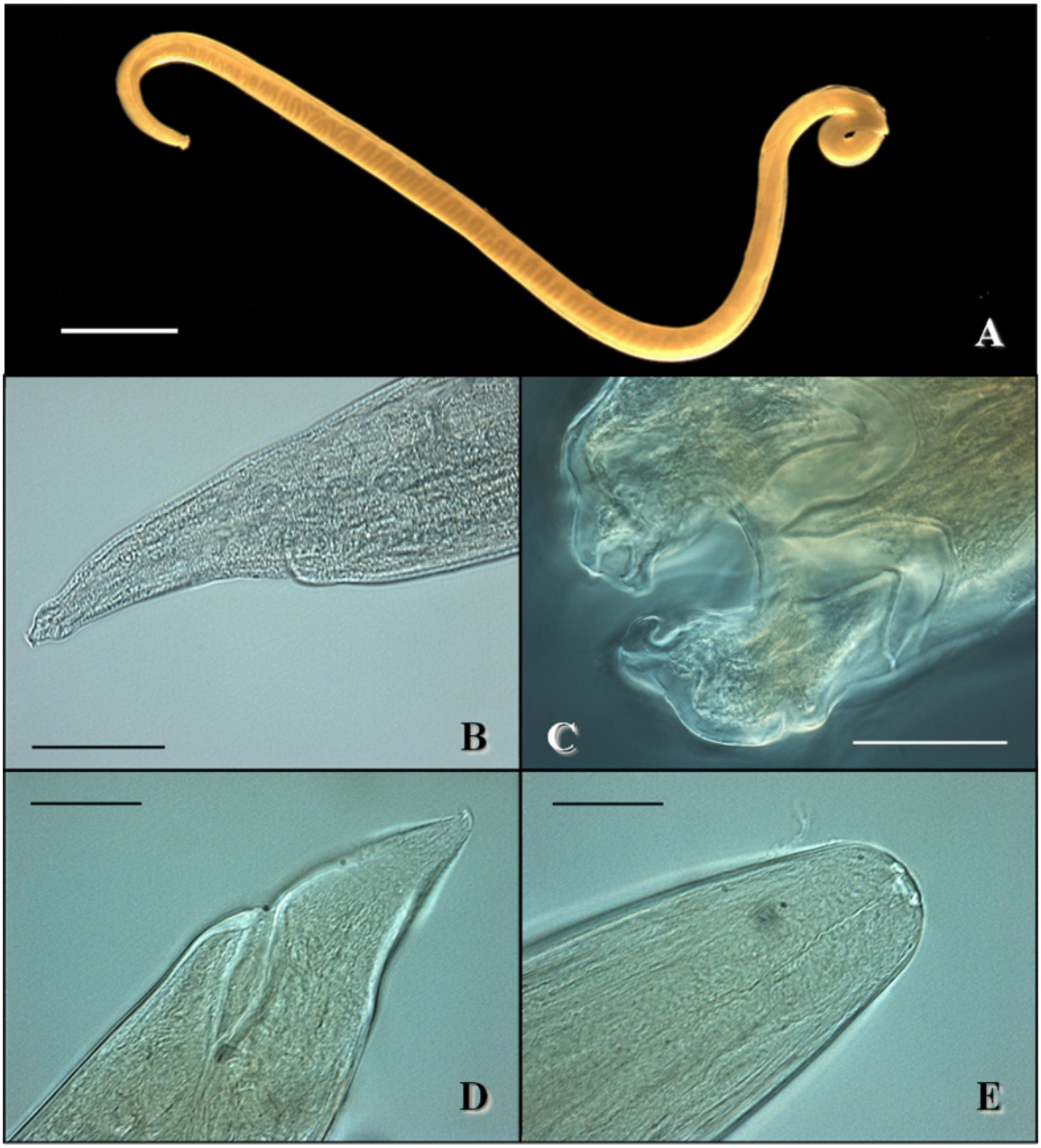


Figure 4.15. *Hysterotylacium seriolae* from *Seriola dumerili* off the western Mediterranean Sea. A, Whole adult specimen. B & C, L4 larva: detail of the tail (B), detail of the lips (C). D & E larva: detail of the tail (D), detail of anterior end (E). Scale bar represent 1000 µm in A; 100 in B; 20 in C and 50 in D& E.

Traditionally, one of the main traits to classify the Mediterranean *Hysterothylacium* spp. has been the ratio between the ventricular appendage and the intestinal caecum. In the current study, this ratio was similar to that reported in Oliveira-Rodrigues *et al.* (1975) for specimens from the Portuguese coast. However, the lengths of ventricular appendage and intestinal caecum were slightly higher in our specimen. Regarding specificity, *H. seriolae* has mainly been reported in *Seriola* spp.: *S. lalandi* from New Zealand (Hewitt & Hine, 1972) and *S. quinqueradiata* and *S. dumerili* from Pacific Ocean (Yamaguti, 1941; Williams & Bunkley-Williams, 1996). However, Oliveira-Rodrigues *et al.* (1975) reported this species in *Beryx decadactylus* Cuvier, 1829, a Beryciform fish. In spite that

different *Hysterotylacium* species have been reported infecting *Seriola* spp. (Deardorff *et al.*, 1982; Hutson *et al.*, 2007a), *H. seriolae* is the only identified to species-level (see Bruce *et al.*, 1994). In the Mediterranean Sea, this species has never been reported while other *Hysterothylacium* spp. are usually found (i.e. *H. aduncum*, *H. fabri* or *H. arnoglossi*, see Petter & Radujkovic, 1989).

***Seasonal occurrence*:**

Monthly occurrence of *H. seriolae* is reported in table 4.2. This parasite species was collected in 8 of the 11 subsamples of the study although adults were only found in April, 2005. During the first year, parasite abundance and intensity appeared to be higher in June and October, 2005 (summer and early autumn). However, during the next year, prevalences were lower and the pattern was not repeated. Thus, seasonal infection patterns were not detected.

Phylum Arthropoda Latreille, 1829

Class Maxillopoda Dahl, 1956: Subclass Copepoda Milne-Edwards, 1840

Order Siphonostomatoida Thorell, 1859

Family Caligidae Burmeister, 1835

Genus *Caligus* Müller, 1785

Caligus aesopus Wilson, 1921

***Localities of collection*:** Majorca, Alicante, Corsica and Sardinia.

***Site*:** Specimens of *C. aesopus* were found attached to the gill filaments.

***Description*:**

Female:

Specimens (N= 10) 3488 ± 415 (2856 - 4313) long and 1668 ± 171 (1496 - 2027) wide (Fig. 4.16 A & B). Cephalothorax 1689 ± 137 (1534 - 1993) long and 1668 ± 171 (1496 - 2027) wide. Lunules 108 ± 3 (105 - 111) long and 168 ± 8 (160 - 177) wide. Fourth pedigerous somite fused with genital complex. Genital complex subtriangular 1063 ± 198 (702 - 1368) long and 880 ± 105 (683 - 1053) wide, with angular postero-lateral corners. Abdomen 424 ± 42 (347 - 461) long and 342 ± 50 (285 - 434) wide. Caudal ramus 60 ± 9 (54 - 71) long and 68 ± 5 (63 - 74) wide, with 6 setae distally located (5 of them feather-like and one simple) and a dorsal small setule. Ovigerous sac 1431 ± 183 (1148 - 1684) long with 14 to 20 eggs (N= 6). Eggs 86 ± 22 (66 -135) long and 329 ± 23 (290 - 354) wide. Furca with tapering tines (Fig. 4.16 F). First leg 3-segmented (Fig. 4 A & B) with a small blunt projection on the proximal segment. Protopod of leg 3 (Fig. 4.17 C) ornamented with a marked ventral patch of 11 to 15 robust spines on inner ventral surface (Fig. 4.17 E) and a longitudinal patch of small spinules on mid-ventral surface (Fig. 4.17 F). First exopodal segment of leg 3 with a robust spine, enlarged at the base and strongly curved, 183 ± 25 (165 - 211) long and 61 ± 3.09 (60 - 64) wide (Fig. 4.17 D). Fourth leg 3-segmented with the innermost terminal spine of the distal segment longer than the 2 outer ones (Fig. 4.17 G).

Male:

Specimens (N= 10) 3092 ± 165 (2726 - 3212) long and 1657 ± 124 (1396 - 1755) wide (Fig. 4.15 D & E). Cephalothorax similar to that of female, 1766 ± 56 (1689 - 1852) long and 1657 ± 124 (1396 - 1755) wide. Genital complex fused with abdomen; genito-abdomen 953 ± 38 (885 - 993) long and 428 ± 38 (376 - 486) wide. Caudal ramus (Fig. 4.16 E) 85 ± 12. (63 - 98) long and 89

± 8. (78 - 99.40) wide, with 5 setae (4 of them feather-like and 1 simple) in the distal margin, 4 small setules in the inner margin and a dorsal simple seta. Legs 1, 3 and 4 as in female.

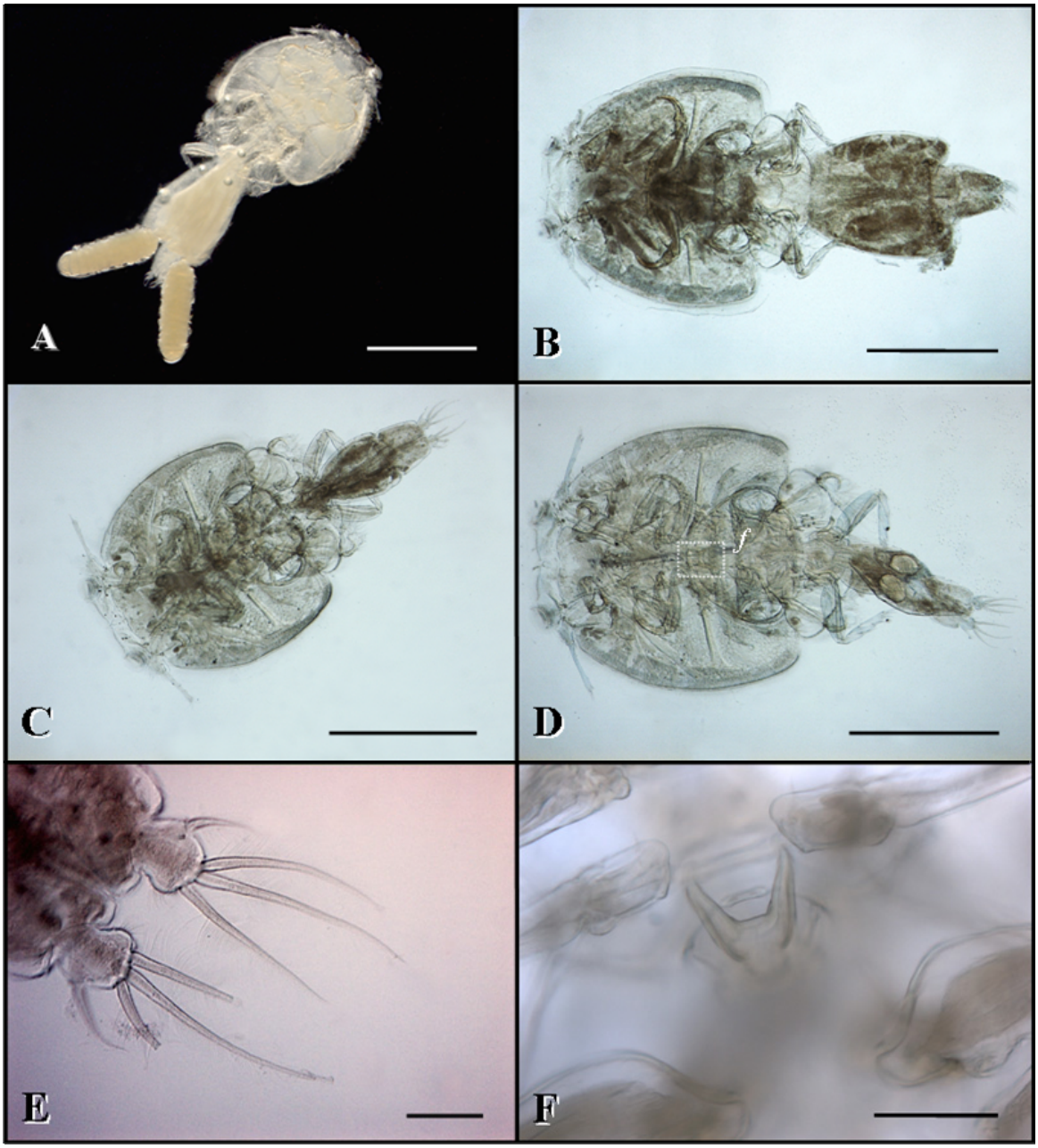


Figure 4.16. *Caligus aesopus* from *Seriola dumerili* off the western Mediterranean Sea. A & B, Female: whole specimens in dorsal (A) and ventral (B) views. C & D, Male whole specimens in dorsal (C) and ventral (D) views; Scale bars represent 1000 µm in A-D, 100 in E and 200 in F.

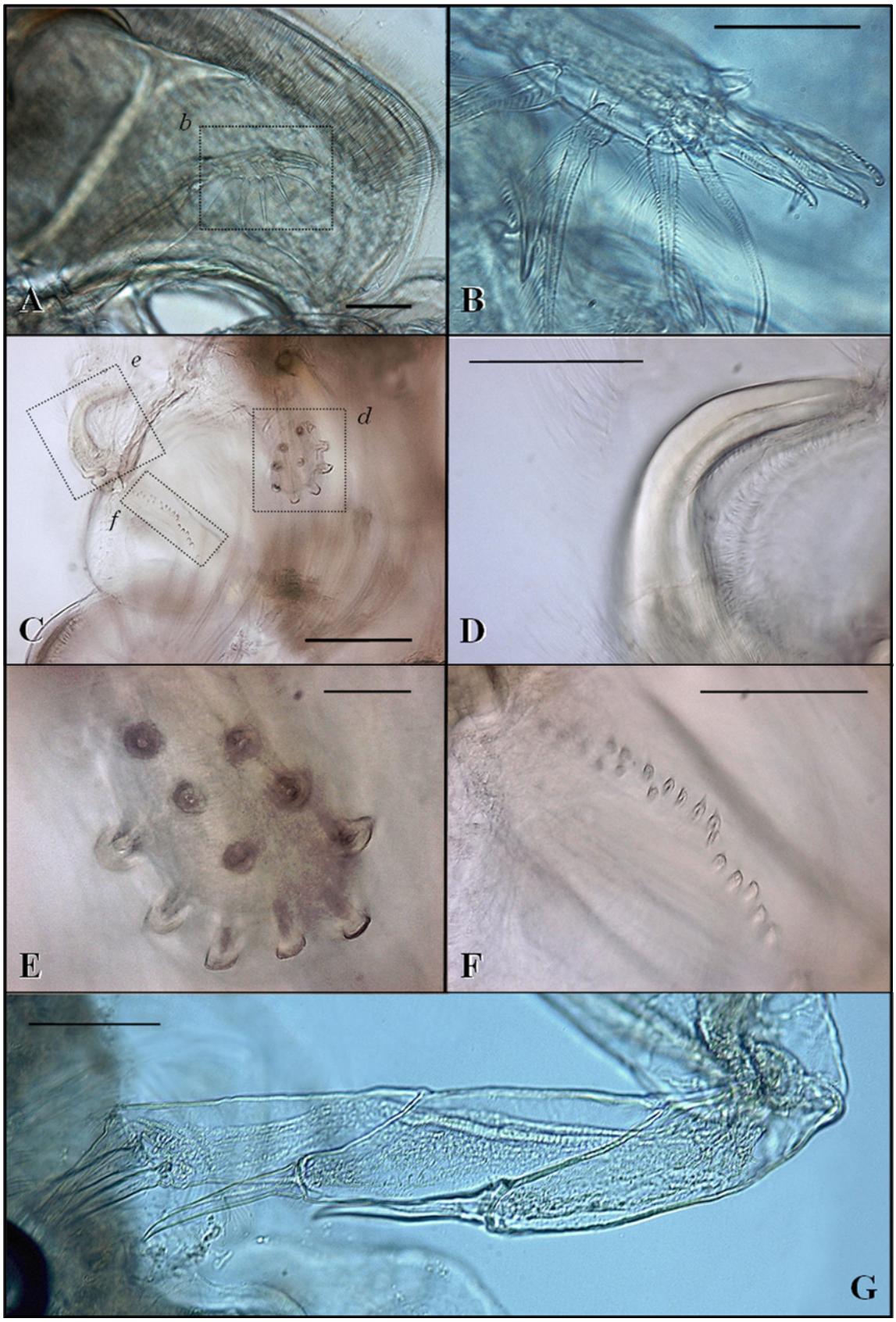


Figure 4.17. *Caligus aesopus* from *S. dumerili* off the western Mediterranean Sea. A & B, First leg: whole (A) and detail of distal segment (B). C-F, Third-leg: whole (C), robust spines in first exopodal segment (D), robust spines in protopod (E), spinules in protopod (F). G, Fourth leg. Scale bars represent 100 µm in A-C, 50 in D, F and G; and 20 in E.

***Remarks*:**

Eight species of *Caligus* have been reported in *Seriola* spp. to date: *C. amblygenitalis* Pillai, 1961; *C. aesopus* Wilson, 1921;, *C. curtus* OF Müller, 1785; *C. diaphanus von Normand*, 1832; *C. lalandei* Barnard, 1948, *C. seriolae* Yamaguti, 1936, *C. spinosus* Yamaguti, 1939 and *C. tenax* (Heller, 1865), (Yamaguti, 1963; Fernandez & Villalba, 1986; Abou-Znada & Ramadan, 1993; Grau *et al.* 1999; Montero *et al.*, 2001b; Hutson *et al.*, 2007a). Only 2 of these species have been reported in *S. dumerili* from the Mediteranean Sea, *C. curtus* and *C. diaphanus* (Grau *et al.*, 1999; Montero, 2001), and the specimens herein described broadly differ from them.

These specimens mostly resemble to 3 species: *C. tennax* which mainly parasitise *Caranx* sp., but also *S. rivoliana* (Abou-Znada & Ramadan, 1993); *C. spinosus*, previously reported in *S. lalandi* and *S. quinqueradiata* from the Pacific Ocean (Hutson *et al.*, 2007a; Cruz-Lacierda *et al.*, 2011); and *C. aesopus* reported in *S. lalandi* off New Zealand and Easter Island and in *S. dumerili* from Taiwan (Lin & Ho, 2007; Choe & Kim, 2010). These three species are mainly distinguished from the rest of *Caligus* by the presence of a patch of large and robust spines in the protopod of the third leg, the dimensions of the spine on the first exopodal segment of the same leg, and the three-segmented fourth leg (Yamaguti, 1963, Choe & Kim, 2010). The specimens found in the greater amberjacks from Majorca were clearly identified as *C. aesopus*. This species differs from *C. tennax* by the absence of the subterminal and inner spine in the distal segment of the fourth leg and the shorter terminal naked seta on the distal segment of the first leg (Yamaguti, 1963). *C. aesopus* also differs from *C. spinosus* although many authors have treated this two species as synonyms (Choe & Kim, 2010). The main features differentiating both species are the small tubercle in the base of the third leg, a patch of less than less than 15 spinules in the protopod of the third, a distinctly long inner and terminal spine on the third exopodal segment of the fourth leg and the number of tubercles in the first maxillipedal segments of males and females (Choe & Kim, 2010).

Features and dimensions of the specimens from greater amberjack coincide with those reported for *C. aesopus* by Choe & Kim (2010) except for the number of spinules in the inner patch of the protopod of the third leg which can reach to 15 instead of to 14. This difference is apparently negligible; however, considering the geographical distributions reported for this species, molecular studies would be recommendable.

***Seasonal occurrence*:**

Monthly occurrence of *C. aesopus* is indicated in table 4.2. Prevalences were similar in all subsamples (from 40 to 60%) except for the peak during December 2005 (87%; see table 4.2), when the highest intensity was also observed. Seasonal infection patterns were not observed.

Sex-ratios (female/male) are depicted in figure 4.18. Females (pre-adult and adult stages) outnumbered males in all subsamples. Sex-ratio apparently increased from February to June (late winter to summer), when water temperatures increased, and decreased from September to November - December (autumn to winter). Gravid females were observed in all subsamples although abundance was higher from November to February.

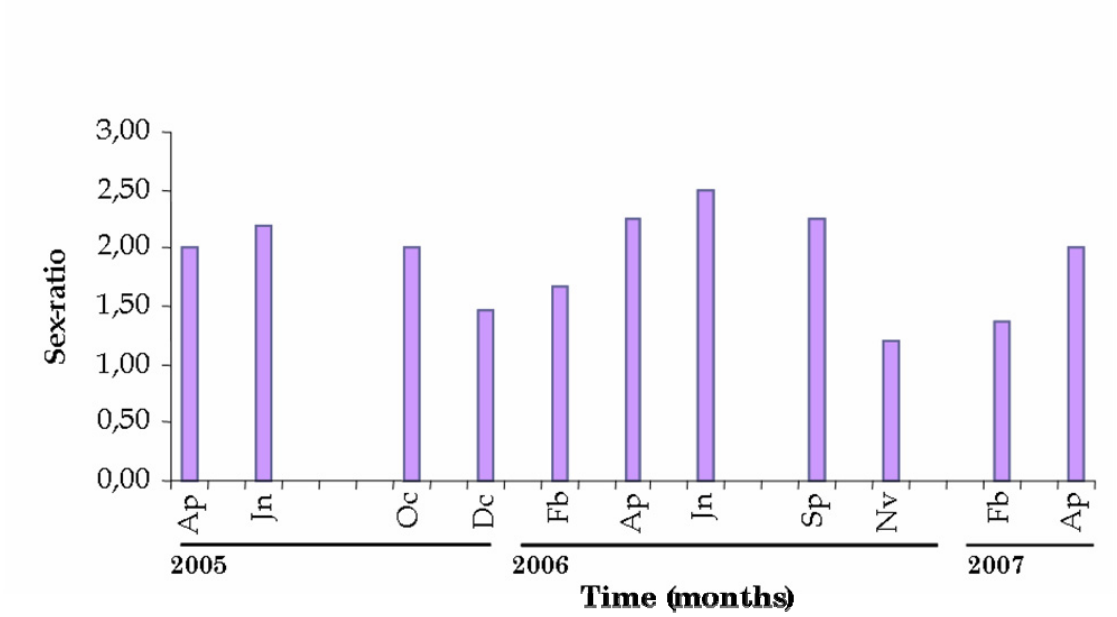


Figure 4.18. Seasonal changes on sex-ratio (females/males) of *Caligus aesopus* infecting *Seriola dumerili* from the Balearic Islands between April 2005 and April 2007.

Genus *Caligus* Müller, 1785

***Caligus* sp.**

***Locality of collection*:** Majorca

***Site*:** The only specimen of *Caligus* sp. was found in the gills.

***Description*:**

Female:

Specimen (N= 1) 3210 long and 1541 wide (Fig. 4.19 A). Cephalothorax trapezoid 1560 long and 1541 wide. Lunules 189 long and 121 wide. Genital complex subtriangular 1095 long and 943 wide, with rounded postero-lateral corners. Abdomen 511 long and 340 wide. Caudal ramus (Fig. 4.19 B) 110 long and 70 wide, with 3 long distal setae, one short subterminal outer seta, and one inner distal setule. Postantennary process digitiform and long (Fig 4.19 E). Dentiform process of maxillule also long and digitiform. Prominent and pointed process (approximately 70 long) near the inner postero-lateral margin of maxillule (Fig 4.19 E). Furca with tapering tines. First leg 3-segmented. Distal segment with 3 long pinnated setae on inner margin and 4 simple terminal setae, 3 of them similar in size and the 4th slightly longer (Fig. 4.19 C). Lateral margin of second endopod covered with fine setules. First exopodal segment of leg 3 with a marginal, robust and digitiform spine and a dorsal club-shaped process on the lateral area of the protopod (Fig. 4.19 F). Fourth leg 2-segmented (Fig 4.19 D).

Remarks:

The specimen herein described is mainly differentiated from the other species of *Caligus* reported in *Seriola* spp. and from most of *Caligus* spp. by the pair of digitiform processes located posterior to maxillulae, the club-shaped dorsal process on the protopod of the third leg and the two-segmented fourth leg. This specimen mostly resembles to *C. seriolae* Yamaguti, 1936, reported from *S. quinqueradiata*, which also have a two-segmented fourth leg (Yamaguti, 1963). However, the special ventral armature of the specimen parasitising *S. dumerili* was not observed *in C. seriolae*. From the other hand, the digitiform spines located posterior to maxillulae have also been described in *C. temnodontis* Brian, 1924 (as *C. mauritanicus*) from *Pomatomus saltarix* (Linnaeus, 1766) off southeastern Atlantic Ocean (Boxshall & El-Rashidi, 2009) and *Dentex dentex* (Linnaeus, 1758) off Aegean Sea (Öktener, 2009a). However, the rest of features and dimensions differed from this species. More specimens (females and males) would be required for identification to species-level.

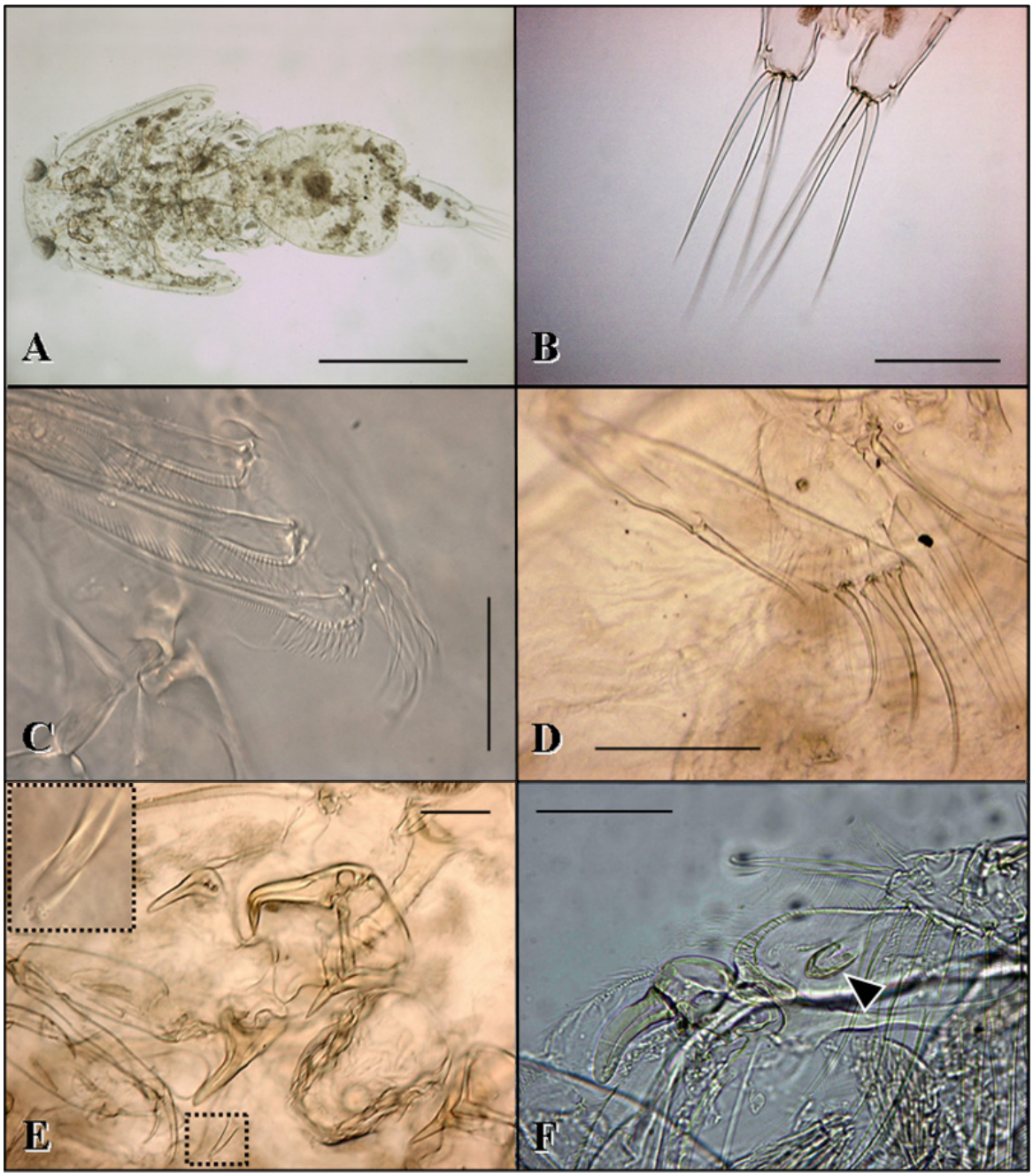


Figure 4.19. *Caligus* sp. in *S. dumerili* off Majorca; A. Whole specimen ventral view; B. Caudal ramus; C. Detail of the distal segment of the first leg; D. Fourth leg; E. Detail of the spines of *Caligus* sp. in ventral view and digitiform process; F. Detail of the marginal digitiform spine (arrowhead) in leg 3. Scale bars represent 1000 μm in A; 100 in B-F.

Seasonal occurrence:

The only specimen of *Caligus* sp. was found in February 2006 (see table 4.2).

4.3.5. Species previously reported in the Mediterranean greater amberjack:

Phylum Platyhelminthes Gegenbaur, 1859
Class Monogenea Carus, 1863
Order Mazocraeidea Bychowsky, 1957
Family Heteraxinidae (Unnithan, 1957) Price, 1962
Genus *Zeuxapta* Unnithan, 1957
Zeuxapta seriolae (Meserve, 1938) Price, 1962

***Localities of collection*:** Majorca, Alicante, Corsica and Sardinia.

***Site*:** Specimens of *Z. seriolae* were found attached to the gill filaments.

***Description*:**

Specimens (N= 10) 5660 ± 873 (4257 - 7011) long and 1004 ± 121 (891 - 1321) wide. Maximum width at haptor. Asymmetrical haptor with 38 to 43 clamps in the long side (43 ± 2 wide) and 29 to 31 in the short side (33 ± 2 wide). Digestive tract bifurcated in two caeca widely ramified. High number of testes (81 - 94) rounded, 87 ± 5 in diameter. Unarmed cirrus opening into a common genital atrium (also unarmed). Germarium arranged anterior to testis. Uterus wide, opening into genital atrium. Vitellarium associated to digestive. Eggs with two polar filaments one of them short and hooked and the other very long. Non-hooked filaments entangled together forming egg-strings.

***Remarks*:**

According to Rohde (1978) and Payne (1990), the diagnosis within the genus *Zeuxapta* is mainly based on the number of clamps and testes. *Z. seriolae* usually parasitises *Seriola* spp. (Meserve, 1938; Yamaguti, 1940; Ogawa & Fukudome, 1994) although it has also been reported and described from different carangid fish (Lamothe-Argumedo, 1970; Rohde, 1978). In the Mediterranean, this species was first recorded in *S. dumerili* from Messina (Italy) in 1996 (Montero *et al.*, 2004) and some years after in Murcia (Montero *et al.*, 2001b) and Majorca (Grau *et al.*, 2003), both in Spain. Mediterranean specimens were described by Montero (2001) who also reviewed previous taxonomical studies on this species. No morphological differences were found between current study specimens and those described by this author.

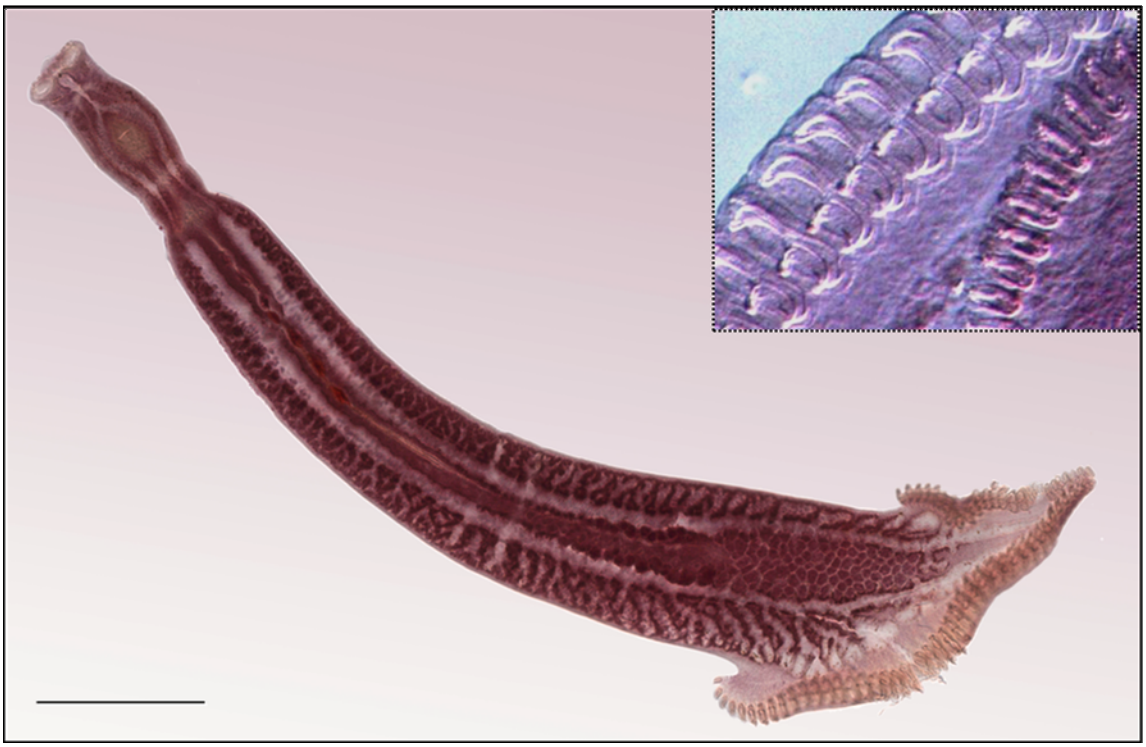


Figure 4.20. *Zeuxapta seriolae* from *S. dumerili* off the western Mediterranean Sea. A, Whole specimen in ventral view; B, detail of the haptor clamps in ventral and lateral views. Scale bar represents 1000 µm.

Seasonal occurrence:

Monthly occurrence of *Z. seriolae* in *S. dumerili* from Majorca is indicated in table 4.2 and seasonality is analysed in chapter 6.

Order Plagiorchiida, La Rue 1957

Family Bucephalidae Poche, 1907

Genus *Bucephalus* Baer, 1826

Bucephalus gorgon (Linton, 1905) Eckmann, 1932

***Localities of collection*:** Majorca, Alicante, Corsica and Sardinia.

***Site*:** Specimens of *B. gorgon* were found in the digestive tract, mostly in the intestine.

***Description*:**

Specimens (N =10, all of them with non-retracted rhynchus) 2477 ± 531 (1762 - 3259) long and 263 ± 62 (189 - 409) wide (Fig. 4.21). Rhynchus 170 ± 58 (100 - 255) long, with 18 tentacles: 12 of them free in medial position and 3 laterally arranged and joined. Ovary pretesticular and rounded 150 ± 15 (134 - 165) in diameter. Two testes rounded to ellipsoidal, similar in size, arranged in tandem, 169 ± 72 (100 - 303) maximum width.

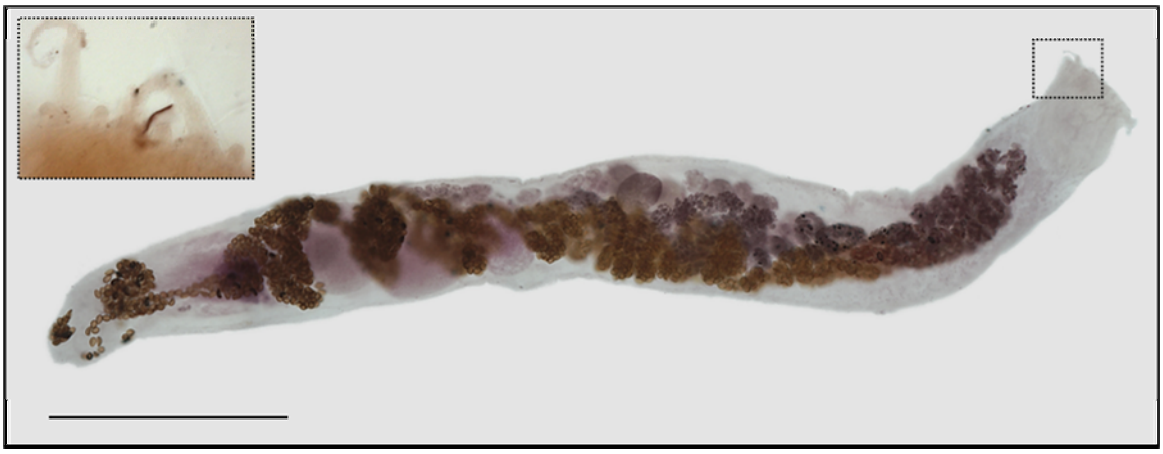


Figure 4.21. *Bucephalus gorgon* from *Seriola dumerili* off the western Mediterranean Sea. Whole specimen in ventral view, with detail of the tentacles of the rhynchus. Scale bar represents 500 µm.

***Remarks*:**

According to Fischthal *et al.* (1982) *B. gorgon* can be distinguished from the rest of *Bucephalus* species by the total body size, the number of tentacles and the egg size. *B. gorgon* has been recently redescribed using specimens recovered from Mediterranean *Seriola dumerili* and morphological comparisons with previous studies have also been reported (Montero 2001; Bartoli *et al.* 2005). Features and dimensions of the specimens herein recorded mostly agree with those reported by these authors although specimens from Bartoli *et al.* (2005) were slightly smaller. From the other hand, Bartoli *et al.* (2005) suggested that it is probable that the bucephalid species

collected in Balearic Sea by Grau *et al.* (1999) corresponded to *B. gorgon* instead of *B. polymorphus* von Baer, 1827, as it is a parasite of freshwater fishes. Present study results clearly support this suggestion, as *B. gorgon* was the only *Bucephalus* species found in Majorca and in the rest of western Mediterranean localities.

***Seasonal occurrence*:**

Monthly occurrence of *B. gorgon* is indicated in table 4.2. Prevalences apparently increased from April to November-December (spring to autumn) and decreased from December to February (during winter) in both years of study. However, prevalences exceeded 50% in all subsamples and Fisher's exact test did not revealed significant differences among them. Intensities and abundances did not follow a clear seasonal pattern, although a decrease in parasite number was observed from November-December to February, the period when the lowest temperatures were reached.

Family Hemiuridae Looss, 1899
Genus *Hemiurus* Rudolphi, 1802

Hemiurus communis Odhner, 1905

***Localities of collection*:** Majorca, Alicante, Corsica and Sardinia.

***Site*:** Specimens of *H. communis* were found in the digestive tract, mostly in the stomach.

***Description*:**

Specimens (N= 10) 2249 ± 338 (1890 - 2863) long and 394 ± 109 (299.51 - 628) wide. Ecsoma usually extended, 502 ± 178 (368 - 598) long and 295 ± 87 (267 - 329) wide (Fig. 4.22 A & B). Oral sucker rounded, 139 ± 24 (114 - 163) in diameter. Ventral sucker rounded, 279 ± 46 (230 - 322) in diameter. Testes 175 ± 27 (141 - 228), arranged slightly oblique. Seminal vesicle bilobed, arranged close to ventral sucker. Ovary 228 ± 33 (209 - 267) diameter. Vitellarium composed by 2 rounded masses. Uterus with descending limbs extending into ecsoma.

***Remarks*:**

Three species of *Hemiurus* have been reported in the Mediterranean Sea to date (Gibson *et al.*, 2005): *H. appendiculatus* (Rudolphi, 1802), *H. communis* Odhner, 1905 and *H. luehei* Odhner, 1905 (syn. *H. rugosus* Looss, 1907; according to Gibson & Bray, 1986). However, *H. communis* is the only species previously reported in *S. dumerili* (Grau *et al.*, 1999) and no more *Hemiurus* spp. have been reported in carangids in this region (Gibson *et al.*, 2005). Dimensions and organ arrangement of the specimens herein recorded coincide with those reported for *H. communis* by Odhner (1905) and Gibson & Bray (1986). According to the latters, *H. communis* can be distinguished from the rest of *Hemiurus* spp. by the ratio among suckers (ventral/oral), which is higher than 1:1, and the arrangement of the seminal vesicle, which is close to the ventral sucker and not well posterior. However, *H. communis* shows a wide variability of morphologies and sizes (Gibson & Bray, 1986) and, therefore, dimensional features must be carefully considered.

***Seasonal occurrence*:**

Monthly occurrence of *H. communis* is indicated in table 4.2. Prevalences increased from September-October to December-February (from autumn to winter) when maximums were reached although high prevalences (60% or higher) were also recorded in the 3 subsamples collected in April. The same pattern was observed for mean abundances and intensities. In general, higher

prevalences and mean intensities were observed when temperatures were lower although Fisher's exact test did not reveal significant differences among subsamples.

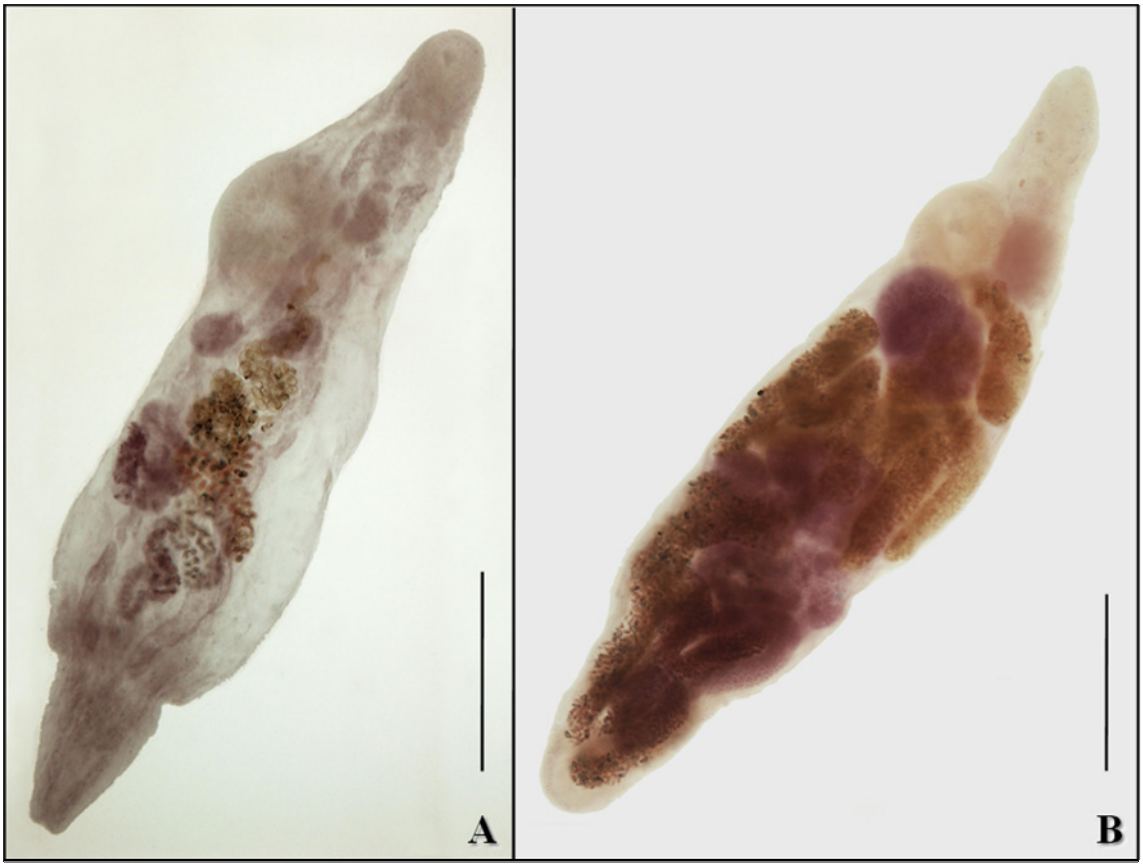


Figure 4.22. *Hemiurus communis* from *Seriola dumerili* off the Mediterranean Sea. Lateral views. A) Mature specimen with few eggs. B) Mature specimen with numerous eggs. Scale bars represent 500 µm.

Family Acanthocolpidae Lühe, 1906

Genus *Stephanostomum* Looss, 1899

Stephanostomum ditrematis (Yamaguti, 1939) Manter, 1947

***Localities of collection*:** Majorca, Alicante, Corsica and Sardinia.

***Site*:** Specimens of *S. ditrematis* were found in the digestive tract, mostly in the stomach and the first section of the intestine.

***Description*:**

Specimens (N= 10**) 6723 ± 1305 (4722 - 8294) long and 321.58 ± 31.27 (276 - 342) wide at level of ventral sucker (Fig. 4.23 A). Oral sucker terminal, cup-like, 106 ± 14 (92 - 120) long and 171 ± 14 (162 - 187) wide, with 36 spines distributed in two rows not interrupted ventrally (Fig. 4.23 E & F). Ventral sucker rounded, 281 ± 30 (251 - 322) in diameter. Pharynx 213 ± 24 (193 - 224) long (Fig. 4.23 B). Ovary 242 ± 41 (202 - 298) long. Two testes similar in size 486 ± 55 (406 - 525) long, separated and arranged in tandem. Distance between testes 140 ± 139 (0 - 332). Eggs 62 ± 3 (59 - 66) long (Fig. 4.23 C & D).

***Remarks*:**

According to Bartoli & Bray (2001) the diagnosis of *Stephanostomum* spp. is mainly based on the number of circum-oral spines, the size of suckers and testes and the arrangement of testes and vitellarium. These authors recently described 4 species of *Stephanostomum* in *Seriola dumerili* off Corsica (Bartoli & Bray, 2004) and also provided detailed information on previous species reported in the Mediterranean Sea. This publication was used to identify the *Stephanostomum* specimens found in the current study.

S. ditrematis was described and reported in greater amberjacks from Murcia (Spain) by Montero (2001) and Montero *et al.* (2001b). Thereafter, this species was redescribed based on specimens from Corsica (Bartoli & Bray, 2004). Features and dimensions of the specimens described here are in accordance with those previously reported (Montero, 2001; Bartoli & Bray, 2004). From the other hand, Grau *et al.* (1999) reported *Stephanostomum pristis* (Deslongchamps, 1824) in *S. dumerili* off Balearic Islands but Bartoli & Bray (2004) suggested that this species could also be *S. ditrematis* as *S. pristis* typically parasitise specimens belonging to other fish families

**Identification of *Stephanostomum* spp. is especially difficult when specimens are obtained from frozen fish because these parasites easily lose their circumoral spines, which are the main diagnostic character. The specimens of *Stephanostomum* spp. used for descriptions were collected from fresh fish and the rest of parasites were classified comparing with described specimens.

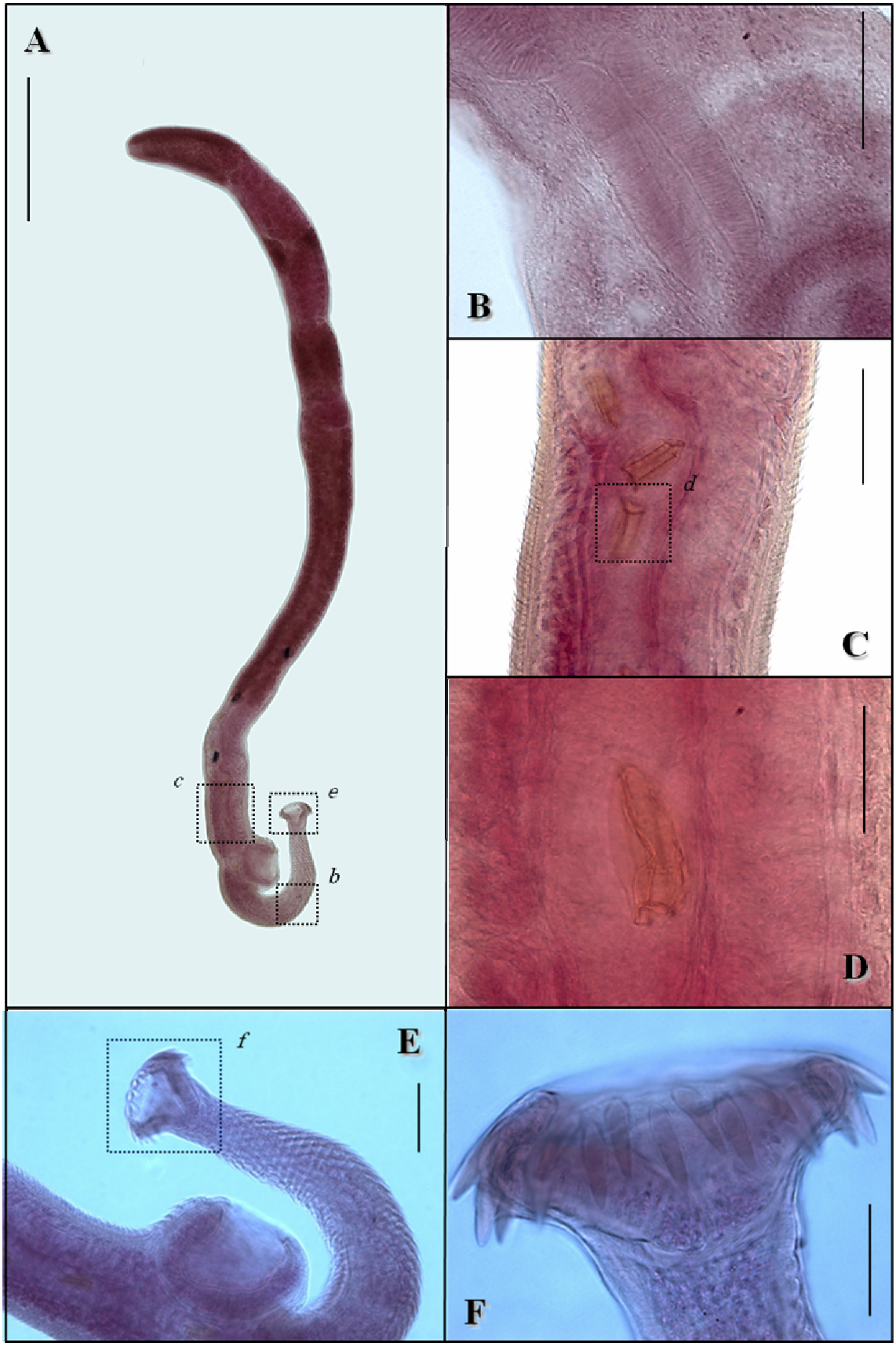


Figure 4.23. *Stephanostomun ditrematis* from *Seriola dumerili* off the Mediterranean Sea. A, Whole specimen in lateral view; B, detail of the pharynx; C, eggs in uterus; D, detail of one egg; E, spined anterior region; F, detail of the circumoral spines and terminal oral sucker; Scale bars represent 1000 µm in A, 100 in B, C & E and 50 in D & F.

(i.e. Gadidae, Lotidae and Moridae). Present study results support this suggestion, as *S. ditrematis* was the most prevalent and abundant species found in Majorca and Alicante waters.

***Seasonal occurrence*:**

Monthly occurrence of *S. ditrematis* is reported in table 4.2. Prevalences apparently increased from February to June and decreased from September to February in accordance with seawater temperature fluctuations. However, no significant differences between subsamples resulted from the Fisher's exact test comparison. Mean abundance and intensity did not follow the pattern observed for prevalence but their lowest values were also recorded in February of both years of study.

Stephanostomum euzeti Bartoli & Bray, 2004

***Localities of collection*:** Majorca, Alicante Corsica and Sardinia.

***Site*:** Specimens of *S. euzeti* were found in the digestive tract, mostly in the intestine.

***Description*:**

Specimens (N= 7) 3342 ± 936 (2499 - 4482) long and 317 ± 26 (285 - 342) wide at level of ventral sucker (Fig. 4.24). Oral sucker terminal, cup-like, widely-opened and large, 198 ± 136 (121 - 295) long and 301 ± 100 (190 - 434) wide, with 49 - 50 spines distributed in two rows not interrupted ventrally. Ventral sucker rounded, 251 ± 45 (209 - 306) in diameter. Pharynx 255 ± 17 (207 - 303) long. Ovary 203 ± 34 (167 - 243) long. Two unequal testes, anterior 347 ± 180 (207 - 445) long; posterior 438 ± 96 (328 - 587) long; contiguous and arranged in tandem. Eggs 65 ± 9 (69 - 63). See footnote in page 79.

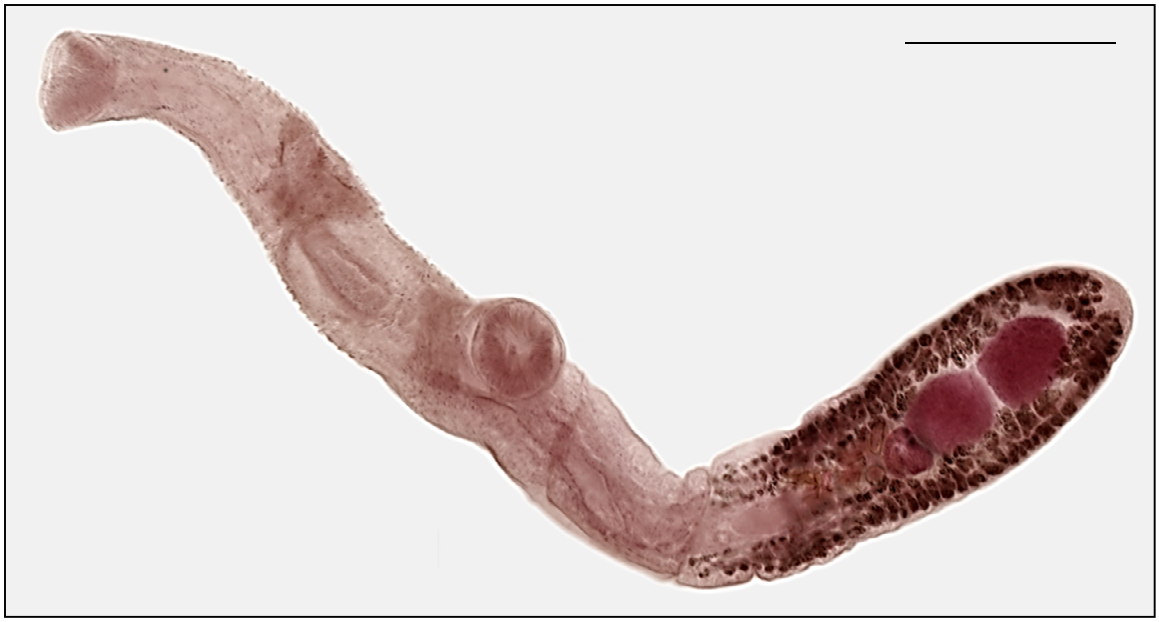


Figure 4.24. *Stephanostomun euzeti* from *S. dumerili* off the Mediterranean Sea. Whole specimen in ventral view. Scale bar represents 500 µm.

***Remarks*:**

As previously stated, specimens of *Stephanostomum* spp. were identified using the descriptions provided by Bartoli & Bray (2004). Moreover, these authors originally described *S. euzeti*. Specimens herein studied were shorter and slightly narrower than those reported in the original description although diagnostic features, dimensions of pharynx and gonads as well as arrangement of gonads and vitellarium, were similar.

***Seasonal occurrence*:**

S. euzeti was only found in 6 out of the 11 monthly subsamples. Prevalence of this species was very low (< 13.3 %) and only 1 parasite per fish was collected. Seasonal infection patterns were not observed.

Stephanostomum filiforme Linton, 1940

***Localities of collection*:** Majorca, Corsica and Sardinia.

***Site*:** Specimens of *S. filiforme* were found in the digestive tract, mostly in the intestine.

***Description*:**

Specimens (N= 10) with body elongated, 7369 ± 1520 (5696 - 9781) long and 372 ± 61 (302 - 468) wide at level of ventral sucker (Fig. 4.25). Oral sucker terminal, cup-like, 168 ± 19 (142 - 192) long and 208 ± 100 (200 - 216) wide, with 44 - 46 spines distributed in two rows not interrupted ventrally. Ventral sucker rounded, 368 ± 25 (323 - 399) in diameter. Pharynx 203 ± 34 (167 - 243) long. Ovary 199 ± 42 (135 - 249) long. Two unequal testes, anterior 513 ± 42 (484.68 - 602.52) long; posterior, 622.88 ± 116.74 (455.21 - 789.28) long, contiguous and arranged in tandem. Eggs 65.41 ± 7.28 (56.30 - 73.57). See footnote in page 79.

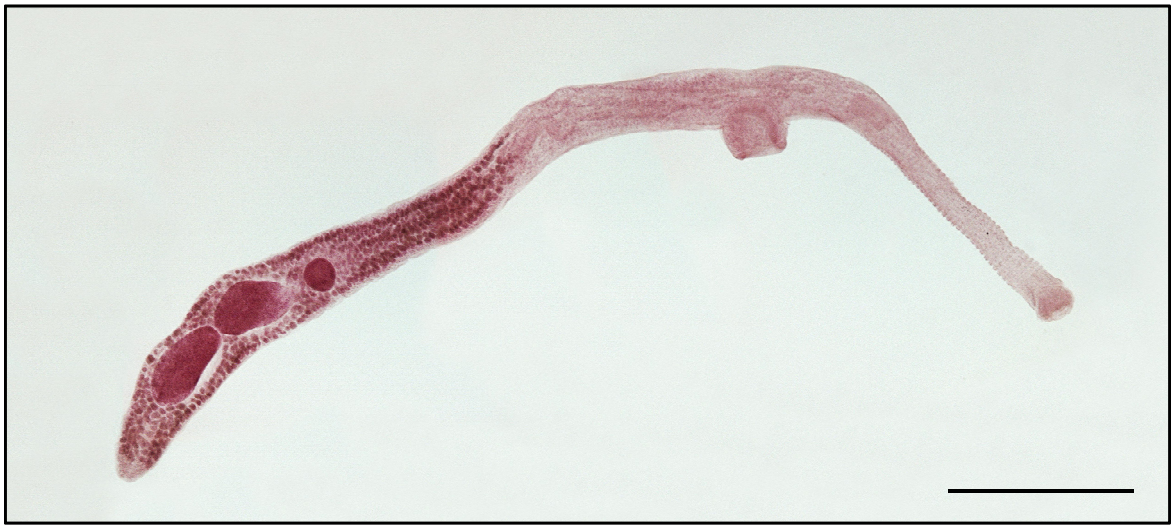


Figure 4.25. *Stephanostomun filiforme* from *S. dumerili* off the Mediterranean Sea. Whole specimen in ventral to lateral view. Scale bar represent 1000 µm.

***Remarks*:**

The morphology of the specimens found in the current study coincide with that reported by Bartoli & Bray (2004) for specimens from Corsica.

Seasonal occurrence:

Monthly occurrence of *S. filiforme* is indicated in table 4.2. This parasite species was found in all fish subsamples although its prevalence was similar and low in all of them (<26.7%). Mean abundances and intensities were also similar in all subsamples. Therefore, seasonal infection patterns were not observed.

Stephanostomum petimba Yamaguti, 1970 **cf** (immature)

***Localities of collection*:** Majorca

***Site*:** Specimens of *S. petimba* were found in the intestine.

***Description*:**

Immature specimens (N= 3) 2025 ± 63 (1950 - 2124) long and 252.26 ± 11.63 (233.78 - 265.50) wide at level of ventral sucker (Fig. 4.26). Oral sucker terminal, cup-like, widely-opened and large, 129 ± 7 (124 - 142) long and 298 ± 9 (287 - 312) wide. Number of spines not available. Ventral sucker rounded, 143.13 ± 19 (125 - 172) in diameter. Pharynx 112 ± 5 (108 - 117) long. Gonads undistinguishable. See footnote in page 79.

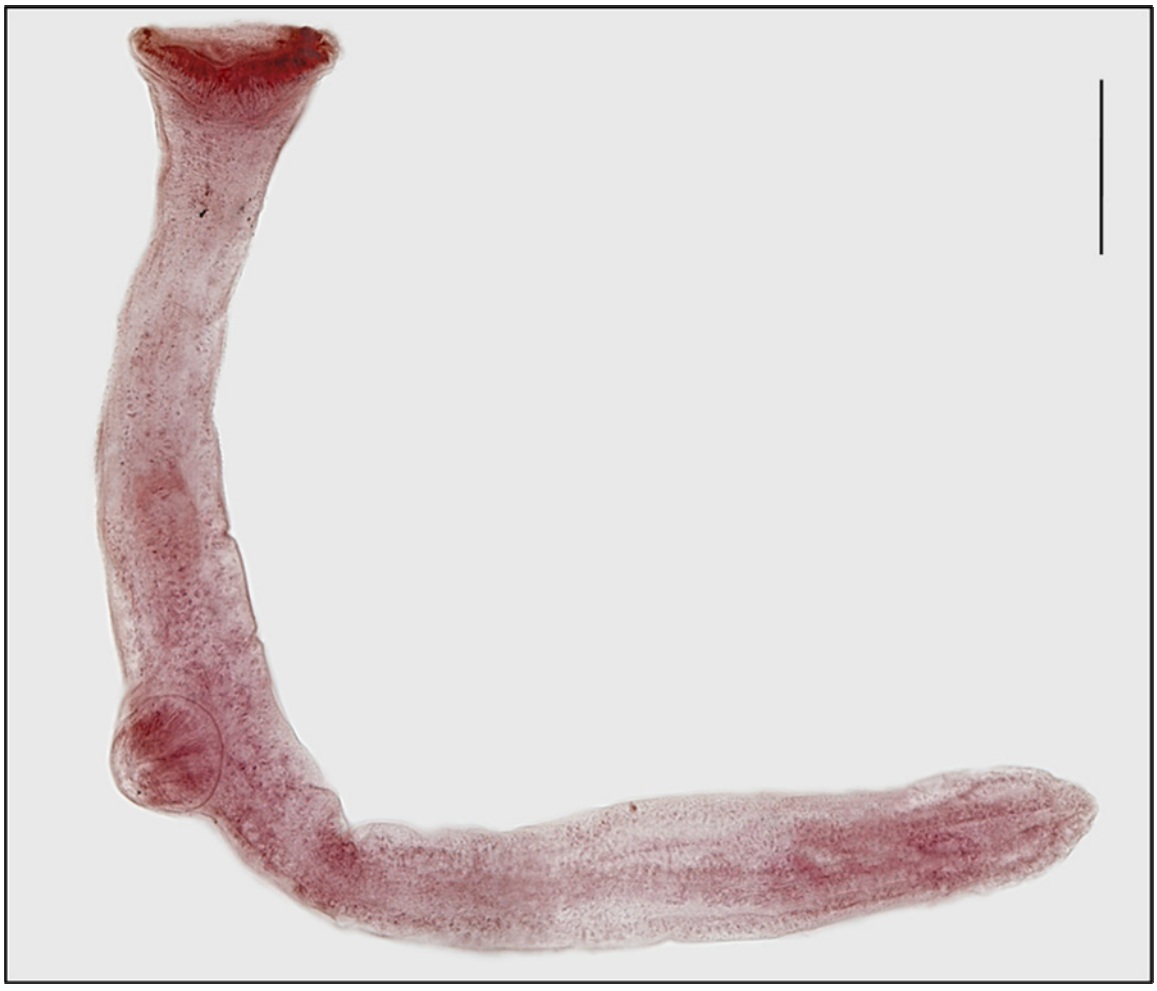


Figure 4.26. Immature *Stephanostomun petimba* cf from *S. dumerili* off Majorca. Whole specimen in lateral view. Scale bar represent 200 µm.

***Remarks*:**

Specimens herein described resembled *S. petimba* by its relatively large oral sucker. However, the low number of individuals and the absence of adults did not allow for a reliable identification. Bartoli & Bray (2004) found adult specimens of this species in fish from Corsica while only larvae have been recorded from Majorca. Adults live in the rectum of greater amberjacks (Bartoli & Bray, 2004) and this section quickly degrades when fish die. Therefore, it is possible that all the contents, including the specimens, also degrade and become undetectable in a short period of time.

***Seasonal occurrence*:**

S. petimba was only found in 3 of the 11 monthly subsamples from Majorca with very low prevalences (< 6.7 %) and intensities (1) (see table 4.2). Seasonal infection patterns were not observed.

Genus *Tormopsolus* Poche, 1926

Tormopsolus orientalis Yamaguti, 1934

***Localities of collection*:** Majorca, Alicante, Corsica and Sardinia.

***Site*:** Specimens of *T. orientalis* were found in the digestive tract, mostly in the intestine.

***Description*:**

Specimens (N= 10) 8910 ± 2093 (5766 - 11952) long and 354 ± 21 (335 - 380) wide at level of ventral sucker (Fig. 4.27). Tegument armed with spines dorsally larger. Oral sucker sub-terminal, 184 ± 28 (156 - 213) long, almost one half of ventral sucker. Ventral sucker rounded and large, 346 ± 63 (328 - 419) in diameter. Ovary 192 ± 66 (149 - 268) long. Two testes almost equal, anterior, 569 ± 100 (491 - 683) long; posterior 636 ± 87 (612 - 729) long; separated and arranged in tandem. Distance between testes 562 ± 75 (428 - 697). Vitellarium absent around testes. Eggs 76 ± 6 (70 - 87).

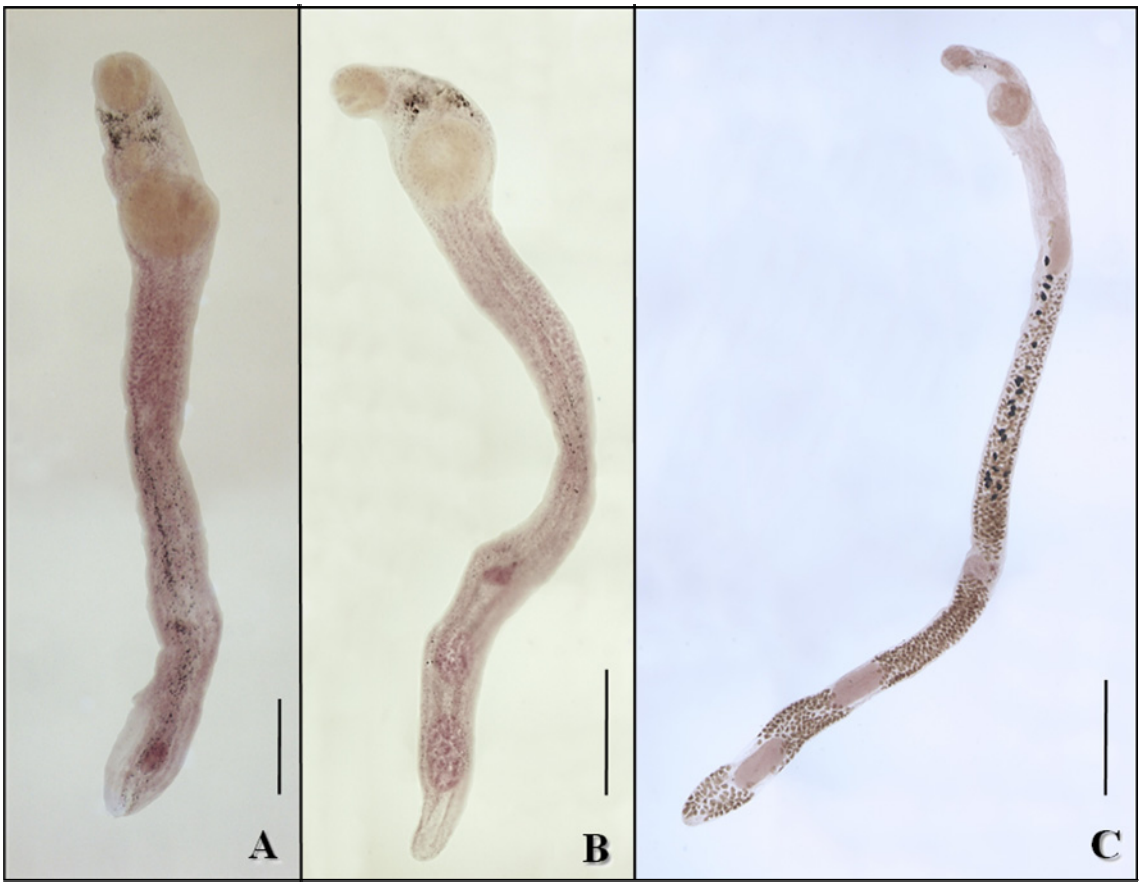


Figure 4.27. *Tormopsolus orientalis* from *Seriola dumerili* off the Mediterranean Sea. Ventral views. A) Inmature specimen; B) inmature specimen with genitalia primordium; C) mature adult. Scale bars represent 500 µm in A and 1000 in B & C.

***Remarks*:**

According to Bray & Cribb (2001) and Bartoli *et al.*, (2004) the main feature differentiating *T. orientalis* from the rest of *Tormopsolus* spp. is the vitellarium arrangement around testes. This species has recently been reported and redescribed in *S. dumerili* from Majorca and other western Mediterranean localities (Bartoli *et al.*, 2004). Features and dimensions of the specimens found in the current study coincided with those previously described.

***Seasonal occurrence*:**

Monthly occurrence of *T. orientalis* is indicated in table 4.2. Prevalences and abundances were high in all subsamples (> 46.7 %; > 1.2 parasites/fish). During the first year, prevalences were apparently higher in autumn and winter. However, this pattern was not observed during the second year.

Phylum Arthropoda Latreille, 1829

Class Malacostraca Latreille, 1802

Order Isopoda Latreille, 1817

Family Gnathiidae Harger, 1880

Genus Gnathia Leach, 1814

***Gnathia vorax* (Lucas, 1849)** (praniza and zuphea larvae)

***Localities of collection*:** Majorca, Alicante, Corsica and Sardinia.

***Site*:** Specimens of G. *vorax* were found attached to the gills and the buccal cavity.

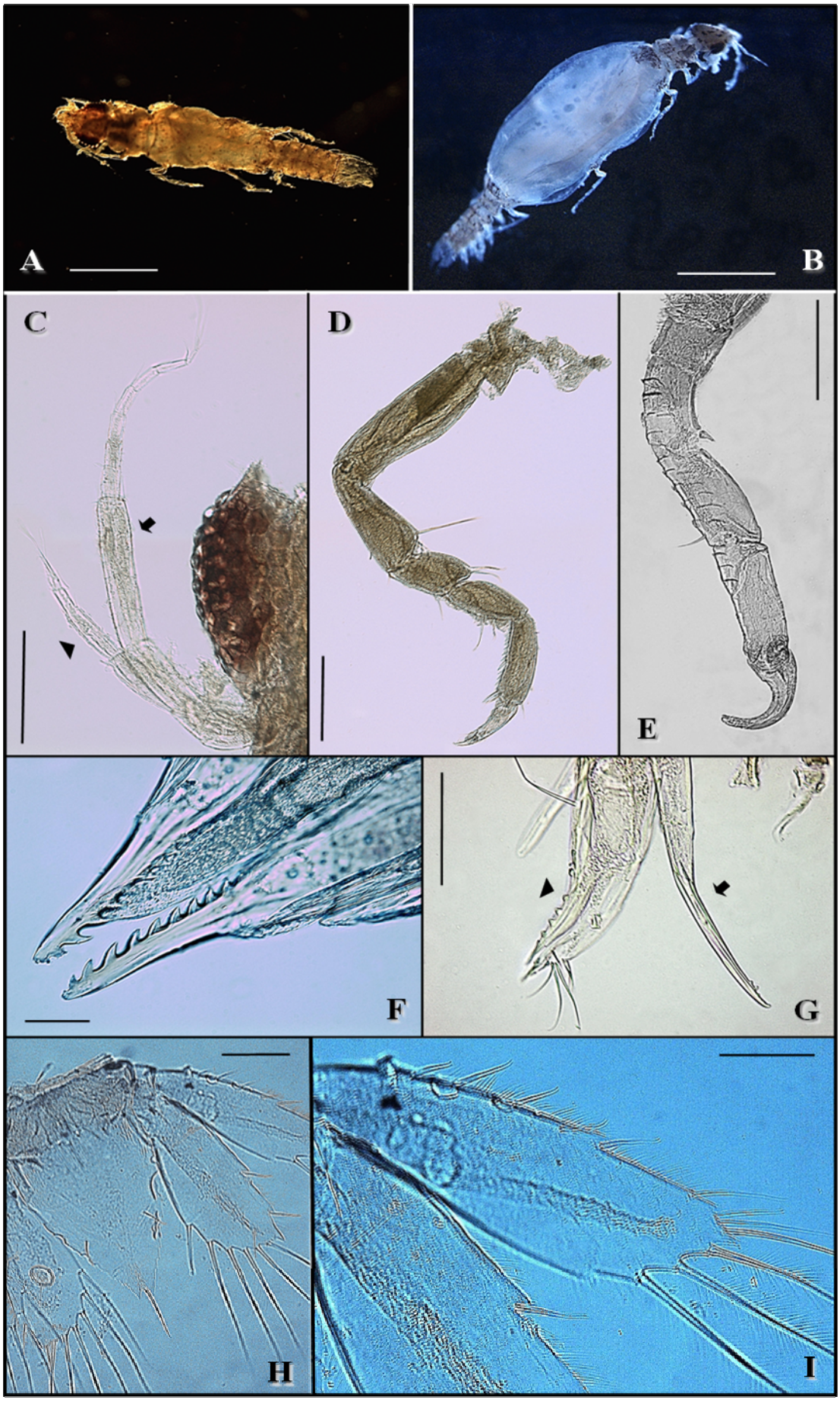
***Description*:**

Praniza larvae (N= 10) 4157 ± 645 (3400 - 5255) long and 1190 ± 204 (1012 - 1416) wide at level of pereion (Fig 4.28 B). Pereion longer than pleon and pleotelson. Pereion 2932 ± 432 (2321 - 3294) long, with an elastic membrane between pereonites 4 and 6. Pleon 1024 ± 106 (885 - 1114) long and 498 ± 55 (440 - 570) wide, with 5 pleonites and one simple setae laterally arranged in each pleonite. Telson triangular, slightly longer than wide, 445 ± 12 (439 - 450) long and 405 ± 17 (390 - 420) wide (Fig 4.28 H); with 2 dorsal setae and serrated margins, terminating in two simple setae distally located (Fig 4.28 I). Antenna 1 (antennula) half length of antenna 2 (antenna) (Fig 4.28 C). Antennula with 3-segmented peduncle, segments increasing distally in length, and flagellum 4-segmented with 3 apical setae. Antenna 2 (antenna) with 4-segmented peduncle, segment 4 being the largest, and flagellum with 7 segments and 4 distal apical simple setae. Sharp mandible with 12 teeth (Fig 4.28 F). Gnathopod, smaller than the pereiopods (Fig 4.28 D), 4-segmented, the 3 most proximal segments with scales (Fig 4.28 E). Maxiliped 4-segmented, ornamented with half-moon scales on the basipodite and at least 6 setae at distal segment (Fig 4.28 G). Maxillule long and slender with 7 teeth on the distal inner margin. Endopod of the uropods larger than exopod, extending beyond distal margin of pleotelson, with 6 feather-like setae in the inner margin, 2 simple setae in the external margin, and 1 simple seta distally located. Two more setae dorsally located. Exopod reaching apex of pleotelson, with 4 feather-like setae in the inner margin and 9 simple setae in the external margin, the 3 most distal arranged together.

Additional measures from zuphea larvae:

Specimens (N= 10) 3915 ± 481 (2985 - 4521) long and 813 ± 142 (670 - 971) wide (Fig 4.28 A). Elastic membrane between pereonites 4 and 6 absent.

Figure 4.28. *Gnathia vorax* from *Seriola dumerili* off the Mediterranean Sea. A, Larva zuphea in dorsal view. B-I, Larva praniza: B, whole specimen in dorsal view; C, antenna 1 (arrowhead) and antenna 2 (arrow); D, pereiopod; E, gnathopod with half-moon scales; F, tips of mandibles; G, maxiliped (arrowhead) and maxillule (arrow); H, telson; I, detail of setation in exopod of peotelson. Scale bar represent 1000 µm in A&B; 100 in C-E, G, H, 20 in F and 50 in I.


A
B
C
D
E
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G
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I

***Remarks*:**

Taxonomy of Gnathiidae spp. is mainly based on free-living adult specimens and gnathiid larvae are rarely identified to species-level (Tanaka, 2007). In fact, the only taxonomical key for larvae was reported by Monod in 1926 and merely included 13 species. Some of the diagnostic features reported in this key are apparently ambiguous, e.g.: length of the second antenna can be longer or shorter than head width but the key does not specify how much and many times it is slightly longer or shorter. Moreover, recent studies have shown that other traits not included in Monod (1926), as the shape of eyes and head or the shape and setation of uropods, could be diagnostically useful (Wilson *et al.* 2011).

First adult specimens of *G. vorax* reported in the Mediterranean were found in Formentera, one of the Balearic Islands (see Monod, 1926; Rodríguez-Sánchez *et al.*, 2000). However, larvae of this species have been reported in several fish species and localities of this region (Monod, 1926; Grau *et al.*, 1999; González-González, 2005). Based on the key for praniza larvae of Monod (1926) larvae could be identified as either *G. vorax* or *G. vetusta* which were undistinguishable by the author. Morphological diagnostic features showed by the specimens found in Majorca were: flagellum of the antenna seven-segmented, long and narrow first antenna (longer or equally long than head width), telson longer than wide but not very sharp ("*postice plus minusve acutus*" in Monod (1926)), endopod with six feather-like setae in the inner margin, and exopod with 4 feather-like setae in the inner margin. The only difference with Monod's (1926) description was found in the number of simple setae reported in the external margin of the exopod, which were three (one of them distally located) in current study and one in the key. Specimens were finally identified as *G. vorax* as this is the only species previously reported in Mediterranean greater amberjacks (Grau *et al.*, 1999).

Recent researches are focused on finding molecular links between adult males, females and larvae of gnathiids, in order to create an ITS2 sequence database for this taxonomically controversial family (Grutter *et al.*, 2000). Nowadays, molecular data of *Gnathia* spp. reported in genbank only refer to the genus and molecular analyses of larvae are not very helpful for taxonomic identification.

***Seasonal occurrence*:**

Monthly occurrence of *G. vorax* is indicated in table 4.2. Prevalences recorded were similar in most of subsamples while abundances oscillate and high values were recorded both, in summer and in winter months. Thus, periodical occurrence patterns were not observed.

4.3.6. Scavenger species:

Phylum Arthropoda Latreille, 1829

Class Ostracoda Latreille, 1802

Order Myodocopida Sars, 1866

Family Cypridinidae Baird, 1850

Genus *Skogsbergia* Kornicker, 1974

Skogsbergia costai Kornicker, 1974

***Localities of collection*:** Majorca and Alicante.

***Site*:** Specimens of *S. costai* were found between gill filaments and within pericardial and abdominal cavities.

***Description*:**

Specimens (N= 10) 3511.76 ± 228.04 long and 2210.83 ± 262.63 height (Fig. 4.29 A-D). Oval caparace with deep curved incisur. Surface smooth, without ornamentation. First antenna with 7 segments. Second antenna with short endopodite 2-segmented. Exopodite with 9 segments, bristle of second segment with 25 ventral spines, bristles of segments 3-9 with natatory elongated and soft setae hairs; distal segment (9th) with 3 long and 1 short bristle. Mandible basal, with 6 bristles in ventral margin (3 proximal, 2 medial and the longest and feather like posterior). Dorsal margin with three bristles, similar in size, 1 medial and 2 subterminal. Seventh limb with approximately 27 bristles (from 25 to 28). Eyes lateral, with 33-35 omatidia. Furca with 10 claws spined in their inner margin. First claw with medial spines distally arranged. Claw-size decreasing from the distal to the proximal position except for claw 4 which is shorter than claw 5.

***Remarks*:**

Morphological features of the specimens agree with those of *Skogsbergia costai* based on Kornicker, (1974) (as *Cypridina mediterranea* in Müller 1894, 1912; see Kornicker, 1974). This species differs from the other congeneric species by the two segments of the endopodite of the second antenna, the number of bristles in the seventh limb (25 - 28), the number of ommatidia in the eyes and the number of furca claws (10).

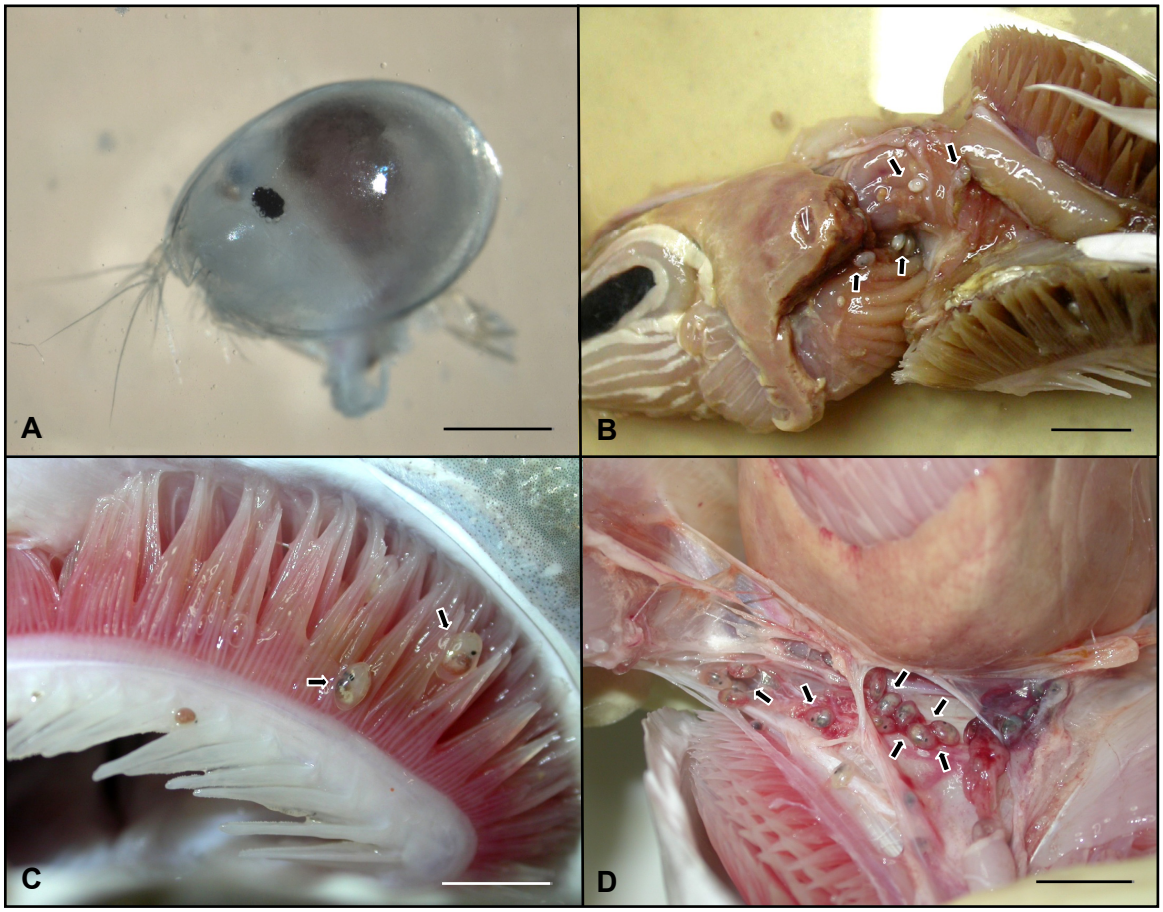


Figure 4.29. *Skogsbergia costai* from *Seriola dumerili* off the Mediterranean Sea. A) *S. costai* specimen full of ingested tissue from the fish; in lateral view. B-D) Specimens (arrows) located in different fish sites: B, specimens on gills and viscera, note that the heart and a part of the liver have been ingested; C specimens on gills; specimens within the pericardial cavity, note that the heart had been devoured and the ostracods were coloured by the ingested tissue. Scale bars represent 1000 µm in A and 10000 in B-D

S. costai was first described in the Gulf of Naples (Italy) and has been frequently reported in the Mediterranean Sea (Kornicker, 1974). Müller (1894) collected the specimens from the sea bottom but this author also found them in the stomach of the shark *Mustelus mustelus* (Linnaeus, 1758) (see Kornicker, 1974). In the current study, *S. costai* was considered a scavenger species which feeds on decayed fish, including *S. dumerili*.

***Seasonal occurrence*:**

This low prevalent species was only found in one subsample from Majorca (December 2006) and one subsample from Alicante (February 2006) (see table 4.1). Therefore, seasonal patterns of occurrence were not observed.

4.4. Discussion

4.4.1. Parasite fauna

A total of 45 parasite species have been reported from Mediterranean greater amberjacks to date (see check-list in the appendix). In the current study, 17 species were added to the set of previously cited species in this area, increasing the richness in a 24% (Grau *et al.*, 1999; Montero *et al.*, 2001b; Bartoli *et al.*, 2004; Bartoli & Bray, 2004; Bartoli *et al.*, 2005). Among these species, 2 are new for science, 1 is a new host and locality record, 8 are new host records of Mediterranean species, 6 are new records in the Mediterranean, and 9 species had already been reported.

Parasite richness of wild *S. dumerili* from western Mediterranean was higher than those previously reported in wild and cultured fish from the same region (Grau *et al.*, 1999; Montero *et al.*, 2001b; respectively). This could be explained by four reasons: (*i*) the erection of new species after 2001 (in the current and in previous studies, e.g. Bartoli *et al.*, 2004); (*ii*) the larger sample size in the current study; (*iii*) the analysis of organs that were not usually examined (e.g. the brain); or/and (*iv*) the reassignment of species previously classified with other names. Moreover parasite richness reported by Montero *et al.* (2001b) should be specially considered as fish in this study were reared in tanks and culture conditions could modify parasite loads (e.g. trophically transmitted parasites would decrease after a period without fresh food supply).

Majorca was the locality with the highest parasite species richness, followed by Alicante, Corsica and Sardinia. This result would be mostly explained by the different number of fish examined in each locality. Trematodes were predominant in the parasite community from western Mediterranean Sea and the most represented family was Acanthocolpidae. All the common parasite species recovered from Majorca, were also found in the rest of localities except for *Paradeontacylix balearicus* which is locally specific (see chapter 5). In contrast, accidental species, which represent the 50% of the total species richness, were only recovered from Majorca. Therefore, most of the differences of parasite diversity among localities were related to particular accidental species. Concerning parasite abundances, common species were also the most abundant and among them, numbers of specimens of *Zeuxapta seriolae* and *B. gorgon* were distinctly high. Both species represent the 84% of the total number of specimens collected.

One of the most remarkable differences among the parasitological studies on *S. dumerili* from the western Mediterranean Sea reported to date was the relatively high nematode richness (four species) recorded here contrasting with the scarce nematode presence (one species) of previous parasite compilations (Grau *et al.*, 1999; Montero *et al.*, 2001a). Moreover, nematode diversity also differed among studies: while previous publications reported *Philometra* sp. or *Philometra globiceps*

(Rudolphi, 1819), species of this genus were not found in the current study (Grau *et al.*, 1999; Montero *et al.* 2001b). Other difference was the absence of two trematode species: *Wedlia bipartita* (Wedl, 1855), previously found in Majorca (Grau *et al.*, 1999) and *Tergestia laticollis* Rudolphi, 1819, previously found in Murcia (Montero *et al.*, 2001b). Most of these species were accidental and this could explain their absence in the following studies. However, more consistent differences as the presence of *Hysterothylacium seriolae*, which is frequently found in the current study or the higher prevalence of some of the trematodes (e.g. *Prosorhynchus* sp.), could also be related to environmental conditions, changes of diet due to temporal variation in prey availability, local intermediate host, etc. Finally, *Gnathia vorax* and *Hemiurus communis*, both common species in all the localities analysed and also found in previous studies in Majorca (Grau *et al.*, 1999), were not found in previous studies from Murcia (Montero *et al.*, 2001b). These absences could be explained by culture conditions.

Most of the species parasitising *S. dumerili* were heteroxenous (N= 21; nematodes, trematodes and cestodes), while only five species were monoxenous (monogeneans, copepods and isopods). Greater amberjack is the definitive host of almost all its heteroxenous parasites, including all nematodes, as larvae four and adults were predominant. This is indeed a species at a high trophic level, at least in the western Mediterranean region. Moreover, greater amberjack is the intermediate host of the two trematode species found as metacercariae, *Cardiocephaloides* sp. and *Galactosomum* sp. (both accidental), and the two larval cestodes, *Floriceps saccatus* and the tetraphyllidean larvae (also accidental). The definitive hosts of these metacercariae are birds, mainly gulls belonging to the genus *Larus* (Pearson & Prévot, 1971), although some species of *Galactosomum* have also been reported from dolphins (Buriola & Ceroni, 1995; Gibson *et al.*, 2005). Definitive hosts of cestodes are elasmobranch fishes and in the case of *F. saccatus* they are known to be sharks, mainly *Carcharhinus* spp., although this species has also been reported in *Notorynchus cepedianus* (Péron, 1807), *Prionace glauca* (Linnaeus, 1758) and *Negaprion brevirostris* (Poey, 1868) (Bates, 1990). The tetraphyllidean larvae belong to a complex of species (Chervy, 2002) and, therefore, their definitive hosts could not be determined.

Many of the parasite species herein described are strict host specialists (eight out of the seventeen non-accidental taxa identified to species-level; see table 4.1). Therefore, phylogenetic relatedness seems to be quite important for parasite-community composition in this region. Among these species, the specificity of *Paradeontacylix* spp. is especially remarkable, as almost all the species of this genus (N= 7) are specialist of different *Seriola* spp. within and outside the Mediterranean Sea. Thus, a long coevolutionary history seems plausible between these blood flukes and its hosts (see discussion in chapter 5). The specificity of those species identified to genus-level could not be

assessed although trematode metacercariae and cestodes reported here are known to be generalist in the intermediate host. In the case of nematodes, *Hysterothylacium seriolae* was the only species identified to species-level. This species was classified as generalist as it has been found in a Beryciform fish (Oliveira-Rodrigues *et al.*, 1975). However, the rest of reports were from *Seriola* spp. (*Seriola lalandi* and *S. quinqueradiata*) which could be alternative definitive hosts (Yamaguti, 1941; Hewitt & Hine, 1982; Williams & Bunkley-Williams, 1996).

4.4.2. Seasonal occurrence

In general, seasonal parasite population dynamics have been associated to environmental variability but could also be related to other density-independent or density-dependent factors as: changes in the host diet or eating rate, competence, parasite-host coordination of transmission, host immune response against the parasite, host or parasite life-span, parasite accumulation in host, etc. (see references in Bush *et al.*, 2001). Moreover, in the case of heteroxenous parasites, adult seasonal occurrence could also be related to dynamics of all previous parasite stages and hosts.

Usually, taxonomical studies on fish parasites disregard their infection dynamics as long-term and periodical studies are required in order to record consistent data on parasite seasonal occurrence. Therefore, seasonal patterns are not usually described and only punctual parasite incidences are provided. In the case of parasites from *S. dumerili* off the Mediterranean, periodical studies have only been carried out for the heteraxinid monogeneans *Allencotyla mchinoshi* and *Zeuxapta seriolae* (Montero *et al.*, 2001b), occurring in mixed infections, and the trematode *Paradeontacylix ibericus* (Montero *et al.*, 2009). Data reported in the current study aim at providing a preliminary description of seasonal dynamics of parasites from *S. dumerili* off Majorca. All patterns described here should be statistically tested, and non environmental factors influencing parasite loads, such as host-size, should be further investigated (see chapter 6 for the case of *Z. seriolae*).

Considering the results reported in the current study, seasonal occurrence patterns could potentially exist for five out of the nine common parasite species recorded from Majorca: *B. gorgon*, *H. communis*, *P. balearicus*, *S. ditrematis* and *Z. seriolae*. Seasonal dynamics of trematodes, copepods and isopods have been discussed separately below. A full section of this discussion has been dedicated to blood flukes in accordance with their importance for aquaculture. Moreover seasonal dynamics of the monogenean *Z. seriolae* has been analysed in chapter 6.

Blood flukes

Ogawa *et al* (1993) suggested that infection with *Paradeontacylix* spp. (*P. grandispinus* and *P. kampachi* mixed) in *S. dumerili* from Japan followed a one-year cycle, based on the disappearance of

eggs from summer to autumn (see fig. 4.30). Thereafter, according to similar evidences, Montero *et al.* (2009) also suggested a one-year cycle for *P. ibericus* from Murcia. In the current study, eggs of *P. balearicus* were found in all monthly subsamples (see fig. 4.30). However, considering that eggs from September to November were old remains from previous generations, the present study observations would mostly agree with those previously recorded in fish from Majorca (Grau, 1992), Murcia (Montero *et al.*, 2009; in 1996-1997 survey) and Japan (Ogawa *et al.*,1993).

In view of the infection dynamics reported here for *P. balearicus* in Majorca, a likely life-cycle in the definitive host has been hypothesised. According to previous studies on aporocotylids, cercaria penetrates into fish and encapsulates (Køie, 1982; Montero *et al.*, 2009). Montero *et al.* (2009) found the schistosomulum of *P. ibericus* in fish from Murcia and stated that this stage mostly developed into adult when water temperatures began to increase. Since then, specimens would mature and release their eggs into the bloodstream. Unfortunately schistosomula of *P. balearicus* were not found in greater amberjacks from Majorca, probably because summer samplings were interrupted by the closed-fishing period. Therefore, a complete series could not be provided from our data. However, at the end of summer (September) most of the eggs were immature and since

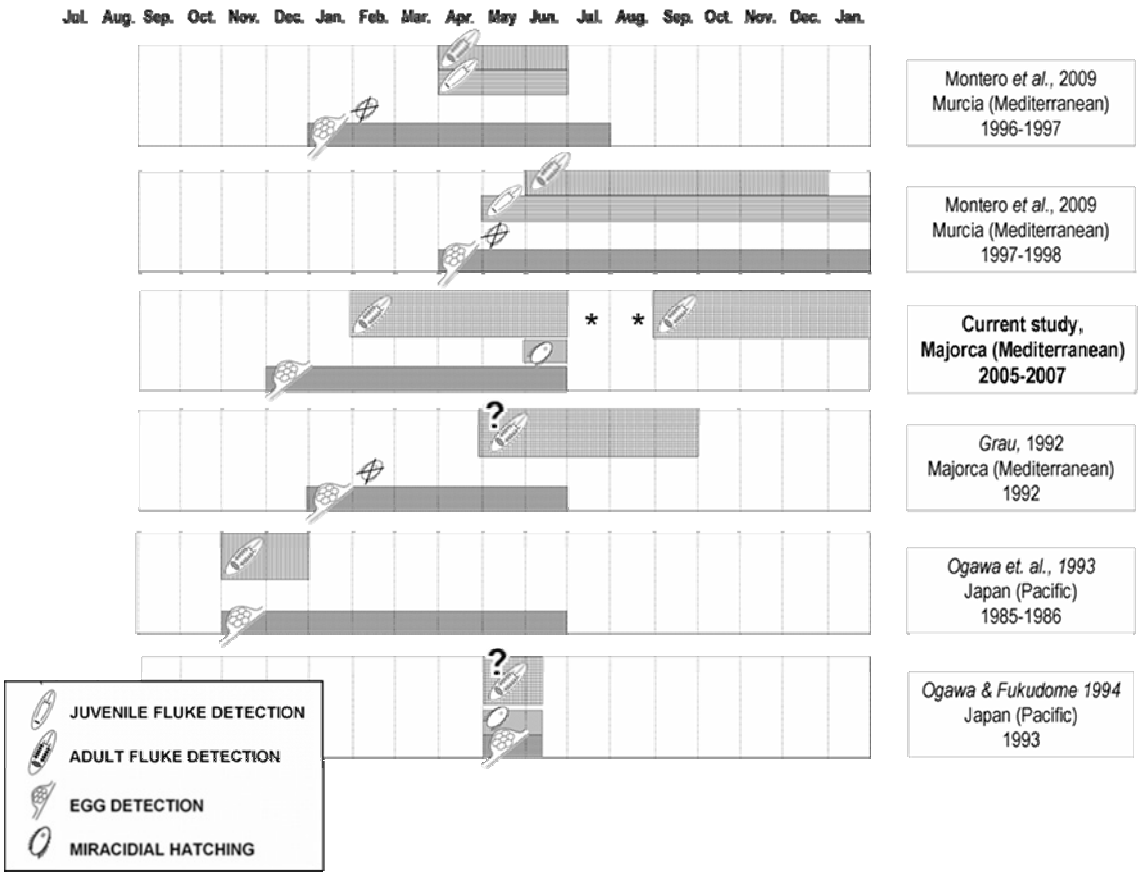


Figure 4.30. Schematic diagram of the annual development of *Paradeontacylix* spp. in different localities and years extracted from different studies. Question marks indicate no data available on the stage of maturity of the parasites. Modified from Montero *et al.* (2009). *Data from July and August not available in current study.

then, their abundance increased gradually until June-July. According to the histological study reported by Grau (1992), most of the *P. balearicus* eggs in fish from Majorca hatch in June. This evidence coincided with the finding of mature eggs during June in the current study. The detection of old degraded eggs during the next months would indicate that not all them hatch in June but some remain and are encapsulated by the host. Additionally, some of the eggs do not arrive to the gills as they get trapped in the capillaries of different organs.

Other trematode species from *S. dumerili* off Balearic Islands

Seasonal dynamics have only been previously reported for one of the trematode species described here (out of blood flukes), the hemiurid *H. communis* (Gibson & Bray, 1986, and references therein). According to Meskal (1967; in Gibson & Bray, 1986) the prevalence of this parasite in *Gadus morhua* Linnaeus, 1758 increased in autumn and spring. A similar pattern was detected by Gibson (1971) in *Platichthys flesus* (Linnaeus, 1758), although this author only observed the peak in autumn (see Gibson & Bray, 1986). In the same way, current study showed that *H. communis* prevalences and abundances peak during winter and then gradually decrease until the next spring. Finally, between spring and summer parasite abundance decreases abruptly. Meskal (1967) suggested that recruitment of young specimens of *H. communis* was continuous from early fall (September) to spring, in accordance with the peaks detected. Thus, in view of the data obtained in the present study, the recruitment of *H. communis* in Majorca would follow a similar pattern although it would begin later in the year, at the end of autumn.

Population dynamics of bucephalids are poorly known and most of the available refer to species parasitising intermediate hosts in freshwater systems (Chubb, 1979; Taskinen *et al.*, 1991; Wang *et al.*, 2001). In greater amberjacks from Majorca, prevalence of adult specimens of *B. gorgon* increased from early autumn to winter when maximums were reached. Previous studies on bucephalid species (i.e. *Dollfustrema vaneyi* Tseng, 1930) associate the seasonal increase in adult prevalence with the increase of metacercariae in intermediate host few months before (Wang *et al.*, 2001). Moreover, experimental studies demonstrate that metacercariae of two Bucephalidae spp. (*Riphidocotyle* spp.) develop into adults one month after being ingested (Taskinen & Valtonen, 1995). Thus, it is likely that the increase of prevalence of *B. gorgon* between autumn and winter is due to an increase of metacercarial recruitment few months before, during summer. As previously stated, variations in this recruitment could be related to seasonal changes in diet or feeding rate as well as to seasonal cercarial emergence and infection of intermediate hosts.

As in the case of bucephalids, studies on seasonal dynamics of adult acanthocolpids are scarce. In the current study, only *Stephanostomum ditrematis* showed an apparent seasonal infection

pattern which might be positively associated with water temperature. Most of the previous studies on annual dynamics of acanthocolpid species (i.e. *S. bicoronatum* (Stossich, 1883) and *S. baccatum* (Nicoll, 1907)) reported the absence of seasonal variations in infection levels (Wolfgang, 1955; Holmes & Bartoli, 1993). In contrast, concerning metacercariae, seasonal fluctuations in the prevalence of *S. lophii* Quinteiro, Tojo, Nunez, Santamarina et Sanmartin, 1993 were reported in many second intermediate fish species from Galicia (Quinteiro *et al.* 1993). More information on the intermediate stages of the acanthocolpid species reported would be required in order to understand the occurrence pattern observed.

Concerning didymozoids, their population dynamics have not been explored to date and they constitute one of the less studied families, comparing with the rest of trematodes. In the current study, *N. periorbitalis* specimens were observed throughout all year although none seasonal pattern was detected. Life-cycles of didymozoids and their intermediary hosts are mostly unknown (Pozdnyakov & Gibson, 2008) and more information would be required to understand adult occurrence in definitive hosts.

Copepods

Infection dynamics of caligid species (e.g. *Caligus curtus* OF Müller, 1785, *C. elongatus* von Nordmann, 1832 *Lepeophtheirus salmonis* (Krøyer, 1837) and *L. pectoralis* (OF Müller, 1776)) have been described in several studies (see Boxshall, 1974; Hogans & Trudeau, 1989; Zagmutt-Vergara, 2005). Among the caligids parasitising *Seriola* spp., seasonal patterns of occurrence have only been reported for *C. spinosus* from *Seriola quinqueradiata* (Cruz-Lacierda *et al.* 2011). In all these studies, a high and positive correlation between parasite prevalence and abundance with temperature is usually recorded (Boxshall, 1974; Hogans & Trudeau, 1989). The authors explain this correlation by the faster completion of parasite reproductive cycle at higher temperatures (Boxshall, 1974; Hogans & Trudeau, 1989). Infection levels of *C. aesopus* did not vary much during the year and no apparent seasonality was recorded. In contrast, a seasonal pattern was observed for reproduction as gravid females mainly occurred from February to April. Reproduction of *C. curtus*, *C. elongatus* and *L. salmonis* off Northwest Atlantic Ocean takes place from late-spring to summer (Hogans & Trudeau, 1989). Thus, the reproduction period of *C. aesopus* would occur earlier than in other species of the family. Such difference could be due to the higher or the earlier spring raise of temperatures in the Mediterranean Sea comparing to the North-Atlantic region.

Sex-ratio also followed a seasonal pattern although females outnumbered males in all subsamples. The proportionally higher female abundance during some periods of the year has already been reported from other caligid species, usually after the reproduction period in summer

(Boxshall, 1974; Hogans & Trudeau, 1989). According to some authors, this seasonal bias could be explained by: (*i*) higher generation of females; (*ii*) higher male susceptibility to winter conditions; or (*iii*) male death after copulation exhaustion (Boxshall, 1974; Hogans & Trudeau, 1989). In this study, sex-ratio apparently increased before summer and decreased during late fall. Unfortunately, samplings from summer, the most likely period after reproduction, could not be analysed due to closed-fishing period. Therefore, none of the previous hypotheses could be rejected and they could also be combined.

Isopods

Life-cycles and population dynamics of gnathiids in the wild have been poorly studied (Naylor, 1972; Fernández *et al.*, 1989; Smith *et al.*, 2003; Tanaka, 2007). According to Smit *et al.* (2003), three different life-cycles have been described from the gnathiids studied to date: *i)* a yearly life-cycle, as that of *Paragnathia formica* (Hesse, 1864), *ii)* a two-years life-cycle, as that of *Caecognathia calva* (Vanhoeffen, 1914), and *iii)* a life-cycle with three or more generations per year as that of *Elaphognathia cornigera* (Nunomura, 1992) (Upton, 1987; Tanaka & Aoki, 2000; Tanaka, 2003; Tanaka, 2007).

The only *Gnathia* sp. examined in Smit *et al.* (2003), *G. africana* (Barnard, 1914), showed several yearly peaks of prevalence and abundance and thus, did not display a clear seasonal pattern. The authors stated that its life-cycle would probably be similar to that of *E. cornigera* which takes place continuously throughout the year (Smit *et al.*, 2003). Thereafter, the same pattern was reported for *G. pilosus* Hadfield, Smit et Avenant-Oldewage, 2008 (Hadfield *et al.*, 2009). As stated in results, peaks of *G. vorax* do not seem to be related to any particular season and this might indicate that its life-cycle is also similar to that of *E. cornigera* but alternative explanations are also plausible. Little is known about the reproductive patterns of gnathiids and their six larval stages (praniza and zuphea 1, 2 and 3) are usually undifferentiated in most publications (Smit & Davies, 2004). In order to better understand the life-cycle and seasonal dynamics of this and other gnathiids, the different developmental stages should be separately considered in future studies (Smit & Davies, 2004).

4.4.3. Potential parasitological risks

The parasite faunas of cultured *Seriola* spp. have been deeply studied. However, parasite risk-assessment has only been performed for species parasitising *S. lalandi* in South Australia (Hutson *et al.* 2007b). Thus, this study aims at providing new data about parasitic infections of concern for greater amberjack aquaculture in the Mediterranean Sea.

According to the parasite pathologies described in previous studies, species found in greater amberjacks from western Mediterranean were classified into three categories. (*I*) Harmful species for greater amberjack in the Mediterranean: monogeneans and aporocotylids that are known to cause severe epizootics, mostly in cultures. (*II*) Potentially dangerous species: metacercariae of heterophyids and strigeids, didymozoids, acanthocolpids, caligids and gnathiids that belong to taxa including harmful species, although no outbreaks of *Seriola dumerili* have been directly related with them. (*III*) Harmless or less hazardous species: other trematodes, cestodes, nematodes and some of the copepods. These species represent a minimal risk for Mediterranean greater amberjacks because of their mild effects or their low prevalence and abundance in this region. The pathogenic potential of the species is discussed in the following sections.

Monogeneans

The most severe outbreaks caused by helminths in cultures of *Seriola* spp. have been associated with monogeneans (Deveney *et al.*, 2001; Grau *et al.*, 2003; Whittington & Chisholm, 2008). In the present study, the only monogenean found in greater amberjack from western Mediterranean was *Z. seriolae* which has been related to epizootic episodes in culture and in the wild (see chapter 6 for review). Pathological effects of this species have been described in detail by Montero *et al.* (2004) and Mansell *et al.* (2005) and resumed in the general introduction of the present study and in chapter 6.

Blood flukes

Aporocotylids of the genus *Paradeontacylix* are the only trematode species that have been associated with outbreaks of greater amberjack in culture conditions (Ogawa & Fukudome, 1994; Crespo *et al.* 1992; Crespo *et al.* 1994). Pathological effects of these parasites have been mainly related to the accumulation of eggs in the circulatory system of fish, especially in the gill vessels (see Crespo *et al.* 1992, chapter 5 and general introduction of this study). Despite that eggs of *Paradeontacylix* spp. from the Mediterranean were similar in size, those of *P. balearicus* seemed to go through narrower gill vessels (see site of infection for these species in results). *P. balearicus* flukes are smaller than *P. ibericus* ones and this would allow them to get into narrower gill vessels and to spread widely through the bloodstream. Therefore, *P. balearicus* specimens would be closer to the interllamellar vessels when they release their eggs, which are irregular and soft-shelled in the uterus. These eggs would reach and enter into the small vessels before the shell is completely developed and become spherical. In contrast, *P. ibericus* eggs would be released from more distant vessels and would get trapped before into the afferent filament arteries where they would become spherical and consistent. Thus, it was suggested that the wider distribution of adults and eggs inside the host

could contribute to the higher pathogenicity of *P. balearicus* observed in culture (see chapter 5 for further explanation).

Other trematodes

Effects of acanthocolpids have been poorly studied as they are considered harmless to fish (Hutson *et al.*, 2007b). However, Grau (1992) reported the destruction of epithelial cells and haemorrhages in the pyloric caeca and anterior portion of the intestine of greater amberjacks associated with the attachment of these parasites (described as Echinostomidae gen. sp.). This statement and the characteristic morphological features of acanthocolpids, spined body or circum-oral spines, suggest that these species could be potentially pathogenic. Moreover, as they are highly abundant in Majorcan greater amberjacks (see references in this and in previous studies; Grau *et al.*, 1999; Grau, 1992), their presence should be considered. However, to our knowledge, acanthocolpids have never been related to outbreaks of any *Seriola* spp. Thus, they do not seem to represent an important threat for greater amberjack in the western Mediterranean Sea. Moreover these parasites could be easily removed with oral anthelminthics in culture conditions and, as they are trophically transmitted, feeding with dry food would avoid new infections.

Regarding didymozoids, their effects depend on the species but also on their location inside the host. Specimens of *N. periorbitalis* live within the connective tissues occupying different fish sites although they are especially found in gills. Perera (1992) reported that effects of a similar Nematobothriinae species (unidentified), which arranged in the space between the basal lamina of primary epithelium and the efferent gill artery of primary lamellae of *Scomber australasicus* Cuvier, 1832, were low, i.e. the stretching of the lateral epithelium and the formation of a layer of columnar epithelial cells. This statement and the absence of important damages associated with this parasite in the current study suggest that *N. periorbitalis* could be considered harmless for greater amberjack. However, this species has also been reported in the flesh or below the eyes of *S. dumerili* (Yamaguti, 1970; Montero, 2001) where its effects could be more deleterious.

Finally, concerning metacercariae, *Galactosomum* sp. and *Cardiocephaloides* sp. are usually located in the brain of fish and their presence should be considered as they could alter host behaviour (Osset *et al.*, 2005). However, in the current study their incidence was low and their effects would be negligible. In addition, despite these species are widespread in the Mediterranean Sea, no pathological effects related with them have been reported on *Seriola* spp. to date. Therefore, these parasites do not seem to represent an important risk for greater amberjacks in the western Mediterranean Sea.

Copepods

Caligid copepods usually cause severe effects in cultured and wild fish, especially in salmonids, which are mostly infected with *Lepeophtheirus salmonis* (Pike & Wadsworth, 2000). Regarding caligids parasitising *Seriola* spp., *Caligus spinosus*, morphologically similar to *C. aesopus*, has been previously reported to cause anaemia in cultured *S. quinqueradiata* from Japan (Egusa, 1983). In contrast, the potential effects of this species and other caligids infecting *S. lalandi* in sea-cages from South Australia, i.e. *C. epidemicus* Hewitt, 1971 and *C. lalandei* Barnard, 1948, were considered low (Hutson *et al.*, 2007b). Such difference could be explained by intensity of infection, which was higher in Japan than in Australia or in the Mediterranean localities sampled in the current study (Egusa, 1983; Hutson *et al.*, 2007b). *C. aesopus* does not seem to represent an important risk for wild greater amberjacks from the western Mediterranean Sea. However, this species should be monitored in cultures as captivity and overcrowding favours transmission of monoxenous parasites (Nowak, 2007).

Isopods

Zuphea and praniza larvae are the only parasitic stages of gnathiids although praniza larvae are the most studied as life-time of zuphea larvae is very short (Monod, 1926; Smit *et al.*, 2003). *G. vorax* is a host-generalist species and its larvae attach to the mouth and gill epithelia of different fish provoking wounds and haemorrhages (Marino *et al.*, 2004). Each praniza larva is able to suck up to 3.07 mg of blood and, specimens severe infections may cause fish anaemia (Marino *et al.*, 2004). *G. vorax* has not been associated with any pathological effect on *Seriola* spp. to date. However, due to the high parasite loads recorded from wild fish in the current study (around 100 specimens per fish), prophylactic treatments against this species should be applied in cultures in order to avoid sedentary populations of free living adults close to the facilities.

Scavenger species, ostracods

In general, ostracods can be herbivorous, filter-feeders, detritus-feeders or scavengers (Vannier *et al.*, 1998) although some species, as *Vargula* spp., are known to be micropredators which can attack living fish (Stepien & Brusca, 1985). Some parasitic ostracods have also been described, as *Sheina orri* Harding, 1966 from *Hemiscyllium ocellatum* (Bonnaterre, 1788) or *Photeros parasitica* (Wilson, 1913) from the gills of *Sphyrna zygaena* (Linnaeus, 1758), *Epinephelus adscensionis* (Osbeck, 1765) and *Caranx crysos* (Mitchill, 1815) (Wilson, 1913; Bennett *et al.*, 1997). However, later studies have demonstrated that the latter species was a carrion-feeder (Cohen & Morin, 2010).

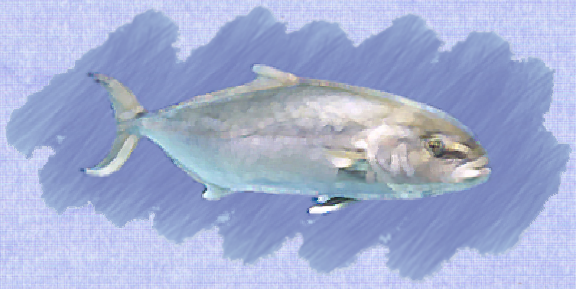
Specimens of *Skogsbergia costai* were found in greater amberjacks with healthy external appearance. In fact, when fish were examined, some of the trematodes and monogeneans were still alive. Thus, these ostracods did not seem to feed on long-term decayed animals and it is possible that fish invasion occur after fish capture but before fish death. The apparently aggregated distribution of *S. costai* also seems to be in accordance with a fast and massive invasion, similar to that occurred in other cypridinid ostracods (see Vannier *et al.*, 1998).

Stepien & Brusca (1985) reported extensive damages associated with other crustacean zooplankton species found in fish caught in gill nets. Invasion of all these ostracods seems to follow the same pattern. (*I*) First, specimens would invade the fish through the mouth and/or the operculum, where wounds were found. Gills did not seem very affected as no wounds were observed on gill filaments or gill rakers. Other studies report a double invasion of decayed fish, through the anus and the operculum (Stepien & Brusca, 1985). (*II*) Subsequently, ostracods would invade the pericardial chamber digging small orifices in the pericardium tissue. In this chamber they would feed on heart, bulbus arteriosus and sinus venosus. (*III*) Thereafter, they would enter into the visceral cavity and devour the liver and other soft tissues. The last affected organs seemed to be intestine and stomach which have strength muscles and might be more difficult to tear. Effects of these ostracods should be especially considered in commercial fish as their presence could alter and devaluate the product.

Finally, it is important to highlight that spatio-temporal coexistence could modify population dynamics and enhance parasite effects on fish. There are different mechanisms that can promote or prevent parasite coexistence as environmental heterogeneity, density-dependence, genetic variation, etc. (see references in Flatt & Sheuring, 2004). In the current study, most of the potentially harmful species were found in the gills and high infection levels of these species coincided in the same seasons, during spring and summer. Interactions among species should be explored in the future in order to evaluate possible synergistic effects.

CHAPTER 5.

Speciation of the *Paradeontacylix* spp. (Sanguinicolidae) of *Seriola dumerili*. Two new species of the genus *Paradeontacylix* from the Mediterranean.



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Speciation of the *Paradeontacylix* spp. (Sanguinicolidae) of *Seriola dumerili*. Two new species of the genus *Paradeontacylix* from the Mediterranean ☆

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ABSTRACT

Two new species of teleost blood fluke belonging to the sanguinicolid genus *Paradeontacylix* are described from the greater amberjack, *Seriola dumerili*, i.e. *Paradeontacylix ibericus* n. sp. from the Iberian Peninsula and *Paradeontacylix balearicus* n. sp. from the Balearic Islands. *P. ibericus* n. sp. and *P. balearicus* n. sp. show morphological similarities with *Paradeontacylix kampachi* and *Paradeontacylix grandispinus* respectively, which occur in mixed infection in *S. dumerili* from Japan. Multivariate analysis of morphometrical data provided statistical evidence for the separation of four species. However, component by component analysis did not show statistically significant differences between *P. balearicus* and *P. grandispinus*. Molecular data based on rITS2 and mCO1 gene sequences also supported the separation into four species. Morphological and molecular data were used to examine phylogenetic relationships between *Paradeontacylix* species from *S. dumerili* and other species in the genus. The results coincided in revealing two main branches with *P. kampachi*+*P. ibericus* and (((*P. grandispinus*+*P. balearicus*) *Paradeontacylix sanguinicoloides*) *Paradeontacylix godfreyi*). *Paradeontacylix odhneri*, for which little data are available, was located basal in a separate branch. This is the only species of *Paradeontacylix* which parasitizes a non-carangid host which might probably explain the separation from the other species. Paired similarities between the Japanese and the Mediterranean species, despite the large geographic distance, could be explained by the speciation of parasite geminate lines before host separation by tectonic events. Consequently, geographic and historical isolation support the morphological and genetic differences leading to the evolution of the new species described here.

1. Introduction

The amberjacks are species of the genus *Seriola* (Carangidae, Perciformes) which have a long tradition in aquaculture and fisheries, especially in Japan [1]. One of the most valuable species for consumption worldwide is greater amberjack *Seriola dumerili* (Risso, 1810). In the Mediterranean area, it has been experimentally cultivated in Italy, Spain, Malta and Greece [1–3]. Attempts involving more intensive culture have been unsuccessful due to high juvenile mortality. One of the most significant causes of loss of 0+ and 1+ *S. dumerili* are parasitic infections caused by blood flukes [4,5] belonging to the genus *Paradeontacylix* (Sanguinicolidae, Digenea).

Mainly, pathologies are related to egg release in the circulatory system. The accumulation of eggs in gills and the lost of branchial epithelium caused by the hatching and leaving of miracidia provokes abnormal blood circulation, haemorrhages, hypoxia and cause fish mortalities. Severe sanguinicolid infections episodes have been reported in some places around the world as far as Majorca in Mediterranean and Japan in Pacific Ocean [4,5].

In Japan, mortalities have been associated with the sanguinicolid *Paradeontacylix grandispinus* Ogawa et Egusa, 1986 occurring in mixed infections with *Paradeontacylix kampachi* Ogawa et Egusa, 1986 [5]. The first experimental cultures of *S. dumerili* in the western Mediterranean were carried out in Andratx (Majorca, Balearic Islands) and in Puerto de Mazarrón (Murcia, Iberian Peninsula). Although sanguinicolidosis was reported in both localities, juvenile fish mortality was only found in Majorca being so high that the culture was discontinued [4]. The blood flukes from the Mediterranean are very similar to those from

☆ Note: Nucleotide sequences data reported in this paper are available in the EMBL, GenBank and DDJB data bases under the accession numbers AM489593–AM489607.

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Japan: those from Murcia were provisionally identified as *P. kampachi* [3,6] whereas the parasites from Majorca were described as *P. grandispinus*-like [7]. Type of culture facilities was also different in both localities: sea-cages in Majorca and tanks in Murcia. Invertebrate intermediaries in sea-cages together with the high density of host in culture conditions could increase the infection abundance levels [8]. Determining the identity of the involved parasite is necessary in order to detect other possible related factors as different species virulence.

This study deals with the morphological and genetic analysis of Mediterranean blood flukes from *S. dumerili* in order to determine the identity of the species of *Paradeontacylix* in Balearic Islands and in the Iberian Peninsula and their relation to the known species of the genus.

2. Materials and methods

2.1. Sample collection

Ninety *S. dumerili* were obtained from two sites off the island of Majorca (Balearic Islands, Spain): between Cala Figuera and Cabrera (South, 39°8′–21′N/2°42′–3°02′E) and between Pollença and L'Alcúdia (North, 39°52′–40°07′N/3°20′–2°53′E). Fifteen fish were obtained from Santa Pola (Iberian Peninsula, Spain, 38°06′–17′N/0°16′–34′W). Herein, the blood flukes from Majorca will be referred to as "Balearic" and those collected from Santa Pola, as "Iberian". Five samples of 15 *S. dumerili* were obtained every 2 months between April 2005 and March 2006 (except in the fishing restriction period, between July and September), from local fish markets in Majorca. One sample of 15 individuals was obtained from Santa Pola in February 2006. The circulatory system and organs of strong blood supply were scrutinized for the presence of blood flukes and eggs including the: sinus venosus, atrium, ventricle, bulbus arteriosus, aorta dorsalis, vena caudalis, gill arches, kidney, spleen and liver. Thoracic and pelvic girdles and the associated muscular tissue were also examined, following the suggestion of Montero et al. *et al.* [6] that blood flukes are frequently found in these microhabitats. The organs were washed with saline, pressed between Petri dishes and observed under the stereomicroscope at 10 magnifications. The presence of fluke eggs was recorded and adult blood flukes were collected and fixed in 70% ethanol for morphological analysis and in 100% ethanol for molecular analysis. Ecological terms according to Bush et al. *et al.* [9].

2.2. Morphological analysis

Worms were stained with iron acetocarmine [10], dehydrated and mounted with Canada balsam. In order to compare the Mediterranean *Paradeontacylix* spp. with morphologically similar species 10 paratypes of *P. grandispinus* (K. Ogawa's collection) and 4 paratypes of *P. kampachi* (K. Ogawa's collection) from Kochi and Kagoshima Prefectures, Japan were analysed. In addition, 10 specimens of *Paradeontacylix* sp. from experimental cultures of *S. dumerili* at the Spanish Institute of Oceanography (SIO) in Puerto de Mazarrón (Murcia, peninsular Spain) were examined. These specimens had been examined previously [7,6] and were obtained from the parasite collection of the Cavanilles Institute of Biodiversity and Evolutionary Biology (ICBIBE, University of Valencia, Spain). Drawings and measurements of adult specimens and eggs were made with the aid of a drawing tube and digital images using Image Tools for Windows 3.00. Co. 1995–2002, UTHSCSA. Measurements are given as the mean in μm, with the range in parentheses.

2.3. Statistical analysis

The morphometrical information obtained was analysed by discriminant analysis and principal component analysis using SPSS® 12.0 (SPSS, Inc., Norusis, 2002). Fourteen morphometric characters were obtained to describe individuals: perimeter/2, maximum body width, posterior marginal spine length, oesophagus length, length of anterior and posterior caeca, ganglion width, length of testicular zone, ovary length and width, post-testicular space, uterus length and wide, length of seminal receptacle and oviduct length. PCA was performed with the discriminant variables resulting from the discriminant analysis. Metrical data was log-transformed and corrected to reduce the differences caused by isometric growth: data of each individual were divided by the geometric average of all variables measured [11]. In order to verify that the PCA assumption of homogeneity of variance distribution was correct, and considering that the analysis had been made with multiple groups, the difference between the variances of the study groups, *P. kampachi*, *P grandispinus*, *Paradeontacylix ibericus* and *Paradeontacylix balearicus*, was tested [12] using the statistical software Philips CPC* (Phillips, P., 1994–7. CPC — common principal component analysis program. University of Texas at Arlington, Texas). Tested hypotheses were: 1, equality of variance; 2, homogeneity of variance; 3, variances with four, three, two or one common components; 4, random distribution of the variance. The eigenvectors resulting from the latter analysis were used to perform a common principal component analysis (CPC); eigenvectors were transformed in components and graphically represented with SPSS 12.0. The relaxation degree of the *P. grandispinus* paratypes was different but all the type specimens were included in the analysis. Thereafter, the average measurements of each species were statistically compared and the significance of the differences between the species by each component was tested [13].

2.4. Phylogenetic analysis based on morphological data

Morphological data of all the *Paradeontacylix* species [*P. kampachi*; *P. sanguinicoloides* McIntosh, 1934; *P. godfreyi* Hutson et Whittington, 2006; *P. grandispinus*; *P. sinensis* Liu, 1997; and *P. odhneri* (Layman, 1930)] [14–18] and the Mediterranean morphotypes were analysed under the criterion of maximum parsimony following the methodology of Wiley et al. *et al.* [19]. The revision of the original descriptions of all known *Paradeontacylix* species showed that *P. sinensis* does not agree with the diagnostic character for the genus *Paradeontacylix*. The main differences are: only one large testis in *P. sinensis*, which reaches near the lateral body margins in contrast to multiple testes between the caeca (*Paradeontacylix*); and genital pores close together in *P. sinensis* whereas they are separated in *Paradeontacylix*. This species should be assigned to a different genus and was consequently excluded from the analysis.

Phylogenetic analyses using PAUP* version 4.0b10 (Swofford, 2001. PAUP 4 — phylogenetic analysis using parsimony. Version 4. Sinauer Associates, Sunderland, Massachusetts) were conducted using a heuristic search algorithm, characters unordered and the ACCTRAN option. Character polarization was done using *Aporocotyle spinosicanalis* Williams, 1958, as outgroup [20]. The outgroup was selected because the type species of the genus *Aporocotyle*, *A. simplex*, was described as a junior synonym of the *Paradeontacylix* genus [21].

Table 1
Morphological data matrix of *Paradeontacylix* species and *Aporocotyle spinosicanalis* as an outgroup

Sanguinicolid species	Morphological characters											Host
	1	2	3	4	5	6	7	8	9	10	11	
Aporocotyle spinosicanalis	0	0	0	0	0	0	0	0	0	0	0	*Merluccius merluccius*
P. kampachi[a]	1	2	1	1	0	0	1	1	0	0	1	*Seriola dumerili*
P. ibericus[a]	1	2	1	1	0	0	1	1	0	0	0	*Seriola dumerili*
P. grandispinus	1	1	0	0	1	1	1	1	0	0	2	*Seriola dumerili*
P. balearicus	1	1	0	0	1	1	1	1	0	0	2	*Seriola dumerili*
P. godfreyi	1	0	0	2	1	1	0	0	0	1	0	*Seriola lalandi*
P. sanguinicoloides	1	1	0	1	1	1	1	0	0	0	2	*Seriola lalandi*
P. odhneri	0	0	0	1	0	0	1	0	1	0	1	*Takifugu porphyreus*

Includes host identity.
[a] Note that *P. grandispinus* and the *P. balearicus* share the same codification.

However *A. spinosicanlis* was the only sequenced species of the genus. Clade support was determined via 1000 bootstrap replicates. Character argumentation: see Table 1.

Eleven characters, 8 binary and 3 multistate characters with a total of 25 character states were used in the analysis. 0 represents the plesiomorphic condition and 1 or 2 an apomorphic one. Categories of quantitative variables were established with one third of the range as step, except for the number of tegumental spines as the range was very small and categories were established with one half of the range as step. 1) Relative position of ovary and uterus: 0, ovary posterior; 1, ovary anterior. 2) Number of testes rows: 0, irregular (no rows); 1, 1 row; 2, 2 rows. 3) Arrangement of testes: 0, contiguous; 1, separated. 4) Number of testes: 0, until 35; 1, between 35 and 70; 2, >70. 5) Ovary shape: 0, "tear-shaped"; 1, "heart-shaped". 6) Large posterior tegumental spines: 0, absent; 1, present. 7) Number of marginal tegumental spines per row: 0, >15; 1, <15. 8) Caeca shape: 0, "H-shaped"; 1, "X-shaped". 9) Vitelline fields extend posterior to ovary: 0, no; 1, yes. 10) Testes posterior to caeca: 0, absent; 1, present. 11) Maximum body size: 0, between 3500 and 7000; 1, >7000; 2, <3500. *P. grandispinus* and *P. balearicus* share all these characters. Consequently, they were joined as a single taxon for the analysis.

2.5. Phylogenetic analysis, based on molecular data

DNA was extracted from two individual worms each from the Iberian and the Balearic *Paradeontacylix* morphotypes from *S. dumerili*; from one specimen each of *P. kampachi* and two specimens of *P. grandispinus* from *S. dumerili* from Japan (Ushine, Kagoshima Prefecture, 31°33′N/130°43′E, June 2006), and from two individuals of *P. godfreyi* from *Seriola lalandi*, from Australia (Port Augusta, South Australia, 32° 42′S/137° 46′E, August 2006).

Fixed specimens were transferred to 300 µl TNES urea (10 mM Tris–HCl (pH 8), 125 mM NaCl, 10 mM ethylenediaminetetra-acetic acid (EDTA), 0.5% sodium dodecyl sulphate (SDS), 4 M urea) and DNA was extracted using a phenol-chloroform protocol as described previously [22]. DNA of two ribosomal gene regions, i.e. the partial 28S and the complete internal transcribed spacer 2 (ITS2), as well as of the mitochondrial cytochrome oxidase 1 (COI) gene was amplified using the primers summarised in Table 2. The forward primer 3S was used for all *Paradeontacylix* spp. except for *P. godfreyi*, for which GA1 was used. Each 30 µl PCR reaction contained 1.5 U of Thermoprime Plus DNA polymerase and 10× 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. Denaturation of DNA (95 °C for 2 min) was followed by 35 cycles of amplification (95 °C for 50 s, annealing temperature for 50 s and 72 °C for 50 s) and terminated by a 4-minute extension (72 °C). Annealing temperature was 56 °C for all reactions but the ones amplifying COI, for which it was set to 50 °C. The PCR products obtained were purified for sequencing using the GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare). Cycle sequencing of the DNA fragments was conducted on a 48 capillary ABI 3730 sequencer (Applied Biosystems) using the BIG Dye Terminator v3.1 Ready Sequencing Kit (Applied Biosystems, USA). Forward and reverse strands were sequenced in all cases. Nucleotide sequence data reported in this paper are available in GenBank™, EMBL and DDBJ databases under the accession numbers AM489593–AM489607.

The obtained sequences were aligned using Clustal X [23] using a gap opening penalty of 10.00, a gap extension penalty of 5.00 and the DNA weight matrix of the International Union of Biochemistry (IUB). The resulting alignments of 28S and ITS2 rDNA sequences as well as of the COI sequences were joined in a combined dataset using BioEdit 4.8.9 [24] and were exported in NEXUS format. Tree inference was carried out by a likelihood-based Bayesian tree sampling procedure (BI) and under the maximum parsimony optimality criterion (MP). BI was conducted using MrBayes v 3.0 [25] with parameters corresponding to the general time-reversible model GTR+I+Γ [26], which was estimated as the best substitution model for the dataset by using Modeltest version 3.7 [27]. BI used the following parameters: nst=6, rates=invgamma, ngammacat=4. The MCMC was allowed to run for 1,000,000 generations sampling every 100th tree. The first 50,000 generations were later discarded as the burn-in period. The maximum parsimony (MP) analysis was conducted with PAUP*, version 4.0b10 using a heuristic search with tree bisection-reconnection (TBR) branch swapping, random addition of taxa (10 replications) and the ACCTRAN option. Gaps were treated as missing data. Clade support was assessed with bootstrapping of 1000 replicates.

3. Results

Eggs and adults of *Paradeontacylix* were frequently found at all Spanish sampling localities. In fish from Majorca eggs were detected all year round with a mean prevalence of 51.3%. The adult mean prevalence throughout the year was 10.2%. *S. dumerili* sampled from Santa Pola (February 2006) showed a prevalence of 53.3% for eggs and 20.0% for adults. Eggs found were located exclusively in the heart and gills. The position of the eggs inside the gills was also different between localities: the eggs from Majorca were located in both the afferent and efferent vessels, as well as in the secondary lamellae, whereas in Santa Pola they were only detected in the afferent vessels of the primary gill lamellae. The comparison of specimens from the Iberian Peninsula sites (Santa Pola and Puerto de Mazarrón) revealed no morphological differences. The Iberian and Balearic blood blood-fluke adults were markedly different but similar to *P. kampachi* and *P. grandispinus* respectively, however according to their morphological and molecular peculiarities two new species are proposed.

3.1. P. balearicus n. sp. (Fig. 1A,B,C)

3.1.1. Description

Based on 7 whole mounted, gravid specimens; metrical data from all the 7 adult worms. Body smooth, elongate, dorsoventrally flattened, lancet-shaped, approximately 10 times as large as wide. Total length 1579 (1318–1901); maximum width 170 (112–219). Lateral margins armed ventrally with elongate marginal tegumental spines distally

Table 2
Primers used for the amplification and sequencing of ribosomal and mitochondrial genes of *Paradeontacylix* spp.

Gene/region	Primer	Primer sequence	Direction	Application	Reference
Ribosomal					
28S	U178	5′-GCACCCGCTGAAYTTAAG-3′	Forward	PCR+seq	Lockyer et al., 2003
28S	L1642	5′-CCAGCGCCATCCATTTTCA-3′	Reverse	PCR+seq	Lockyer et al., 2003
28S	LSU1200R	5′-GCATAGTTCACCATCTTTCGG-3′	Reverse	Seq	Lockyer et al., 2003
ITS2	3S	5′-GGTAACGGTGGATCACGTGGCTAGTG-3′	Forward	PCR+seq	Bowles et al., 1993
ITS2	ITS2.2	5′-CCTGGTTAGTTTCTTTTCCTCCGC-3′	Reverse	PCR+seq	Cribb et al., 1998
ITS2	GA1	5′-AGAACATCGACATCTTGAAC-3′	Forward	PCR+seq	Anderson and Baker, 1998
Mitochondrial					
COI	JB3	5′-TTTTTTGGGCATCCTGAGGTTTAT-3′	Forward	PCR+seq	Bowles et al., 1993
COI	JB4.5	5′-TAAAGAAAGAACATAATGAAAATG-3′	Reverse	PCR+seq	Bowles et al., 1993

hooked, distributed in 341 (321–361) rows surrounding entire body margin. Each row with 8–12 spines, decreasing in number towards posterior end until it reaches 2–3. Large posterior tegumental spines 23 (22–24) long and 3 (2–4) wide (Fig. 1C) gradually decreasing in length towards anterior end. Body marginal tegumental spines 2.5 (2–5) in length and 1 (0.5–1.5) in width. Nerve-cord laterally extended 1516 (1278–1872) long, 18 (14–22) maximum wide, 29 (20–35) from body margin in mid-body, connecting in posterior end of body. Ganglion arranged perpendicular to mid-line of body connecting dorsolateral nerve-cords, 89 (66–118) or 5–6% of body length from anterior end of body, 56 (48–69) across width of worm 33 (41–27) in diameter.

Mouth anteroventral subterminal. Oesophagus 403 (241–546) long or 15–34% of body length, 15 (11–20) in maximum width. Oesophagus ventral to nerve ganglion, medial, winding smoothly and extending posteriorly along mid-line for 1/3–1/4 of body length, complete extension surrounded by gland-cells, most abundant in area 204 (160–230) long or 42–66% of oesophagus total length, up to approximately. Intestinal caeca X-shaped, caecal intersection 573 (523–638) or 34–39% from anterior end of body. Anterior caeca 50 (36–62) long or 3% of body length, 19 (14–24) wide; posterior caeca 522 (437–645) long or 33–34% of body length, 15 (13–17) wide. Posterior caeca located laterally along testes zone and ending blindly anterior to ovary.

Testes 22 (20–26), rounded or ellipsoidal, in a single row between posterior caeca. Testicular zone 496 (403–586) in length, occupying 34% of total length, 102 (87–117) wide or 53–78% of body width, 4.6–5.0 longer than wide. Post-testicular space 515 (381–613) long or 30–32% of body length. Vasa efferentia not observed. Vas deferens, 750 (619–886) long, 37 (27–72) wide, originating in anterior testes, extending sinistral, dorsally to ovary, leading to seminal vesicle enclosed in cirrus sac. Seminal vesicle 106 (86–124) long, 35 (23–48) wide. Cirrus 25 (11–40) long or 13–32% of seminal vesicle length. Male genital pore dorsal, 272 (204–339) or 15–18% of body length from posterior extremity of body, 15 (7–21) from sinistral body margin and 149 (97–193) from dextral body margin.

Ovary heart-shaped, distally elongate, 111 (95–142) long or approximately 7% of body length, 123 (105–158) wide or 70–90% of body width, 1.1–1.3 wider than long. Located immediately posterior to testis, between nerve-cords, anterior to seminal vesicle and ventral to vas

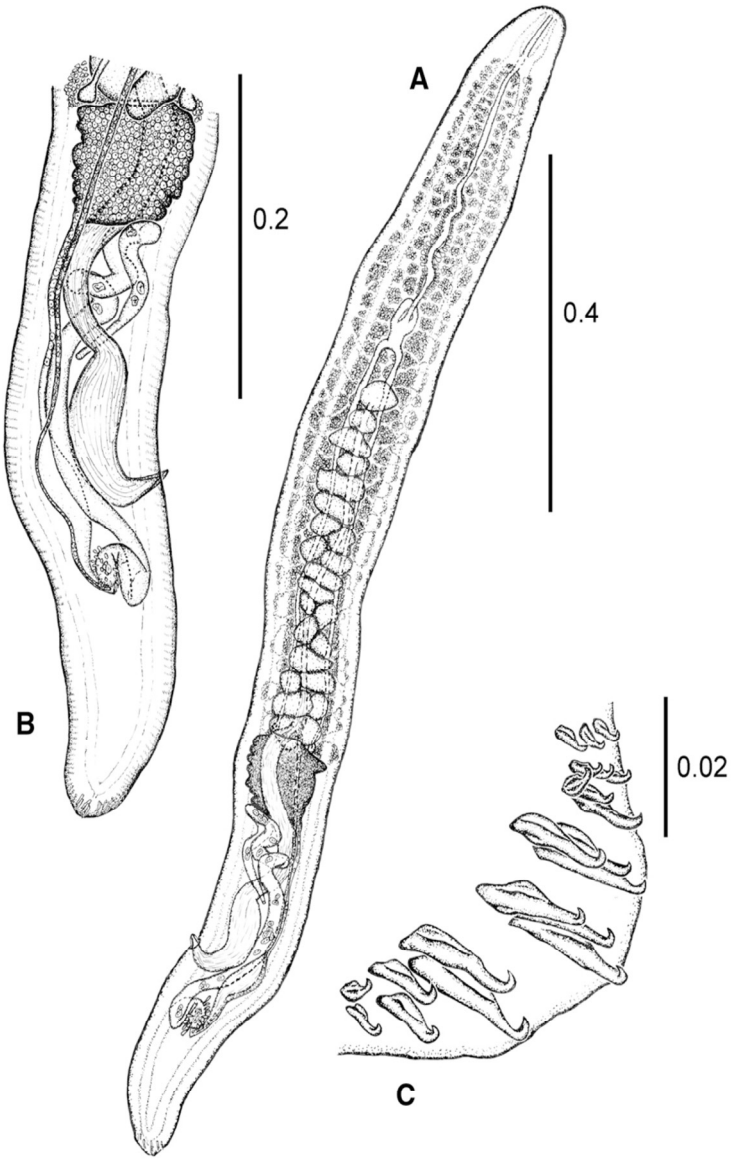

Fig. 1. *Paradeontacylix balearicus* sp. n. A. Holotype, whole mount, dorsal view. B. Posterior end, ventral view showing post-ovarian region. C. Large posterior tegumental spines, ventral view. Scale bars in millimetres.

deferens. Oviduct originates at dextral posterior convex part of ovary. Oviduct 170 (103–251) long, 8 (6–11) wide. Posterior portion of oviduct forms seminal receptacle 148 (120–192) long, 28 (22–41) wide. Seminal receptacle narrowing progressively and joining vitelline duct before forming oötype. Oötype 32 (15–49) long and 21 (11–27) wide, surrounded by Mehlis' gland. Vitelline follicles extending along body from posterior margin of nerve commissure to ovary, with much lower density close to lateral margins. Vitelline duct in anterior portion of testes area running ventrally until reaching seminal receptacle and oötype. Uterus folded 467 (348–586) long and 29 (24–38) wide, extending along post-ovarian zone, short portion descending posterior to oötype connecting to ascending portion slightly dextral to body mid-line ending with ventral curvatures near the ovary, terminating with the descending portion 88 (71–119) long or 20% of uterus length, 12 (9–19) wide. Female genital pore opening dorsally at 307 (229–368) of posterior body margin, 96 (69–119) of sinistral body margin and 49 (33–86) of dextral body margin. Uterine eggs amorphous, spherical to ellipsoidal, measuring 8–14 long and 4–11 wide or 16–37% of uterus maximum width (Fig. 1B).

3.1.2. Taxonomic summary

Type-host: *Seriola dumerili* (Risso, 1810) (Carangidae).

Site: Afferent gill vessels and heart.

Type-locality: Cabrera — Cala Figuera (South, 39°8′–21′N/2°42′–3°02′E), southern Majorca, Balearic Islands.

Other locality: L'Alcúdia — Pollença (North, 39°52′–40°07′N/3°20′–2°53′E), northern Majorca, Balearic Islands.

Type specimens: Holotype MPM Coll. No. 18871-A5123, 1 Paratype MPM Coll. No. 18871-A5124; 1 Paratype NHM 2008.5.9.1. GenBank accession numbers: AM489594 (28S rRNA), AM489599 (ITS2), AM489604 (COI).

Collectors: A. Repullés (University of Valencia) and F.E. Montero (Autonomous University of Barcelona).

Infection details: Number of infected fish=43 (eggs)/8 (adults); prevalence 51.3% (eggs); mean intensity 1 (adults); infected host size 32.50–42.50 cm TL (32.00–44.50 cm TL, *n*=90).

Etymology: The species name refers to the host origin, the Balearic Islands.

3.1.3. Remarks

Paradeontacylix McIntosh, 1934 (*sensu* Smith, 1907). *P. balearicus* n. sp. displays all the diagnostic characteristics of the genus *Paradeontacylix*. It is differentiated from the other species of the genus by the combination of: short length (the shortest species ranging from 1318 to 1901), no more than 26 testes arranged in one row, ovary heart-

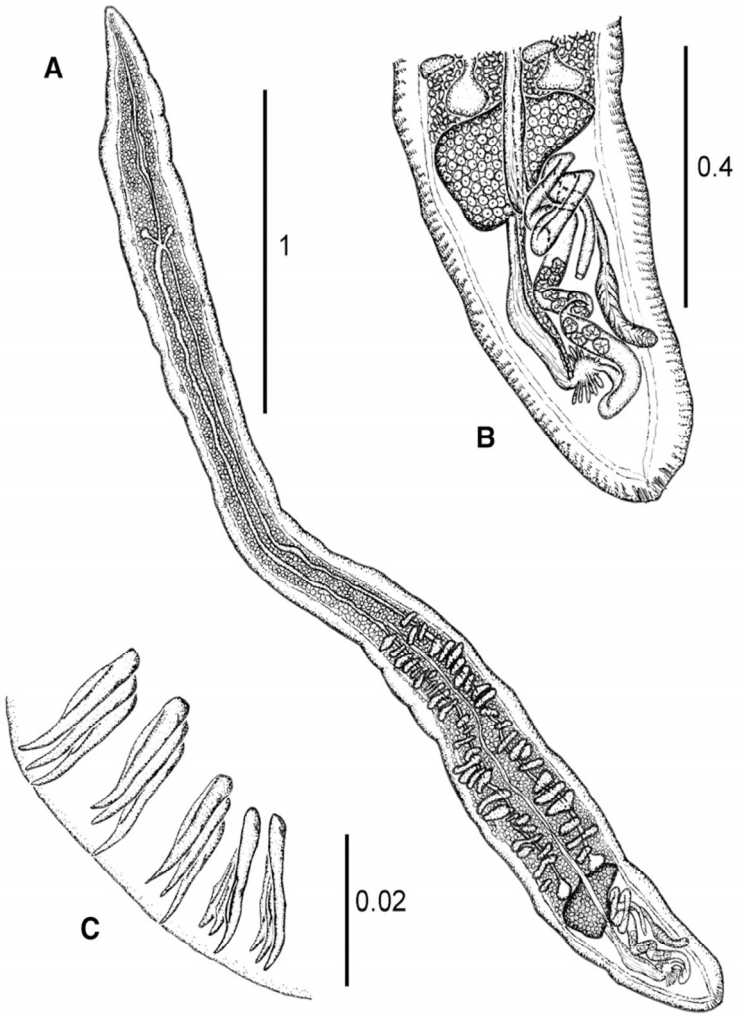

Fig. 2. *Paradeontacylix ibericus* sp. n. A. Holotype whole mount. B. Posterior end, showing post-ovarian region. C. Large posterior tegumental spines. Ventral views. Scale bars in millimetres.

shaped, uterus post-ovarian, female genital pore slightly dextral near mid-line, from 8 to 12 spines per row and large posterior tegumental spines. *P. balearicus* most closely resembles *P. grandispinus*, as both species share a similar number and arrangement of testes, 19 to 32 testes in *P. grandispinus* and 20 to 26 testes in *P. balearicus* arranged in one row; also share very large elongate marginal posterior tegumental spines, 21 to 26 in *P. grandispinus* and 22 to 24 in *P. balearicus*. However, *P. balearicus* differs from *P. grandispinus* in that its dimensions and proportions are halved. Furthermore, the marginal posterior tegumental spines are relatively longer: posterior spine length/total body length: 1/60–1/80 in *P. balearicus*; 1/93–1/128 in *P. grandispinus*. The discriminant analysis and the host distribution are also disparate.

3.2. *P. ibericus n. sp.* (Fig. 2A,B,C)

3.2.1. Description

Based on 13 whole mounted, gravid specimens from Santa Pola (n=3) and from Puerto Mazarrón (n=10); metrical data from 10 adult worms. Body smooth, elongate, dorsoventrally flattened, lancet-shaped, approximately 14 times as long as wide. Total length, 4090 (2480–5700); maximum width 340 (290–460). Lateral margins armed ventrally with elongate marginal tegumental spines distally hooked, distributed in 678 (577–746) rows, which surround entire body margin except anterior extremity. Each row with 12–14 spines, decreasing in number towards the posterior end until reach 3–5. Large posterior tegumental spines 13 (8–18) long and 3 (2–4) wide (Fig. 2C) gradually decreasing towards the anterior end. Body marginal tegumental spines 5 (4–6) long and 1 (0.5–1.5) wide. Nerve-cord laterally extended 4039 (2438–5652) long, 15 (13–19) maximum wide, 43 (31–52) from body margin in mid-body, connecting in posterior end of body. Ganglion arranged perpendicular to mid-line of body connecting dorsolateral nerve-cords, 130 (114–152) or 2–5% of body length from anterior end of body, 61 (54–67) across width of worm 33 (29–43) in diameter.

Mouth anteroventral subterminal. Oesophagus 700 (580–1140) long, 14–28% of body length, 15 (10–18) in maximum width. Oesophagus ventral to nerve ganglion, winding smoothly and extending posteriorly along mid-line for 1/5–1/6 of body length, surrounded the complete extension by gland-cells, most abundant in area 368 (179–510) long or 30–44% of oesophagus total length, up to approximately. Intestinal caeca X-shaped, caecal interseccion intersection 854 (800–952) or 17–32% from anterior end of body. Anterior caeca limbs 90 (40–130) long or 2% of body length, 18 (14–24) wide; and posterior caeca limbs, 2483 (2143–2914) long or 51–86% of body length, 15 (13–19) wide; ending blindly anterior to ovary. Posterior caeca extended laterally along testes zone and ending blindly anterior to ovary.

Testes 46 (34–65), ellipsoidal, arranged in two rows, distributed along and ventral to distal third of posterior caeca. Testicular zone 905 (550–1280) in length, occupying 22% of total body length, 298 (227–382) wide or 78–83% of body width, 2.4–3.4 longer than wide. Post-testicular space 577 (382–724) long or 13–15% of body length. Vasa efferentia not observed. Vas deferens, 893 (643–1383) long, 31 (24–37) wide, originating in anterior testes, extending medial, dorsally to ovary, leading to seminal vesicle enclosed in cirrus sac. Seminal vesicle 133 (108–153) long, 26 (19–34) wide. Cirrus 47 (26–64) long or 24–41% of seminal vesicle length. Male genital pore dorsal, 323 (211–401) or 7–9% of body length from posterior extremity of body, 39 (25–64) from sinistral body margin, 236 (180–289) from dextral body margin.

Ovary kidney-shaped, laterally widened, 130 (70–190) long or approximately 3% of body length, 190 (140–270) wide or 48–58% of body width, 1.4–2.0 wider than long. Located immediately posterior to testis, between nerve-cords, anterior to seminal vesicle and ventral to vas deferens. Oviduct begins at the dextral posterior part of ovary. Oviduct 220 (150–360) long, 11 (7–18) wide. Wider posterior portion of oviduct forming seminal receptacle, measuring 150 (70–220) long, 30 (20–38) wide. Seminal receptacle narrowing progressively and joining vitelline duct before forming oötype. Receptacle narrowing progressively joining and terminating in oötype together with vitelline duct. Oötype 57 (48–69) long and 36 (31–43) wide surrounded by the Mehlis' gland. Vitelline follicles filling space from anterior body margin to anterior margin of ovary, extending to body margin laterally. Vitelline duct originates in anterior portion of testes, running ventrally until seminal receptacle and oötype. Uterus folded 751 (547–905) long and 31 (24–43) wide, extending in the post-ovarian zone, short portion descending posterior to oötype connecting to ascending medial with dorsal slight curvatures and anteriorly, near the ovary, ventral curvatures, terminating with the descending portion 100 (83–127) long or 15% of uterus length, 15 (10–24) wide. Female genital pore opening dorsally at 321 (223–417) of posterior body margin, 130 (95–164) of sinistral body margin and 198 (114–261) of dextral body margin. Uterine eggs amorphous, spherical to ellipsoidal, measuring 23–31 long and 12–30 wide or 72–96% of uterus maximum width (Fig. 2B).

3.2.2. Taxonomic summary

Type-host: *Seriola dumerili* (Risso, 1810) (Carangidae).

Site: Heart, gill and girdles.

Type-locality: Santa Pola (38°06′–17′N/0°16′–34′W), Iberian Peninsula, Mediterranean sea.

Other locality: Puerto de Mazarrón (37°29′–34′N/1°9′–15′W).

Type specimens: Holotype MPM Coll. No. 18872-A5125, 2 Paratypes MPM Coll. No. 18872-A5126 from Puerto de Mazarrón; 2 Paratypes NHM 2008.5.9.2 from Puerto de Mazarrón. GenBank accession numbers: AM489593 (28S rRNA), AM489598 (ITS2), AM489603 (COI).

Collectors: A. Repullés (University of Valencia) and F.E. Montero, (Autonomous University of Barcelona).

Infection details: Santa Pola: Number of infected fish=8 (eggs)/3 (adults); prevalence 57% (eggs); mean intensity (adults) 1; host sizes 36.50–42.00 cm TL (n=15). Puerto de Mazarrón (additional

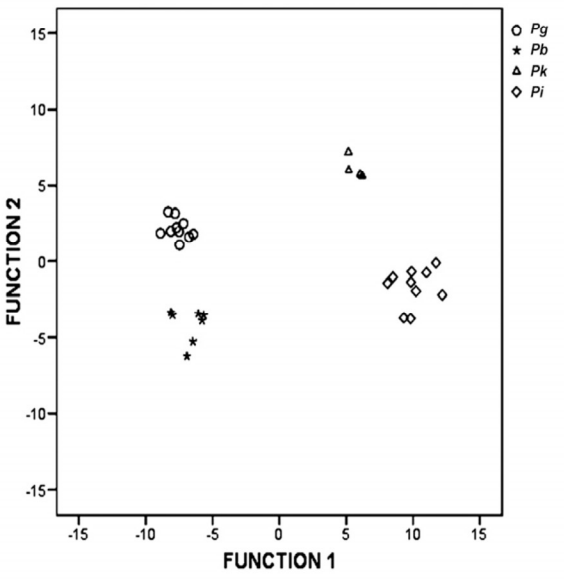

Fig. 3. Scatterplot of the discriminant scores performed on 14 morphometrical variables of 31 *Paradeontacylix* spp. specimens collected from *Seriola dumerili* (*Pg*, *Paradeontacylix grandispinus*; *Pb*, *P. balearicus*; *Pk*, *P. kampachi*; *Pi*, *P. ibericus*).

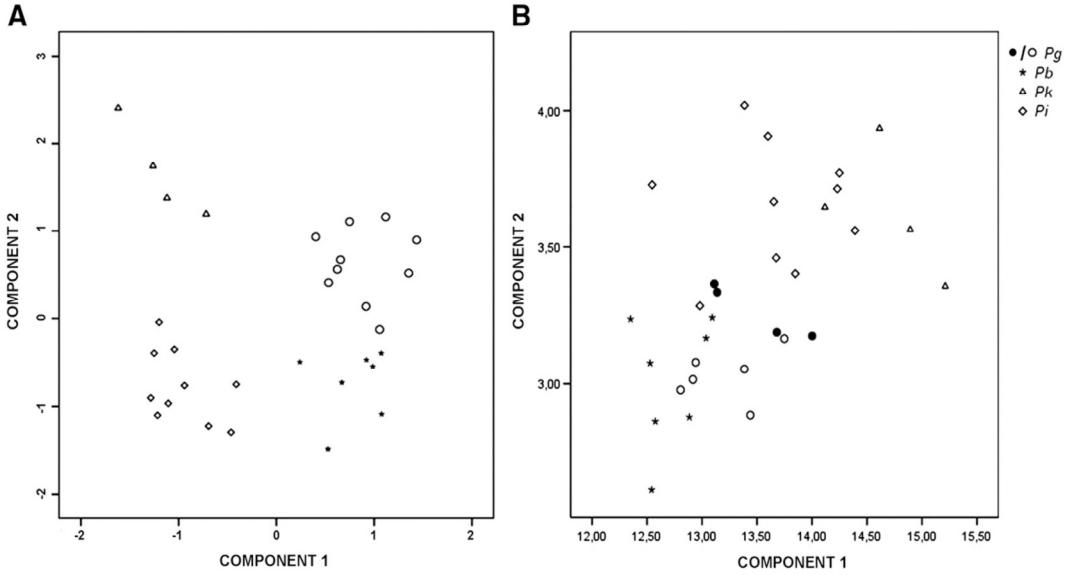

Fig. 4. Scatterplot of the 2 first principal components performed on 7 morphometrical and discriminant variables of 31 *Paradeontacylix* spp. specimens collected from *Seriola dumerili*. *Pg*, *Paradeontacylix grandispinus*; *Pb*, *P. balearicus*; *Pk*, *P. kampachi*; *Pi*, *P. ibericus*. A. Principal components analysis. B. Common principal components analysis. Non-contracted *P. grandispinus* are represented with solid circles.

data in [6,7]): number of infected fish = 94 (eggs)/43 (adults); prevalence 96.9% (in months when infection was detectable); mean intensity (adults) 3 (1–78); infected host sizes 21.00–43.00 cm TL (16.50–46.00 cm TL, $n = 137$).

Etymology: The species name refers to the host origin, the coasts of the Iberian Peninsula, emphasizing the separation from the species from the Balearic Islands.

3.2.3. Remarks

Paradeontacylix (McIntosh, 1934) (*sensu* Smith, 1907). *P. ibericus* n. sp. exhibits the characters of *Paradeontacylix* genus. It is distinguished from the other species of the genus by the combination of total length from 2480 to 5700, testes arranged in two rows and ovary kidney kidney-shaped, uterus post-ovarian, marginal tegumental spines distributed in 678 rows (577–746), and short posterior tegumental spines, from 8 to 18. The morphology is almost identical to that of *P. kampachi*, described from *S. dumerili* in Japan: short posterior tegumental spines 8 to 18; two rows with separated testes; and a teardrop-shaped ovary. The number of tegumental spines per row in *P. ibericus* (12–14) is the same as in *P. kampachi* specimens, which was found different from the original description (7–10 spines). *P. ibericus* can be distinguished from *P. kampachi* in the smaller body length 2480 to 5700 in *P. ibericus* and 4680 to 8100 in *P. kampachi* and relatively longer posterior marginal tegumental spines: posterior spines length/total body length 1/310–1/316 in *P. ibericus* vs. 1/360–1/480 in *P. kampachi*. Other noticeable differential characteristics of the new species are the higher total number of spine rows, 577 to 746 in *P. ibericus* and 510 to 590 in *P. kampachi*; and the narrower ovary 140 to 270 in *P. ibericus* and 305 to 530 in *P. kampachi* (see also the discriminant analysis in the statistical results). The host geographic distribution of *P. ibericus* and *P. kampachi* is dissimilar.

3.3. Statistical analyses

The four species, *Pg*, *Pb*, *Pk*, *Pi* appear clearly separated in the scatterplot of the discriminant analysis. The selected discriminant variables were: perimeter/2, maximum width, posterior marginal spine length, length of testicular zone, oviduct length, ovary width and uterus width. Function 1 separated the groups (*Pg*+*Pb*) and (*Pk*+*Pi*) and the variables that contributed more significantly to this function were perimeter/2, oesophagus length and posterior marginal spine length. Function 2 separated the groups (*Pg*+*Pi*), *Pb* and *Pk* based on the ovary width, ganglion length and posterior marginal spine length (Fig. 3). PCA allowed the differentiation of two clearly defined groups with 2 subgroups each: *P. kampachi* (*Pk*) and *P. ibericus* (*Pi*) and; *P. grandispinus* (*Pg*) and *P. balearicus* (*Pb*) (Fig. 4A). Variance distribution within the groups was no homogeneous although 4 common components emerged between the variances of the four data groups (*p*-values between 0.065 and 0.160 higher than 0.050). CPC analysis showed that *Pk* and *Pi* overlapped only slightly while *Pg* and *Pb* exhibited more common ground (Fig. 4B). However, in the scatterplot of CPC results the separation of *Pg* and *Pb* was improved markedly when we only focus on the more relaxed paratypes of *Pg* marked as solid circles in the representation.

The Levène test for variance of the 4 common components indicated that *p*-values of the first three (0.596, 0.561, 0.050) were high enough to obtain a reliable evaluation. However, the *p*-value of the fourth component was lower than 0.05, and was not taken into account. Table 3 shows the *p*-values for the pairwise comparisons of the species for each component. Three differentiated subgroups for component 1 were established using ANOVA: *Pg* and *Pb*; *Pg* and *Pi*; and *Pk*. Component 2 separates 2 subgroups, *Pg*–*Pb* and *Pk*–*Pi*. Notably, the *p*-value for the combination *Pg*–*Pb* in component 1 is a little higher than 0.05.

Table 3
ANOVA *p*-values for the pairwise comparison of *Paradeontacylix* species in *Seriola dumerili* for components 1 and 2

Pair of species	Component 1	Component 2
Pg–*Pb*	0.057	0.691
Pg–*Pk*	0.000*	0.002*
Pg–*Pi*	0.362	0.000*
Pb–*Pi*	0.001*	0.000*
Pb–*Pk*	0.000*	0.000*
Pk–*Pi*	0.003*	0.997

Significant values are indicated with an asterisk. *Pg*, *Paradeontacylix grandispinus*; *Pb*, *P. balearicus*; *Pk*, *P. kampachi*; *Pi*, *P. ibericus*.

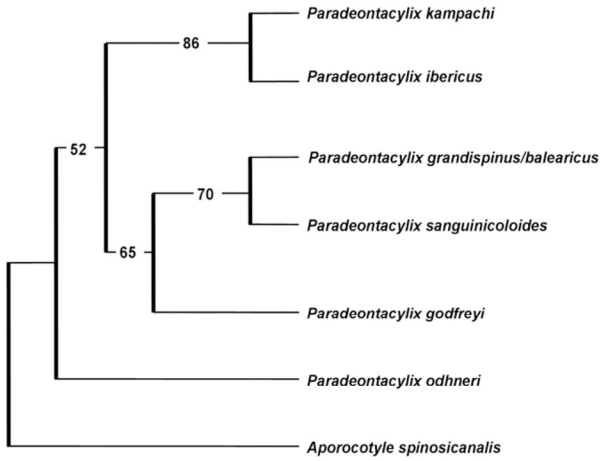

Fig. 5. Phylogenetic tree based on morphological data treated as unordered (11 characters; TL=17, CI=0.82, HI=0.18, RI=0.77, RC=0.63). *Aporocotyle spinosicanalis* as outgroup. Numbers represent bootstrap values of branches (1000 replications).

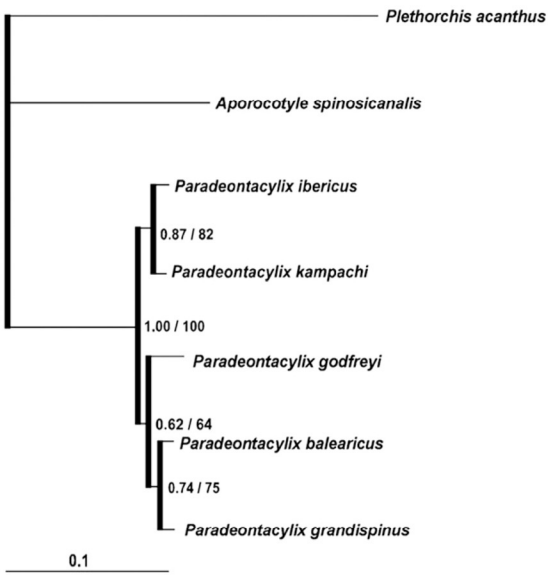

Fig. 6. Phylogenetic tree based on partial 28S, partial 5.8S, complete ITS2 ribosomal, and COI mt sequences, using *Aporocotyle spinosicanalis* (AY222177) and *Plethorchis acanthus* (AY222178+AY465875) as outgroup. Numbers at the nodes show: above, clade posterior probability, i.e. the proportion of the trees sampled containing that branch (BI), and below, percent of bootstrap support of 1000 replicates for the MP analysis.

3.4. Phylogenetic results from morphological data

The cladistic analysis resulted in a single phylogenetic tree with a consistency index of 0.81 (Fig. 5). This tree shows 2 clades: (*Pk*+*Pi*) and (((*Pg*/*Pb*)+*P. sanguinicoloides*)+*P. godfreyi*). *P. odhneri* was located basal to all these species in a separate branch. Bootstrap levels for the branch (*Pk*+*Pi*) are high so these 2 species are clearly related. Bootstrap levels for the branch relating (*Pg*/*Pb*) and *P. sanguinicoloides* is 70% pointing to a relationship between these 3 species. The remaining branches presented bootstrap values lower than 70%; these are shown for a comparison with molecular results.

3.5. Phylogenetic results from molecular data

With the exception of *P. kampachi*, all genes from all *Paradeontacylix* spp. used for molecular analysis were sequenced from two specimens each. The replicate sequences obtained for each species were found to be 100% identical.

Partial 28S DNA sequences of 1613 bp (GenBank accession numbers AM489593–97) complete ITS2 sequences (including partial 5.8S sequence) of 540 bp (AM489598–02), and complete mt COI sequences of 420 bp (AM489503–07) were obtained for all species. The sequence difference matrices (Table 4) show that the percent sequence difference increases from 28S over ITS2 to COI. 28S rDNA sequences show little variation up to only 0.9% between the *Paradeontacylix* species. The ITS2 and COI sequences allowed better species discrimination with sequence differences between 2.5–12.5% (ITS2) and 6.3–16.0% (COI) between different species. Low sequence differences between all genes were found between *P. grandispinus* and *P. balearicus* (0.2%/2.5%/6.3%), and to a slightly smaller extent between *P. kampachi* and *P. ibericus* (0.2%/4.7%/7%). However, ITS2 sequence differences of *P. kampachi* with *P. grandispinus* and with *P. balearicus* (4.1% and 4.3%) are slightly lower than between *P. kampachi* and *P. ibericus*. Based on the ITS2 and COI data, *P. godfreyi* showed the highest percentage of sequence difference with all other species, with values as high as 12.5% (ITS2) and 16.0% (COI).

Table 4
Sequence differences (percentage) of the aligned 28S and ITS2 ribosomal as well as the mitochondrial COI sequences obtained for *Paradeontacylix* spp. Smallest sequence divergence marked in bold and grey

	P.iber	*P.kamp*	*P.bale*	*P.grand*	*P.godf*
Partial ribosomal 28S sequences (1613 bp)					
P.iber	0	**0.2**	0.9	0.9	0.6
P.kamp		0	0.8	0.9	0.5
P.bale			0	**0.2**	0.7
P.grand				0	0.7
P.godf					0
Ribosomal ITS2 sequences (including partial 8.5S; 540 bp)					
P.iber	0	**4.7**	6.0	6.9	10.5
P.kamp		0	**4.3**	**4.1**	12.5
P.bale			0	**2.5**	10.0
P.grand				0	11.8
P.godf					0
Mitochondrial COI sequences (420 bp)					
P.iber	0	**7.0**	11.5	11.7	16.0
P.kamp		0	11.5	11.5	15.1
P.bale			0	**6.3**	13.7
P.grand				0	13.9
P.godf					0

The ITS2 sequence of the *Paradeontacylix* spp. contains an area of at least 10 bases of repetitive GT sequences. At the beginning of this region, an expansion segment of 20 bases occurs (mainly AT repetitions) in *P. godfreyi* and *P. kampachi*. Furthermore, *P. godfreyi* shows a unique addition of 30 repetitive AT/GT sequences following the GT rich region, which differentiates it from all other *Paradeontacylix* spp.

The result of the phylogenetic analysis using all sequence data obtained shows that the *Paradeontacylix* species split into two well supported clades, separating (*P. ibericus*+*P. kampachi*) from (*P. godfreyi*+(*P. balearicus*+*P. grandispinus*)) (Fig. 6).

4. Discussion

4.1. Identity of the Mediterranean Paradeontacylix species

In the current study, two new species of *Paradeontacylix* are described, *P. balearicus* from the Balearic Islands and *P. ibericus* from the Iberian Peninsula. Each species could be distinguished morphologically from the other species of the genus by size or shape. However, *P. grandispinus* and *P. balearicus* differences are not statistically significant.

The results of morphologically based matrices depend on the method of character coding and any set of features favoured by one author may yield a widely different range of phylogenetic estimates depending on the coding strategy employed (see review by [28]). To redress the conflict and increase resolution molecular data have been sought as an independent estimate of phylogeny and were thus used in this study to support the interpretation of the morphological data. Thereby, it was decided to use a combination of more conservative and more variable gene regions, as this has been shown to improve phylogenetic estimates considerably (e.g. [29]).

Molecular analysis of five *Paradeontacylix* spp. showed that 28S rDNA sequences were not informative with regard to intrageneric phylogeny as they show only 0.2–0.9% sequence divergence between species. The 28S rDNA has many divergent domains or expansion segments and is useful for the phylogenetic estimation of general evolutionary events into the Cenozoic [30]. The ITS2 region of the rDNA gene shows a much higher degree of divergence between taxa and has been successfully used to distinguish digeneans on a species level (see review by [31]). Thus, as expected, more divergent sequences were obtained from the ITS2 region (2.5–11.2%). Morgan and Blair [32] showed that mt DNA evolves more quickly than ITS, and is even more useful for distinguishing among closely related species. This can be confirmed in the current study as mt COI sequences differed by 6.3–16.0%. The levels of interspecific sequence variation is typically ≤1% for ITS and a fraction of a percent up to 2% for mtDNA (see review by [33]). It is thus suggested that, molecularly, the Mediterranean species of *Paradeontacylix* are clearly distinct from the Japanese ones. However, all gene regions showed high sequence identities between *P. ibericus*/*P. kampachi* and *P. balearicus*/*P. grandispinus*. The ITS2 rDNA of *Paradeontacylix* spp. was found to have a fast evolving, highly variable region with repetitive inserts, resulting in a similar sequence divergence between the Mediterranean and the Japanese species. Thus, with regard to *Paradeontacylix* spp., the mt COI sequences seem to be more suitable for phylogenetic analyses. [34,35] used the ITS2 region for phylogenetic analysis of various sanguinicolid genera, i.e. *Cardicola*, *Braya*, *Phthinomita* and *Ankistromeces*. Their sequences lack the *Paradeontacylix* spp. specific expansion segment of base repeats and thus seem to be suitable for phylogenetic comparison. Nolan and Cribb [34,35] considered sequence divergences as low as 0.3%, i.e. a single base e.g. for *Cardicola lafii* and *Cardicola parilus* or for *Phthinomita littlewoodi* and *Phthinomita jonesi* as sufficient for species separation. Although morphological differences were present between the *Cardicola* spp., the two *Phthinomita* species were morphologically almost identical. However, the single base was consistently different in 25 specimens sequenced for *P. jonesi* and 8 for *P. littlewoodi*, including from different sympatric hosts, supporting the interpretation and different species designation of Nolan and Cribb [35]. According to [35] the sequence divergence between species of different genera shows variable degrees, however, as *Cardicola* and *Paradeontacylix* are molecularly very closely related, the degree of divergence should be comparable within the two genera. It is thus concluded that the difference of 2.5% between *P. ibericus*/*P. kampachi* and 4.7% between *P. balearicus*/*P. grandispinus* justifies the distinct species status of the Mediterranean forms, especially as this is supported by some morphological differences.

Habitat of the two new species was found to be different. Eggs of *P. balearicus* were present in the afferent and efferent gill vessels, as well as in the secondary lamellae. In contrast, *P. ibericus* eggs were only found in the afferent vessels. The distribution of eggs in the former species increases their dispersion, which could be related to the higher pathogenicity previously observed in fish infected with *P. balearicus* in comparison to fish infected with *P. ibericus* [4,7]. No adult *P. balearicus* were found in girdles of Balearic fish whereas this was the site of maximum adult infection in fish from Puerto de Mazarrón [6]. The distribution of eggs and adults of *P. ibericus* was similar to that of *P. kampachi*, as for *P. balearicus* and *P. grandispinus* [36,6, present study].

4.2. Isolation between the Paradeontacylix spp. populations

The Balearic Islands are separated from the Iberian Peninsula by approximately 300 km and 1900 m of maximum depth. This study shows that, despite inhabiting the same host, the two species of *Paradeontacylix* remain isolated. There are no reports of mixed infections of *S. dumerili* by the two species in either of the two geographical localities [4,7,6, present study]. However, Ogawa and Egusa [16] found mixed infections of *P. kampachi* and *P. grandispinus* in *S. dumerili* off Japan.

Although adult greater amberjacks are pelagic and migratory [37] and could potentially move between the peninsular coastal waters and the islands, juvenile *S. dumerili* have a coastal habitat, restricted mobility and are usually associated with floating objects. Previous studies on Mediterranean *S. dumerili* have not reported the presence of *Paradeontacylix* sp. in adult fish [7]. It appears that in the Mediterranean *Paradeontacylix* spp. only infect juvenile amberjacks. Although the close proximity of the localities would imply merging of the Balearic and Iberian host populations, present data suggest they remain isolated during fish youth.

4.3. Phylogeny of the genus, a global study

The phylogenetic study of *Paradeontacylix* spp. worldwide is preliminary as molecular data of *Paradeontacylix* spp. is limited. However, as the morphological clustering is strongly supported by the molecular data available, the information obtained allows some conclusions: The tree is divided in two main branches, separated from *P. odhneri*, one allocating *P. kampachi* and *P. ibericus* and the other *P. grandispinus*, *P. balearicus*, *P. sanguinicoloides* and *P. godfreyi*. All *Paradeontacylix* spp. infect fish of the genus *Seriola* (see Table 1) except *P. odhneri* parasite of *Takifugu porphyreus*, (Teleostei: Tetraodontiformes). This is the species most similar to the outgroup, *A. spinosicanalis*. However, the description of *P. odhneri* is brief [14] and there are no deposited specimens available.

P. sanguinicoloides, *P. godfreyi*, *P. grandispinus* and *P. balearicus* share several characters that differentiate these species from the *P. kampachi*/*P ibericus* group. Interestingly, *P. sanguinicoloides* and *P. godfreyi* parasitize *S. lalandi*, whereas *P. grandispinus* and *P. balearicus* parasitize *S. dumerili*. It is suggested that the speciation of the geminate species of the two parasite groups could have occurred after the speciation of *S. lalandi* and *S. dumerili*.

Concerning *Paradeontacylix* spp. from *S. dumerili*, the trees show clustering of *P. kampachi*/*P. ibericus* in a different clade than *P. grandispinus*/*P. balearicus*, associating Japanese species with Mediterranean ones in both groups. Considering the isolation observed between parasite populations of very close localities in the western Mediterranean, a greater degree of isolation would be expected between the populations in regions as distant as Japan and the Mediterranean. In addition, the distribution of *S. dumerili* is circumglobal, but restricted to warm and temperate zones. Only an insignificant interchange between the populations could have recently existed *via* the Suez Channel and, even less probably, via the Panama Channel. A likely explanation for the paired morphological and genetic similarities between the current species could be that the ancestral species of *P. kampachi*/*P. ibericus* and *P. grandispinus*/*P. balearicus* groupings existed before the separation of host populations. The isolation of the Mediterranean and Japanese host populations could have taken place during middle Miocene, 14 or 15 million years ago, when the Tethys sea divided, separating the Paratethys (Indian Ocean) and the Mediterranean [38]. Similarly, individuals of *P. sanguinicoloides* have been recovered from *S. lalandi* from two very isolated areas, i.e. southern Australia (Pacific, Southern and Indian Oceans) [18,39] and Florida (Atlantic Ocean) [15]. It would be necessary to investigate the molecular relationships between *P. sanguinicoloides* from the two localities since morphological differences between sanguinicolid species are not always apparent.

The worldwide diversity of *Paradeontacylix* species in *Seriola* spp. contrasts with the recently reported high cosmopolitan similarity of the parasite faunas in tunas (*Thunnus* spp.), although both genera include large pelagic and migratory fish species. [40] reported the same blood-fluke species, *Cardicola forsteri*, in *T. thynnus* from the Mediterranean and in *T. maccoyii* from Australia. Interestingly, the *Thunnus* spp. parasites were collected from adult migratory fish and the *Paradeontacylix* spp. from *S. dumerili* seem only to parasitize the sedentary juvenile amberjacks. Sedentarism could have contributed to the isolation of hosts. The presently described Mediterranean species show small morphological and more prominent genetic differentiation along possibly 14–15 million years of geographical separation from the Japanese species.

Note Added in Proof

The authors would like to report the existence of 2 nomina nuda, synonyms of the 2 species published in the present paper, and previously reported in Repullés-Albelda, A., Montero, F.E., Holzer, A.S., Cuadrado, M. and Raga, J.A. (2007) Differences in the pathogenicity of four *Paradeontacylix* spp. (Trematoda, Sanguinicolidae) from cultured greater amberjack (*Seriola dumerili*). 13th international conference of the EAFP, 24th–28th September, Viterbo Italy.

/Paradeontacylix balearicus/ Repullés-Albelda, Montero, Holzer, Ogawa, Hutson et Raga, 2008.

Syn:

/Paradeontacylix balearicus/ Repullés-Albelda, Montero, Holzer, Cuadrado et Raga, 2007. nomen nudum

/Paradeontacylix ibericus/ Repullés-Albelda, Montero, Holzer, Ogawa, Hutson et Raga, 2008.

Syn:

/Paradeontacylix ibericus/ Repullés-Albelda, Montero, Holzer, Cuadrado et Raga, 2007. nomen nudum

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CHAPTER 6.

Seasonal population dynamics of *Zeuxapta seriolae* (Monogenea: Heteraxinidae) parasiting *Seriola dumerili* (Carangidae) in the Western Mediterranean.

Seasonal population dynamics of *Zeuxapta seriolae* (Monogenea: Heteraxinidae) parasitising *Seriola dumerili* (Carangidae) in the Western Mediterranean

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Abstract

We examined the seasonal and yearly population dynamics of the monogenean pathogen *Zeuxapta seriolae* on juvenile fish from wild populations of *S. dumerili*. The study is based on bimonthly monitoring between April, 2005 and April, 2007 off Majorca, and newly obtained monogenean population data for juvenile fish from three additional localities in the Western Mediterranean (off Alicante, Corsica and Sardinia). We documented the highest intensities and abundances of *Z. seriolae*, with mean abundance values similar to or higher than those reported in the single case of wild fish mortalities reported to date. There was a recurrent pattern of seasonal change in infection with *Z. seriolae* in the populations of *S. dumerili* off Majorca, with substantially higher parasite loads during the warm season (April to June). Mean parasite abundance was significantly correlated with seawater temperature and associated with higher proportions of juvenile worms in the parasite populations, thus suggesting increased transmission rates at higher temperatures. There was a significant negative association between abundance of *Z. seriolae* and fish length. Comparisons with the samples of younger and older fish off Majorca indicated that whereas infection parameters gradually increased in the first year of juvenile fish life, larger/older fish (> 43 cm; 1+) were much lightly infected than the smaller/younger (<30 cm; 0+) juvenile fish examined in the same season. The observed increases in abundance during the warm weather months were invariably associated with sharp increases in monogenean aggregation levels and this was in contrast with the markedly low levels for both parameters during the cold season months. These data, coupled with the strong negative correlation between the levels of aggregation of *Z. seriolae* and mean fish total length, indicate that heavily infected individuals are being rapidly removed (i.e. within 2-3 months) from the host population thus reducing the heterogeneity of parasite distribution as fish grow. We discuss parasite-induced host mortality and other mechanisms that may account for observed recurrent patterns in monogenean abundance and spatial aggregation and review the data available on the spread of *Z. seriolae* infections in the Mediterranean.

Keywords:

Zeuxapta seriolae, *Seriola dumerili*, monogenea, population dynamics, Western Mediterranean

6.1. Introduction

The greater amberjack, *Seriola dumerili* (Risso) (Perciformes: Carangidae), is a pelagic fish with a worldwide distribution along temperate and tropical areas, living in inshore waters of the continental shelf and over the continental slope (Smith-Vaniz, 1986). This fish species, like the other amberjacks of the genus *Seriola* Cuvier, is much valued for consumption in many countries, especially in Spain and Japan, where it is appreciated and frequently consumed. The high market value and the fast growth rate of the greater amberjack have encouraged the development of its culture in worldwide temperate regions. However, attempts to develop experimental cultures in the Mediterranean have often failed due to parasite infections carried out by juvenile fish being captured from wild populations and reared in the aquaculture facilities (Crespo *et al.*, 1994; Montero *et al.*, 2001a; 2007).

Heteraxinid monogeneans (Polyopisthocotylea: Heteraxinidae) represent one of the main parasite groups causing disease outbreaks on cultured *Seriola* spp. and have been reported from Australia (Ernst *et al.*, 2002; Tubbs *et al.*, 2005; Hutson *et al.*, 2007b), New Zealand (Diggles & Hutson, 2005), Japan (Ogawa, 1996; Ogawa and Yokoyama, 1998; Hutson, 2007), Italy (Montero *et al.*, 2004), Malta (Nielsen *et al.*, 2003) and Spain (Grau *et al.*, 2003; Montero *et al.*, 2001a,b; 2004). Heteraxinid monogeneans have also been reported from wild fish from off New Zealand (Sharp *et al.*, 2003), Australia (Rohde, 1978, 1981; Hutson *et al.*, 2007b) and the Mediterranean [off Turkey (Genç *et al.* (2007) and Italy (Lia *et al.*, 2007)]. However, data on population dynamics of heteraxinid monogeneans parasitising *Seriola* spp. are lacking, and the available information mainly comprises reports on infection levels in cultured fish or isolated records of intensity and prevalence in wild fish populations. Although most studies provide seasonal reference for the observed infection levels, there is a lack of information on the seasonal and annual dynamics of the monogenean populations especially in the Mediterranean region. To date, three heteraxinid species have been recorded in *S. dumerili* in the Mediterranean: *Allencotyla mcintoshi* Price, 1962 (see Montero *et al.*, 2001a,b; Nielsen *et al.*, 2003; Repullés *et al.*, 2005); *Heteraxine heterocerca* (Goto, 1894) (see Grau *et al.*, 1999); and *Zeuxapta seriolae* (Meserve, 1938) (see Montero *et al.*, 2001a,b; 2004; Grau *et al.*, 2003; Genç *et al.*, 2007; Lia *et al.*, 2007).

These monogeneans utilise single host (monoxenous) life-cycles and infect fish directly and are, therefore, able to proliferate rapidly in either captive or wild *Seriola* spp. populations profiting from the high host densities in the aquaculture facilities and host's tendency to form temporary life-stage specific congregations (Riera *et al.*, 1999), respectively. Monogenean life-cycle is directly affected by seawater temperature, the population growth being promoted at elevated seawater temperatures (Whittington & Chisholm, 2008) *via* decrease in egg-hatching times and parasite ages

at maturity to decrease (Kearn, 1986; Tubbs *et al.*, 2005). These temperature-related effects on parasite population dynamics result in increased infection levels and epizootics in aquaculture during the warm weather periods (Ernst *et al.*, 2002; Tubbs *et al.*, 2005; Whittington & Chisholm, 2008).

A typical feature of the culture of *S. dumerili* in the Mediterranean, and of *Seriola* spp. in general, is its reliance on the collection of wild-caught juveniles (Nakada, 2000; Watanabe & Vassallo-Agius, 2003). However, juvenile fish may support abundant monogenean populations prior to their transfer for grow-out in aquaculture facilities, especially at elevated temperatures. This may represent a prerequisite for disease outbreaks that would jeopardize the future expansion of aquaculture of *S. dumerili*. Knowledge on the monogenean population dynamics and infection parameters may contribute to decreasing the risk of potential problems in aquaculture by means of identification of suitable geographical locations and periods with low population densities of *Z. seriolae* in wild juvenile fish populations of *S. dumerili*. The aim of this study was two-fold: (i) to analyse the seasonal and yearly dynamics of *Z. seriolae* populations in juveniles from wild populations of *S. dumerili* in the Western Mediterranean based on intensive sampling off Majorca; and (ii) to provide comparative parasite population data for juvenile fish from other localities in this area. We also review the data available on the spread of *Z. seriolae* infections in the Mediterranean after its first record in 1996.

6.2. Materials and methods

A total of 245 specimens of *S. dumerili* from the Western Mediterranean were examined between 2005 and 2007. One hundred and sixty-five specimens were collected from off Majorca (Balearic Islands) between Cala Figuera and Cabrera (39°80'-39°21'N; 2°42'-3°02'E). These fish, further referred to as 'main sample' represented juvenile fish [young-of-the-year and 1+; total length (TL) ranging between 33-42 cm; age estimation after Kozul *et al.*, (2001)]. The main sample comprised of 11 distinct subsamples of 15 specimens each, collected bimonthly between April, 2005 and April, 2007 (except for the fishing restriction period between July and September) from fish markets in Majorca (see Table 6.1 for details). The main sample was used to determine the relationships between fish size and parasite abundance, fish condition and mean seawater temperature and to assess the seasonal and annual variations in population density of *Z. seriolae*. Two additional samples of fish with different size (Table 6.1) were collected from off Majorca: (i) 10 small juveniles (TL < 30 cm, further referred to as 'smaller fish' sample); and (ii) 10 large juveniles (TL > 43 cm, further referred to as 'larger fish' sample). Comparisons between these and the main

sample were carried out using infection levels of distinct subsamples collected during the same (or the nearest) month.

Four comparative samples of 15 fish each were collected and examined from three different localities in the Western Mediterranean (Table 6.2): two samples from off Alicante (Spain; 38°11'N, 0°33'W); one sample from off Corsica (France; 41°55'N, 8°44'W); and one sample from off Sardinia (Italy; 39°12'N, 9°60'W). A global comparison of the abundance of *Z. seriolae* in these samples with the main sample from off Majorca was carried out using pooled subsamples of the latter (collected during the cold and the warm season). Fish were measured, weighed and labelled. Fulton's condition factor ($K=[\text{weight (g)}/\text{length (cm)}^3] \times 100$; Anderson & Neumann, 1996) was calculated for each fish. Four fish of each sample were analysed fresh and the rest were kept frozen and examined within the following days. Gill arches were isolated for examination under the stereomicroscope at up to 100× magnification. All monogeneans were identified and counted and representative samples from each fish were fixed in hot 70% ethanol for morphological analysis; these were stained with iron acetocarmine, dehydrated and mounted in Canada balsam.

Prevalence, mean and median abundance and intensity were calculated as defined by Bush *et al.* (1997). Data on abundance of *Z. seriolae* were ln (x+1) transformed and those on fish total length (TL) and condition factor (K) were ln-transformed prior to General Linear Model (GLM) analyses. To account for the significant correlation detected between fish size and parasite abundance and fish condition factor (see Section 3), the residuals from the regressions of the latter parameters vs host size (TL) were used as dependent variables in the ANOVA analyses. Seasonal and yearly effects on size-corrected parasite abundance were assessed in a factorial ANOVA. Coding for factor 'season' included two categories, cold (October-February) and warm (April-September) weather months. Prevalences were compared with Fisher's exact test. Spearman's rho (r_s) was used to test for significant correlations between estimates of abundance and minimum, maximum and mean monthly seawater temperatures off Majorca (data obtained from the State Ports System of the Ministry of Development, Spanish Government). All analyses were carried out with Statistica 8.0 (StatSoft, Inc., Tulsa, OK, USA).

6.3. Results

Infected fish examined from off Majorca only harboured *Z. seriolae* on gills. A total of 25,831 monogenean individuals were counted on the gills of 149 fish of the main sample (overall prevalence of 90.3%). Parasite intensity ranged from 1 to 1,182 (mean intensity ± standard deviation, SD = 173.36 ± 261.13; mean abundance ± SD = 156.55 ± 253.34). However, in spite the fish was sampled within a narrow size range (33.5-42 cm; mean TL ± SD = 37.8 ± 2.3 cm), there

were significant differences in fish size among the subsamples (ANOVA, $F_{(10, 154)}$=15.13; p<0.0001). Generally, fish sampled during the cold season of 2005 (October-February) were larger whereas those sampled in April of all three years were smaller (Tukey HSD post-hoc test, Table 6.1). Fish condition factor K also varied significantly among subsamples (ANOVA, $F_{(10, 154)}$=3.65; p=0.0002) due to K-values for fish sampled in October, 2005 being significantly higher than those for fish sampled in April of 2005 and 2006 (Tukey HSD post-hoc test, Table 6.1). However, there was a significant negative association between fish total length and the levels of K ($F_{(1, 163)}$=14.84; p=0.0002; Beta=-0.289), larger fish having generally lower parasite abundance and K-values. After accounting for fish size none of the differences in K between samples remained significant. Both infected and uninfected fish showed external healthy general appearance and colour. However, heavily parasitised fish examined during the warm season had pale gills and liver; gill mucus hyper secretion was also observed in the latter.

Levels of infection of *S. dumerili* with *Z. seriolae* studied bimonthly off Majorca between April, 2005 and April, 2007 are shown in Table 6.1. Prevalence was similar and high in all subsamples (range 73.3-100%) and this resulted in fairly close values for the mean abundance and mean intensity (note almost identical values for the medians in Table 6.1). The highest infection levels were observed in fish sampled during the warm season (April-June) in all three years of study. Most of the specimens of *Z. seriolae* found in the winter samples (December-February) represented adults with no eggs *in utero*. From the beginning of spring (April), the number of gravid adults progressively increased and juveniles showed increased prevalence and abundance until reaching maximum numbers c. 65% of all specimens) in summer (June). During autumn (October-November), the abundance of both juvenile and adult worms gradually decreased until reaching the low winter levels.

There was a significant negative association between fish total length and the abundance of *Z. seriolae* ($F_{(1, 163)}$=23.58; p<0.0001; Beta=-0.355). Using the residuals of the regression of parasite abundance vs fish total length, we found significant differences between the subsamples (ANOVA R^2=0.85, $F_{(10, 154)}$=41.23, p<0.0001) that revealed a clear seasonal dynamics of infection with *Z. seriolae*. Infection levels increased significantly in the warm season (between April and June; range 1-1,182; 283.30 ± 287.24 on average) as opposed to the distinctly lower levels in the cold season (between October and February; range 1-21 parasites; 4.45 ± 4.94 worms per fish on average) (Fig. 6.1). Increased infection levels coincided with extremely high levels of parasite aggregation in the samples examined in the warm season (range for variance-to-mean ratio 25 to 289; this ratio fell down to 3-9 in the samples collected during the cold season) (Fig. 6.2).

Table 6.1.
Infection levels of *Zeuxapta seriolae* in *Seriola dumerili* from Majorca (Balearic Islands) and data on fish length (TL) and condition factor (K) and the temperature of seawater at the month of sampling (T°C).

	Seawater temperature (°C)	**Fish length (TL, cm)**	**Fish K**	**Prevalence (%)**	**Parasite abundance**		**Parasite intensity**		
	Min-Max	Range (Mean ± SD)	Range (Mean ± SD)		Mean ± SD	Median	Range	Mean ± SD	Median
April 05	12.0 - 16.5	33.5-36.0 (35.0 ± 0.9)	0.91 - 1.18 (1.04 ± 0.08)	100	628.87 ± 262.04	618	99 -1,076	628.87 ± 262.04	618
June 05	20.0 - 25.0	34.5 - 40.5 (37.9 ± 2.4)	0.84 - 1.17) (1.09 ± 0.09	100	455.80±363.20	400	5 - 1,182	455.80 ± 363.20	400
October 05	18.3 - 23.9	37.5 - 42.0 (40.4 ± 1.4)	1.11 - 1.40 (1.23 ± 0.09)	93.3	3.93±4.13	3	1 - 17	4.21 ± 4.14	3
December 05	9.5 - 18.4	36.0 - 41.5 (38.5 ± 2.2)	0.99 - 1.37 (1.18 ± 0.11)	86.7	5.60 ± 5.54	5	1 - 20	6.46 ± 5.46	5
February 06	9.6 - 15.9	38.0 - 42.0 (40.4 ± 1.3)	0.98 - 1.24 (1.09 ± 0.07)	73.3	3.40 ± 3.13	3	1 - 9	4.64 ± 2.73	5
April 06	12.6 - 21.4	34.0 - 38.0 (35.9 ± 1.2)	0.88 - 1.47 (1.07 ± 0.14)	100	247.47 ± 134.93	200	79 - 588	247.47 ± 134.93	200
June 06	17.6 - 27.0	36.0 - 42.0 (38.8 ± 2.1)	0.95 - 1.35 (1.12 ± 0.10)	100	273.47 ± 137.47	234	88 - 532	273.47 ± 137.47	234
September 06	17.3 - 23.3	36.0 - 41.5 (38.4 ± 2.2)	0.91 - 1.35 (1.14 ± 0.13)	73.3	5.47 ± 6.09	3	1 - 21	7.45 ± 5.97	7
November 06	15.0 - 17.7	36.0 - 38.5 (37.2 ± 1.2)	0.96 - 1.35 (1.12 ± 0.11)	80.0	3.53 ± 3.60	2	1 - 11	4.42 ± 3.50	3.5
February 07	10.4 - 18.6	35.0 - 39.5 (36.9 ± 1.9)	0.88 - 1.35 (1.09 ± 0.15)	86.7	5.80 ± 7.25	2	1 - 21	6.69 ± 7.41	2
April 07	12.8 - 20.8	35.0 - 38.5 (36.7 ± 1.2)	0.95 - 1.35 (1.10 ± 0.10)	100	88.73 ± 47.06	98	1 - 179	88.73 ± 47.06	98
Smaller fish (September 05)	20.2 - 26.5	26.5 - 29.5 (27.9 ± 1.2)	-	50.0	1.10 ± 1.60	0.5	1 - 5	2.20 ± 1.64	2
Larger fish (July 06)	24.0 - 28.7	43.5 - 48.5 (45.5 ± 1.9)	-	70.0	7.40 ± 5.20	9	6 - 12	10.57 ± 2.44	12

Table 6.2.
Summary infection data for *Zeuxapta seriolae* in *Seriola dumerili* sampled at three localities in the Western Mediterranean.

Locality	**Sample date**	**Fish length (TL, cm)**	**Fish K**	**Prevalence (%)**	**Parasite abundance**		**Parasite intensity**		
		Range (Mean ± SD)	**Range (Mean ± SD)**		**Mean ± SD**	**Median**	**Range**	**Mean ± SD**	**Median**
Alicante	November 05	37.5 - 41.0 (39.2 ± 1.3)	1.15 - 1.65 (1.30 ± 0.11)	80	8.47 ± 7.94	7	1 - 22	10.58 ± 7.48	8
Alicante	February 06	38.0-42.0 (40.1 ± 1.3)	1.07 - 1.42 (1.24 ± 0.08)	87	15.47 ± 13.61	15	1 - 49	17.85 ± 13.04	15
Sardinia	July 06	33.0 - 37.5 (34.7 ± 1.3)	0.99 - 1.22 (1.12 ± 0.06)	80	10.40 ± 9.64	7.5	3 - 30	13.00 ± 9.02	10
Corsica	August 06	37.5 - 45.0 (39.7 ± 2.2)	-	100	90.47 ± 63.34	89	5 - 254	90.47 ± 63.34	89

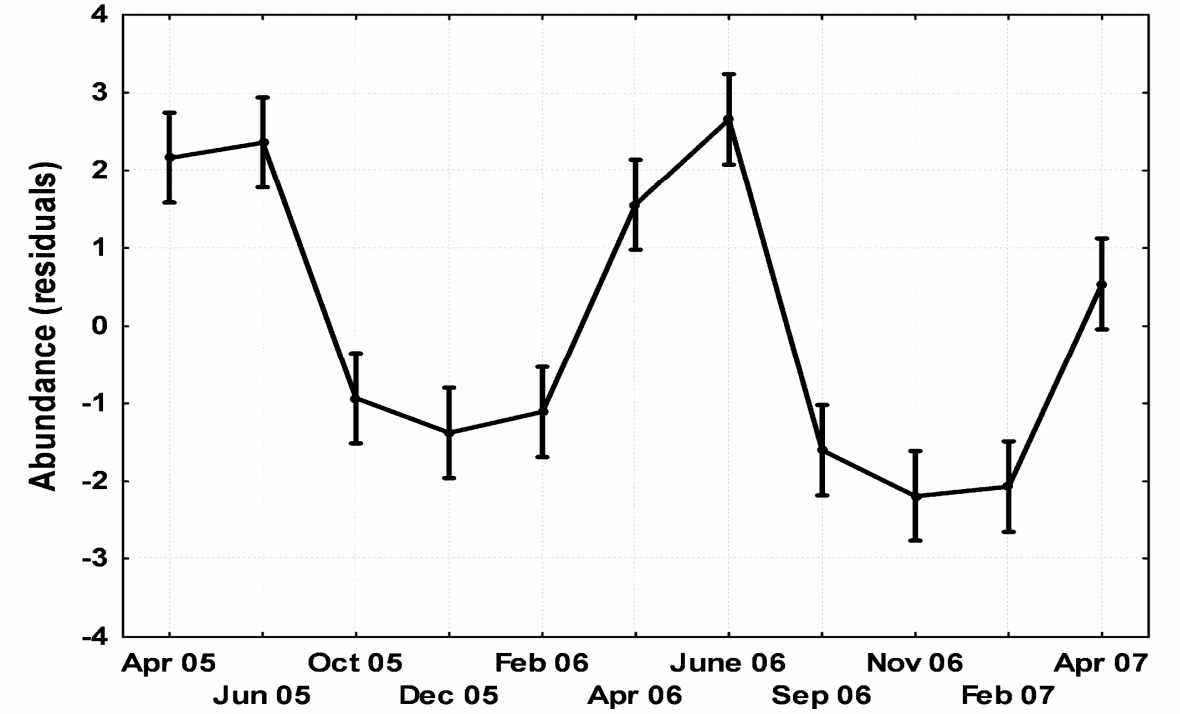

Figure. 6.1. Seasonal dynamics of abundance of *Zeuxapta seriolae* on juvenile *Seriola dumerili* studied off Majorca between April, 2005 and April, 2007. Plotted are means per month of the size-corrected residuals obtained from a regression of raw monogenean abundance against fish total length.

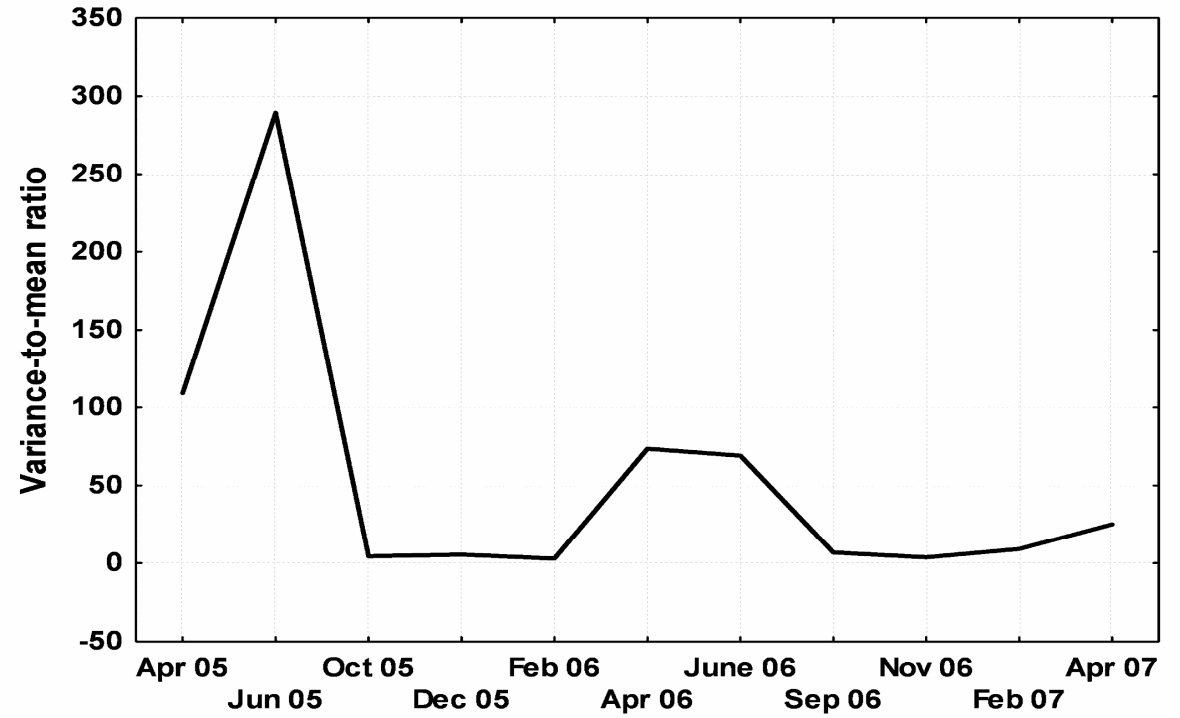

Figure 6.2. Patterns of aggregation of *Zeuxapta seriolae* on juvenile *Seriola dumerili* studied off Majorca between April, 2005 and April, 2007. Spatial aggregation is assessed by the variance-to-mean abundance ratio in the bimonthly samples.

We found a strong negative but marginally significant correlation between the levels of aggregation of *Z. seriolae* and mean fish total length (r_s = -0.601; p=0.05). The estimates of abundance of *Z. seriolae* exhibited a significant correlation with the mean and maximum monthly seawater temperatures (r_s = 0.306 and 0.382, respectively; p<0.01). Finally, there was a trend of decrease in monogenean abundance from 2005 to 2007 (Table 6.1) and ANOVA carried out on size-corrected data revealed highly significant effects of both season ($F_{(1, 161)}$=147.38, p<0.0001) and year ($F_{(2, 161)}$=10.25, p<0.0001) on parasite abundance (Fig. 6.3). 'Smaller' fish sampled off Majorca in September, 2005 exhibited distinctly lower levels of infection than the fish from the main sample (Table 6.1). A comparison with a subsample collected in the same month (September, 2006) revealed significant difference in abundance (ANOVA $F_{(1, 23)}$=4.90, p=0.037). Similarly, 'larger' fish sampled off Majorca in July 2006 was much lightly infected than the fish from the subsamples of the main sample collected in June, 2005 and 2006 (ANOVA $F_{(2, 37)}$=42.18, p<0.0001; Table 6.1).

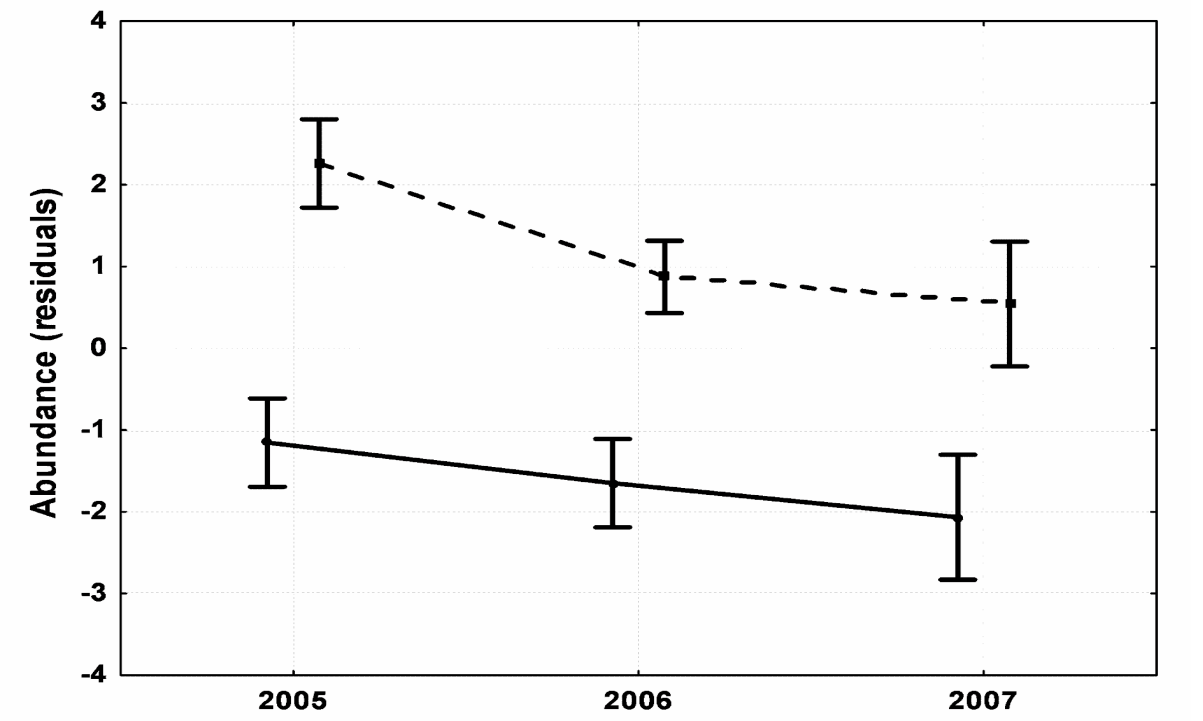

Figure 6.3. Analysis of variance interaction plot showing aggregate differences in mean abundance of *Zeuxapta seriolae* on *Seriola dumerili* studied off Majorca among warm and cold seasons and between years. Data are size-corrected residuals obtained from a regression of raw monogenean abundance against fish total length. Separate plots based on data from the warm (uninterrupted line) and cold (interrupted line) season.

Levels of infection with *Z. seriolae* in the fish examined from three additional localities in the Western Mediterranean (off Alicante, Sardinia and Corsica) are shown in Table 6.2. A comparison of these samples with the two pooled samples of fish collected off Majorca during the cold and warm season, respectively, revealed highly significant overall differences (ANOVA $F_{(5, 219)}$=51.23, p<0.0001). Fish collected in the warm season from off Majorca and Corsica exhibited

the highest infection levels compared with those observed in fish of the cold season samples from off Majorca and Alicante and the sample from off Sardinia collected in July 2006 (Fig. 6.4, Table 6.2).

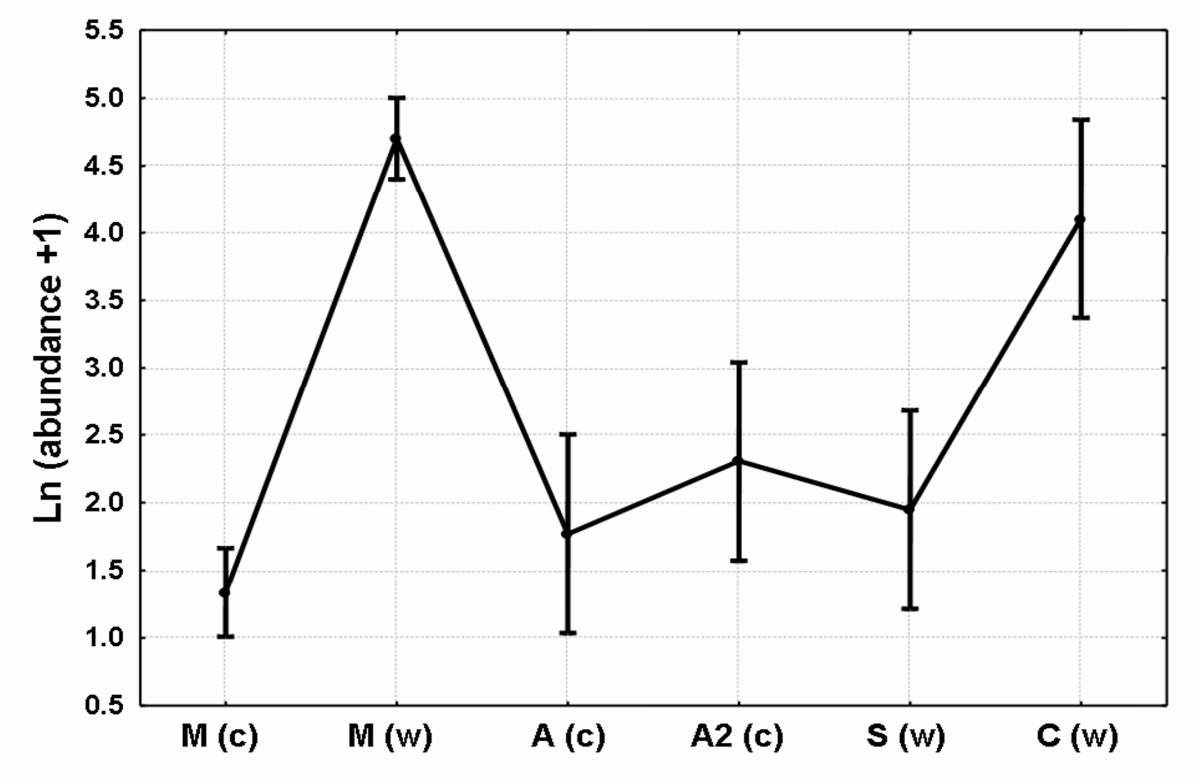

Figure 6.4. Geographical variation in abundance of *Zeuxapta seriolae* on *Seriola dumerili* studied at four localities in the Western Mediterranean. Plotted are sample means. Abbreviations for samples: M, Majorca; A, Alicante; S, Sardinia; C, Corsica; c, cold season; w, warm season.

6.4. Discussion

To the best of our knowledge, the present study reports the highest intensities and abundances of *Z. seriolae* recorded in wild or cultured juvenile fish. Although *Z. seriolae* is a species with a worldwide distribution, massive infections with this and/or other heteraxinid monogeneans in *S. dumerili* have predominantly been recorded in aquaculture conditions in the Mediterranean Sea (Grau *et al.*, 2003; Repullés *et al.*, 2005) and other regions with Mediterranean and temperate climate i.e. Australia, New Zealand, Chile, Japan (Ogawa & Yokoyama, 1998; Wilson *et al.*, 2001; Sharp *et al.*, 2003; Hutson, 2007; Hutson *et al.*, 2007b; Whittington & Chisholm, 2008). This can be explained by the fact that amberjack cultures have been more intensive in these regions. Recently, Lia *et al.* (2007) reported fish mortalities in a wild Mediterranean population of *S. dumerili* (Gulf of Taranto, Ionian Sea, off Italy) associated with high levels of infection with *Z. seriolae*. Infection levels observed by us in the main sample off Majorca were similar to or higher than those

reported in epizootics of this parasite in cultured *S. dumerili* of comparable size in other Mediterranean localities (Montero *et al.*, 2004) or in cultured *S. lalandi* (see Mansell *et al.*, 2005). The mean abundance values observed in *S. dumerili* studied in April and June, 2005 in particular, were similar to or higher than those reported in the single case of wild fish mortalities documented to date (mean abundance range 455.8-628.9 vs 476.3) the maximum intensity reaching almost two-fold higher levels (1,182 vs 640 parasites/fish; see Lia *et al.*, 2007).

One important result of our relatively long-term population study is the recurrent pattern of seasonal change in infection with *Z. seriolae* in the populations of *S. dumerili* off Majorca, with substantially higher parasite loads during the warm season (April to June). These were consistent, in spite of the significant annual variation, and significantly correlated with the increases in mean and maximum seawater temperatures and associated with higher proportions of juvenile worms in the parasite populations, thus suggesting increased transmission rates at higher temperatures. Temperature is perhaps the most important abiotic factor with measurable effects on all aspects of the monogenean life-cycle that produce seasonal patterns of occurrence and abundance of monogenean populations in both natural and aquaculture conditions (Chubb, 1977; Kearn, 1986; Gannicott & Tinsley, 1997, 1998a,b; Tubbs *et al.*, 2005 and references therein). In a laboratory experiment Tubbs *et al.* (2005) have shown that the rates of egg production, egg-hatching, growth and maturation of *Z. seriolae* are temperature-dependent, being significantly lower at low environmental temperatures (13-18^{o}C compared with 21^{o}C).

Our first record on seasonal change in levels of parasitism in wild populations of *S. dumerili* is therefore, in agreement with both the constrains on transmission revealed in this laboratory-based study and the fact that epizootics of *Z. seriolae* are typically associated with increasing seawater temperatures at the onset of summer (Ernst *et al.*, 2002; Tubbs *et al.*, 2005). The data obtained from the three additional localities in the Western Mediterranean (off Alicante, Sardinia and Corsica) generally agree with the seasonal pattern of abundance of *Z. seriolae* observed off Majorca, the only exception being the low parasite loads in the population sampled off Sardinia in July, 2006. These were in contrast to the high infection levels in the closely located populations of *S. dumerili* sampled in August of the same year off Corsica. These results indicate that the geographical variation of the distribution and abundance of *Z. seriolae* on *S. dumerili* in the Mediterranean requires further exploration.

Knowledge on the geography of infection levels may help identify suitable geographical locations with low population densities of *Z. seriolae* in wild juvenile fish populations of *S. dumerili* and thus contribute to decreasing the risk of potential problems in aquaculture. Previous studies on fish assemblages around FADs (floating aggregating devices) in three Mediterranean localities,

Majorca, Sardinia and Sicily, reported that geographic differences were highly significant for young of the year greater amberjacks (Addis *et al.*, 2006). Dissimilarity in community structure around FADs was highest between Majorca and Sardinia, especially during summer and autumn. In accordance with the authors, temperature could be an important factor determining the temporal and spatial variations of fish assemblages in FADs, mainly between Majorca and Sardinia (Addis et al., 2006). Moreover, different parasite abundances among Corsica and Sardinia could also be partially related to the effects of temperature on fish, as specimens from Corsica were collected in August, when water temperatures are slightly higher than in July.

Unfortunately, *Z. seriolae* infection levels in wild adult *S. dumerili* are unknown. Adult fish are known to be migratory and, in Majorca, they only approach to the coast during the spawning period from late spring to summer (May to July) (Lazzari and Barbera 1988; Grau 1992). During the rest of the year they are only sporadically captured, thus explaining the absence of parasitological records. Studies on adult migration routes and stability of their local populations are lacking. However, juveniles have been more studied as they are known to be associated to floating objects during their first months of life (Andarolo et al., 2005). Thereafter, they migrate to reef areas where they find a better food supply (Andarolo et al., 2005; Sinopoli et al., 2007). Thus, young fish seem to temporally remain in the same region. Moreover, Repullés-Albelda et al., (2008) also suggested isolation of juvenile fish populations from Majorca based on the finding of an endemic and specialist parasite species, *Paradeontacylix balearicus*.

Traditionally, pathological effects of polyopisthocotylean monogeneans on wild fish have been considered mild as high parasite loads are not frequent (Whittington & Chisholm, 2008) and this is in contrast with the evidence accumulated recently (predominantly in aquaculture conditions) that polyopisthocotylean monogeneans induce serious gill damage; they have been also implicated in anaemia-induced host mortality (e.g. Rubio-Godoy & Tinsley, 2008 and references therein). The fish examined at high abundance situations (warm season) exhibited typical effects induced by *Z. seriolae* and other monogeneans in severe infections such as gill and liver paleness suggesting anaemia, and hyper secretion of gill mucus (Montero *et al.*, 2004; Mansell *et al.*, 2005; Whittington & Chisholm, 2008). We found no statistically significant effect of parasite infection on Fulton's condition factor K and fish external appearance was healthy, and this is in agreement with previous observations in epizootics of cultured *Seriola* spp. (Montero *et al.*, 2004; Mansell *et al.*, 2005). It is worth noting that Montero *et al.* (2004) reported that infected fish, which appeared to be healthy, died very quickly and suggested that outbreaks occur probably too fast to affect fish condition factor.

Although mortalities in wild fish populations are almost impossible to detect (but see Lia *et al.*, 2007), three lines of evidence obtained in our study indicate a possibility that parasite-induced host mortality might take place in juvenile populations of *S. dumerili* off Majorca. First, there was an overall significant negative association between the distributions of abundance of *Z. seriolae* and fish length. This is in contrast with the typical pattern of population increase with host size observed in various wild fish-monogenean associations (Frankland, 1954; Paperna *et al.*, 1984; Rohde *et al.*, 1995; Buchmann, 1989; Grutter, 1998). Comparisons with the samples of 'smaller' and 'larger' fish off Majorca indicated that whereas infection parameters gradually increased in the first year of juvenile fish life, larger/older fish (> 43 cm; 1+) were much lightly infected than the smaller/younger (<30 cm; 0+) juvenile fish examined in the same season. These size-associated abundance patterns suggest that the majority of the population abundance of *Z. seriolae* is supported by 0+ juvenile fish populations. Secondly, the observed increases in abundance during the warm weather months were invariably associated with sharp increases in monogenean aggregation levels and this was in contrast with the markedly low levels for both parameters during the cold season months. Thirdly, the strong negative correlation between the levels of aggregation of *Z. seriolae* and mean fish total length indicates that heavily infected individuals are being rapidly removed (i.e. within 2-3 months) from the host population thus reducing the heterogeneity of parasite distribution as fish grow. The distinctly lower levels of infection observed in the sample of 'larger' (older) fish off Majorca also support this suggestion.

Two other mechanisms may have also contributed to the observed recurrent patterns in monogenean abundance and spatial aggregation: parasite mortality due to host immunity and seasonality in parasite transmission. Unfortunately, studies addressing the question whether *S. dumerili* parasitised by *Z. seriolae* develop acquired immunity have not been carried out. However, Leef & Lee (2009) have shown experimentally that infection status of the host had no effect on survival of *Z. seriolae* in either serum or mucus of *S. lalandi* thus indicating that the blood-feeding *Z. seriolae* may tolerate various immune parameters expressed within the serum of the host. The temperature dependency of the monogenean life-cycle in general, and that of *Z. seriolae* in particular, indicate that seasonal effects on parasite transmission cannot be ruled out as evidenced by the increased proportions of gravid adults and juveniles in the populations of *Z. seriolae* during the warm season months. The three mechanisms may act synergistically to produce the patterns of infection and parasite aggregation observed in the wild juvenile fish populations off Majorca.

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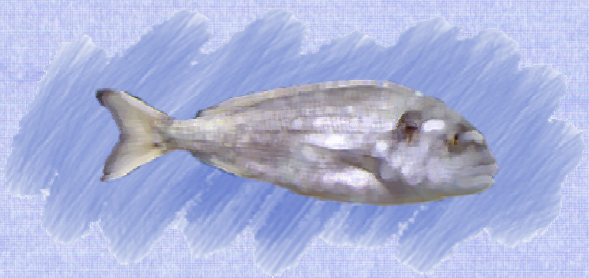
CHAPTER 7.

Oncomiracidial development, survival and swimming behavior of the monogenean *Sparicotyle chrysophrii* (Van Beneden et Hesse, 1863).



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Oncomiracidial development, survival and swimming behaviour of the monogenean *Sparicotyle chrysophrii* (Van Beneden and Hesse, 1863)

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ABSTRACT

Oncomiracidial development of *Sparicotyle chrysophrii*, a monogenean parasite of *Sparus aurata*, was analysed using 450 eggs. Parasite morphological changes in time, data on hatching success, as well as oncomiracidial survival and swimming behaviour were recorded. Eggs were maintained at 20 °C and exposed to LD 12:12. They were observed under the stereomicroscope every 8 h until they hatched. Thereafter, 155 oncomiracidia were isolated in separate wells and observed every 2 h until their death. Most of the hatchings occurred in a short period of time (approximately 24 h). Hatching success was 87% and the hatching period ranged from 5 to 10 days after deposition, with most of the hatchings occurring during darkness (>75%). Oncomiracidial survival time ranged from 2 to 52 h, although only 10% of the oncomiracidia lived more than 24 h. No significant Spearman's correlation was found between incubation and oncomiracidial survival times. Survival times of the oncomiracidia emerging during darkness period were significantly higher than survival times of those emerging during light. Seven oncomiracidial behaviour patterns were recorded: vertical swimming (fast/slow), horizontal swimming close to the bottom (fast/slow), continuous whirling, alive without ciliary movement and dead. A highly significant correlation was found between survival and swimming times ($r = 0.911$, p-value = 0.000). The mean swimming speed of oncomiracidia was 3.97 mm/s ($n = 10$). The complete life cycle of *S. chrysophrii* (from egg to egg) at 20 °C lasts approximately 50 days: 5–10 days of incubation, 2–3 days of oncomiracidial survival time and 36 days of worm maturation. Treatment involving two applications, first for adults and juveniles and later (after 12 to 15 days) for larvae, would allow long periods without monogenean infection.

1. Introduction

The gilthead seabream (*Sparus aurata* L.) is one of the main species in Mediterranean aquaculture, with Greece, Turkey, Spain, and Italy being the major producers (APROMAR, 2011). The culture of this species has experienced an extraordinary growth during the last 10 years. In fact, in Spain cultures provide 95% of consumed gilthead seabream (APROMAR, 2011). Culture conditions are often associated with the emergence of parasitic diseases, especially those caused by pathogens with direct life cycles such as monogeneans (Nowak, 2007). High densities in cultures promote increasing parasite loads and multiply the pathological effects of parasites, leading finally to fish death (Buchmann and Bresciani, 2006). In the case of the gilthead seabream, one of the most harmful pathogens is the microcotylid monogenean *Sparicotyle chrysophrii* (Van Beneden and Hesse, 1863) which has been frequently reported in the Mediterranean area (Álvarez-Pellitero, 2004; Athanassopoulou et al., 2005; Sanz, 1992; Sitjà-Bobadilla et al., 2009). Infections by *S. chrysophrii*, within the "winter syndrome" (Tort et al., 1998) are responsible for the main economical losses in Mediterranean gilthead seabream cultures. These infections are recurrent and epizootic episodes are frequently reported, mostly during spring and summer (Faisal and Imam, 1990; Reversat et al., 1992; Sitjà-Bobadilla and Alvarez-Pellitero, 2009; Sitjà-Bobadilla et al., 2009).

Two parasite stages are responsible for monogenean transmission, eggs (passively) and oncomiracidia (actively), with the eggs being the most resistant to anthelmintic agents. Monogenean reproductive and developmental cycles have been reported to be coordinated with host behaviour (Kearn, 1986), and both are affected by environmental changes (Tubbs et al., 2005; Whittington and Chisholm, 2008). Despite their obvious importance for infection dynamics, these cycles have been poorly studied for most monogeneans. The current work deals with the study of the early stages of *S. chrysophrii*. Oncomiracidial developmental processes and patterns and their chronology were studied in vitro. Oncomiracidial survival and swimming behaviour were also studied. The aim of this study is to improve control of these infection epizootics, paying special attention to the most resistant stage, the egg.

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2. Material and methods

2.1. Oncomiracidial development and hatching

S. chrysophrii eggs were obtained from gravid worms collected from cultured gilthead seabream (*S. aurata*) (TL: 10.1–10.7 cm; weight: 14.6–15.7 g) off Sagunt, Spain (39° 40′ N 0° 14′ W). Worms were isolated from the gills using transmitted light. Gravid specimens of *S. chrysophrii* had been observed to release eggs when they were gently disturbed; thus, gravid worms were deposited in a Petri dish with sea water and energetically shaken in order to release the eggs. Thereafter, the eggs were separated randomly into groups of 15 to 25 eggs, each group being placed in a well, with 3 ml of seawater. The wells were maintained at 20 °C and exposed to alternating 12 h periods of fluorescent light and dark (LD 12:12), referred to below as light and dark periods. Water was renewed every day. Eggs were examined using a stereomicroscope and a research microscope with bright field and DIC optics.

In order to study the oncomiracidial development of *S. chrysophrii* within the egg, two preliminary replicates (R1 and R2) were developed obtaining the approximate timings of the main developmental events and two definitive replicates (R3 and R4) were performed with detailed observations at shorter periods of time (Table 1). "Time 0" was artificially established as the moment when the eggs were deposited. Time periods will be expressed below as hours post-deposition (hpd) or days post-deposition (dpd). The preliminary replicates (R1 and R2) were carried out with 250 eggs (see Table 1) released by 5 specimens of *S. chrysophrii* which were observed on a slide every 24 hpd until 5 dpd. Thereafter, eggs were observed every 6 h until the earliest egg hatching, as *S. chrysophrii* eggs are reported to hatch after 6 days at 22 °C according to Euzet and Noisy (1979). In the definitive replicates (R3 and R4), 200 eggs from 7 adult worms were observed on a slide every 8 h until 5 dpd (see Table 1). Thereafter, eggs were observed every 4 h until the earliest oncomiracidium had emerged. In all the replicates, eggs were observed every 2 h after the first hatching and the number of hatchings recorded during each period.

Oncomiracidial morphological changes were documented and hatching success was determined by counting eggs found empty with the operculum open. Number of oncomiracidia emerging during light and darkness was also recorded every 2 h. Differences between the number of oncomiracidia emerging during light and those emerging during darkness were statistically analysed using the Mann–Whitney test (M–W). Differences between number of hatchings recorded during each of the different light or dark periods were also analysed, using the Kruskal–Wallis test (K–W). These differences were tested for all the replicates together and for each replicate.

2.2. Oncomiracidial survival and swimming behaviour

Those oncomiracidia successfully emerging during the hatching studies were collected and maintained for survival and behavioural studies. In the course of the preliminary study (R1 and R2), most of the oncomiracidia (77%) became trapped in the water surface film and died there. This fact has also been reported in previous studies (Tubbs et al., 2005; Whittington and Kearn, 1988) and results in inexact estimates of oncomiracidial survival. To avoid this in the definitive replicates (R3 and R4), the study of oncomiracidial behaviour was conducted with each well completely full of water and covered with a coverslip to exclude air. As a result of this new design none of the oncomiracidia was lost in this way. Therefore, death on the surface film in R1 and R2 was considered accidental in the current study, and oncomiracidia from this replicates were not considered in the survival and swimming behaviour statistical analyses and figures. Only oncomiracidia that died on the bottom of the wells appear in the table (1/3 of the R2 and none in R1, see Table 1).

One hundred and fifty-five oncomiracidia were used for the survival and swimming behaviour studies. After hatching, specimens were collected with glass pipettes, distributed in separate wells with approximately 400 μl of seawater in each, and observed every 2 h using the stereomicroscope. These time periods will be expressed below as hours or days post-hatching (hph and dph). Data on survival time and swimming period for each specimen were recorded. The Spearman's rank-order correlation was calculated for incubation time and survival time or swimming period. Differences in survival times or swimming periods between replicates were analysed using the Kruskal–Wallis test. The same differences were analysed for those oncomiracidia emerging during light and those emerging during darkness (M–W), as well as for those emerging during each of the different light or dark periods (K–W test, Bonferroni-corrected). All these differences were also tested for each replicate individually.

Three types of categories were established to describe the behaviour of the oncomiracidia at each observation. Some categories (type 1) were related to swimming speed and vitality: i) fast swimming, ii) slow swimming, iii) cilia moving but not swimming, iv) alive but cilia not moving, v) dead. Others (type 2) were related to swimming trajectories: i) up and down (vertical), ii) only on bottom (horizontal), iii) only at top, and iv) "whirling" movement (slight spiralling on or close to the bottom). Finally, some categories (type 3) were related to ciliary activity: cilia of all ciliated cell groups moving (i) only anterior cilia moving (ii), only lateral cilia moving, (iii) or only posterior cilia moving (iv). To characterise the vitality of oncomiracidia, the categories previously recorded were grouped in 7 behaviour patterns (from 0 to 6, numbered from low to high activity): 0, dead oncomiracidia; 1, alive without cilia movement; 2, whirling or static but all or some ciliary movement; 3, slow swimming on the bottom; 4, fast swimming on the bottom; 5, swimming slowly up and down; 6, swimming fast up and down. The proportion of specimens in each category was calculated every 2 hph for all replicates together. Swimming speeds of 10 oncomiracidia with apparently normal swimming were calculated using Petri dishes of 5 cm diameter with a minimum seawater volume in order to minimise depth and, therefore, the vertical swimming. During these observations a drawing

Table 1
Egg hatching and oncomiracidia survival and swimming times in each replicate and total. Hpd: hours post-deposition; hph: hours post-hatching; Ht 50%: moment when the 50% of the eggs have hatched.

Replicate	EGG						Oncomiracidia							
	N	Hatching					N	Survival period			Vertical swimming period			
		Mean ± SD eggs/2 hpd	Ht 50% (hpd)	N (days + nights = total)	Range (hpd)	Success (%)		Mean ± SD (hph)	Median (hph)	Range (hph)	Mean ± SD (hph)	Median (hph)	Range (hph)	
R1	110	3.31 ± 5.28	152	(20 + 76 = 96)	(138–194)	87.27	0							
R2	140	7.65 ± 16.64	172	(16 + 114 = 130)	(164–196)	92.80	58[a]	13.83 ± 8.57[a]	12[a]	(4–50)[a]	7.41 ± 7.96[a]	6[a]	(0–44)[a]	
R3	70	6.85 ± 6.76	144	(9 + 39 = 48)	(124–168)	68.57	46	18.17 ± 11.67	17	(2–52)	12.35 ± 11.43	8	(0–46)	
R4	130	4.95 ± 6.81	178	(30 + 89 = 119)	(168–238)	91.53	109	9.37 ± 6.14	8	(2–26)	5.84 ± 5.78	4	(0–22)	
Total	450	5.17 ± 9.62	172	(75 + 318 = 393)	(124–238)	87.33	155[b]	11.9 ± 9.09[b]	10[b]	(2–52)[b]	7.68 ± 8.26[b]	6[b]	(0–46)[b]	

[a] Data not included in statistical analyses.
[b] R1 and R2 not included.

tube was used to draw the trajectory of oncomiracidia during two periods of 30 s. Statistical analyses were performed using SPSS® 15.0 (SPSS, Inc., 1989–2006).

3. Results

3.1. Oncomiracidial development

Oncomiracidia from all replicates showed a similar developmental pattern. Eggs of *S. chrysophrii* were released in bundles of at least 20 eggs. Each egg had two polar filaments, one short and hooked and other long and gradually thinner. The eggs were usually tangled together by their long polar filaments. At the beginning of the study (time 0), eggs were full of vitelline material, and the embryo could not be distinguished (Fig. 1A). Between 8 and 56 hpd (0 to 2 dpd), most of the vitelline material appeared to be progressively displaced to the egg poles. The embryo became visible in the middle of the egg, flanked by dispersed vitelline material (Fig. 1B–D). At the end of this period the shape of the embryo could be clearly distinguished: the anterior end was slightly dorsoventrally flattened, and the developing haptor was discernible, delimited by a small constriction at one third from the posterior end. The haptor was always located at the end of the egg bearing the hooked polar filament. Between 48 and 64 hpd (2 to 3 dpd), vitelline material was mostly arranged in two lateral, longitudinal and elongated masses. These masses were sometimes joined at the egg poles. Vitelline material decreased during the period of observation. The haptor sclerites became visible between 72 and 88 hpd (3 dpd) (Fig. 1E and F). The first sclerites to appear were the primordia of the hamuli and later the hooklets: first the posterior, then the posterolateral and finally the lateral ones. Simultaneously, at the anterior end, the cephalic adhesive glands were distinguishable (Fig. 1G and H). The terminal globule (Fig. 1F) and a group of laterally aligned ciliated cell primordia were also visible during this period. From 88 hpd cilia were observed (Fig. 1I and J). Ciliary activity was first observed as sequential oscillations in the anterior ciliated cell groups and later in the lateral and posterior ones. Between 88 and 100 hpd (3 to 4 dpd) eyespots were first visible as small and scattered accumulations of pigment which progressively aggregated to become well defined eyespots (Fig. 1G and I). From 120 hpd (5 dpd) the lateral hooklets were completely formed (Fig. 1I) and the pharynx could be distinguished, firstly with diffused margins and later acquiring its definitive shape. On the fifth day almost all egg opercula were differentiated at the end of the egg where the head of the larva was located, the remains of the vitelline material had been almost completely consumed and the oncomiracidia already showed strong mobility (Fig. 1K). No larval rotations within the eggs were observed. Before hatching, spasmodic and undulant oncomiracidium body movements were observed, together with the ciliary activity, until the operculum was opened and fell. Once the operculum opened, the oncomiracidium hatched in 1 to 2 min. The opercular opening was narrow and the oncomiracidium got out contracting the body and propelling with the cilia.

3.2. Hatching

The first oncomiracidium emerged at 124 hpd = 5 dpd (in R3, see Fig. 1L) and the last one at 238 hpd, i.e. after almost 10 dpd (R4) (Table 1, Fig. 2). Total hatching success for the four replicates was 87.3%, ranging from 68% (R3) to 93% (R2). The hatching period lasted from 32 (R2) to 70 (R4) hours (R1, 56 h; R3, 42 h). Most of the hatchings occurred during the first 24 h since first hatching (R2, 89%; R1, 83%; R4, 75%; and R3, 65%; Fig. 2) and more than 75% of them occurred during darkness in all the replicates with a maximum of 87% in R2 (Fig. 2). Dark periods 1 (26%) and 2 (44%) were those with the highest hatching numbers (Fig. 3). The first larvae emerged during a light period in replicates R1 and R2 while they first emerged during a dark period in replicates R3 and R4. Significant differences were found between total hatchings during periods of light and darkness (M–W: p-value = 0.020 < 0.050). Significant differences were also found between the number of hatchings each of the different dark periods (K–W: p-value = 0.001 < 0.005). However, no significant differences were found between the numbers of hatchings during each light period. In 3 replicates (R1, R2 and R4), most of the hatchings occurred during the first dark period while in R3 most hatchings occurred during the second dark period (Fig. 2). The only replicate where the number of hatchings during the first dark period was significantly different from those of the rest of the dark periods was R4, (K–W: p-value = 0.014 < 0.016). A large number of hatchings were observed to occur during the 4 h period after light changes (light to darkness or darkness to light).

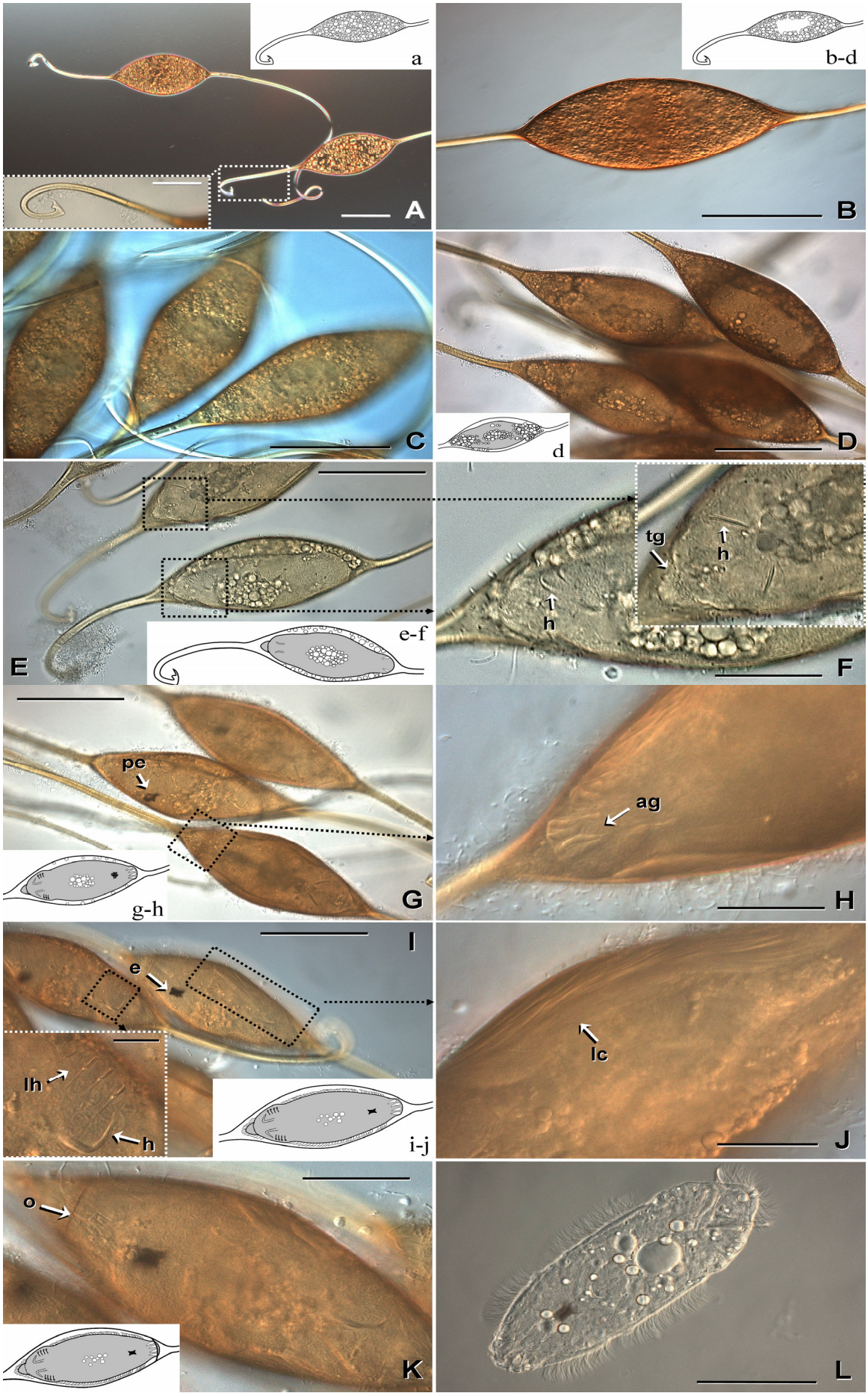
3.3. Oncomiracidial survival

As explained in the Material and Methods, during the preliminary replicates most of the oncomiracidia got trapped and died accidentally on the water surface film (all of them in R1 and two thirds of them in R2, see Table 1) and they could not be used for the survival and behavioural studies. The ranges of oncomiracidial survival time as well as mean survival time are indicated in Table 1. Percentage of surviving oncomiracidia is plotted against different time intervals in Fig. 4. Maximum survival time was 52 hph and mean survival time 11.9 hph, although only 3 out of 155 oncomiracidia lived more than 40 hph. Fifty-four percent of the oncomiracidia had died after 12 h and only 13% of them were alive after 24 h. Most oncomiracidia lived from 7 to 12 hph (28%) and from 0 to 6 h (26%), although survival times from 13 to 18 hph were also frequent (23%). No clear relation was observed between mean survival time and the incubation time (Fig. 5A) and no correlation was found between these two variables. Significant differences with regard to survival times were found between replicates (M–W: R3–R4, p-value = 0.000). Specimens with the longest survival time were found in R3.

Significant differences were found between survival times of individuals that had emerged during light or darkness (M–W: p-value = 0.001), with higher survival times amongst oncomiracidia that hatched during darkness (Fig. 6). These differences were also significant within individual replicates R3 (M–W: p-value = 0.027) and R4 (M–W: p-value = 0.006). In general, mean survival time was observed to decrease in the consecutive dark periods post-first-hatching (see Fig. 6). The same pattern was observed for light periods. Analysis of survival times for individuals emerging on different dark periods revealed significant differences (K–W: p-value = 0.001 < 0.005), with the first and second dark periods being those with higher oncomiracidial survival times. In contrast, no significant differences between survival times of individuals emerging on different light periods were found.

3.4. Oncomiracidial behaviour

Swimming patterns of the oncomiracidia during their active period were mainly of two types: vertical swimming in the water column (patterns 5 and 6), and horizontal swimming close to the bottom (patterns 3 and 4). Oncomiracidia could continuously change their speed between slow and fast. One hundred and forty-four oncomiracidia (93%) were able to swim vertically after emerging (see Fig. 7). After 6 hph approximately 53% of the oncomiracidia continued to swim vertically, but after 16 hph this declined to 11%. On average, oncomiracidia were able to swim vertically between 62% and 67% of their survival time. The longest vertical swimming period detected was 46 hph (almost 2 days, n = 1) and the average was of 7.7 hph. After vertical swimming, some oncomiracidia continued swimming close to the bottom (range, 0–50 h; mean, 8.6 ± 6.7 hph, including vertical swimming period) or showed a whirling movement.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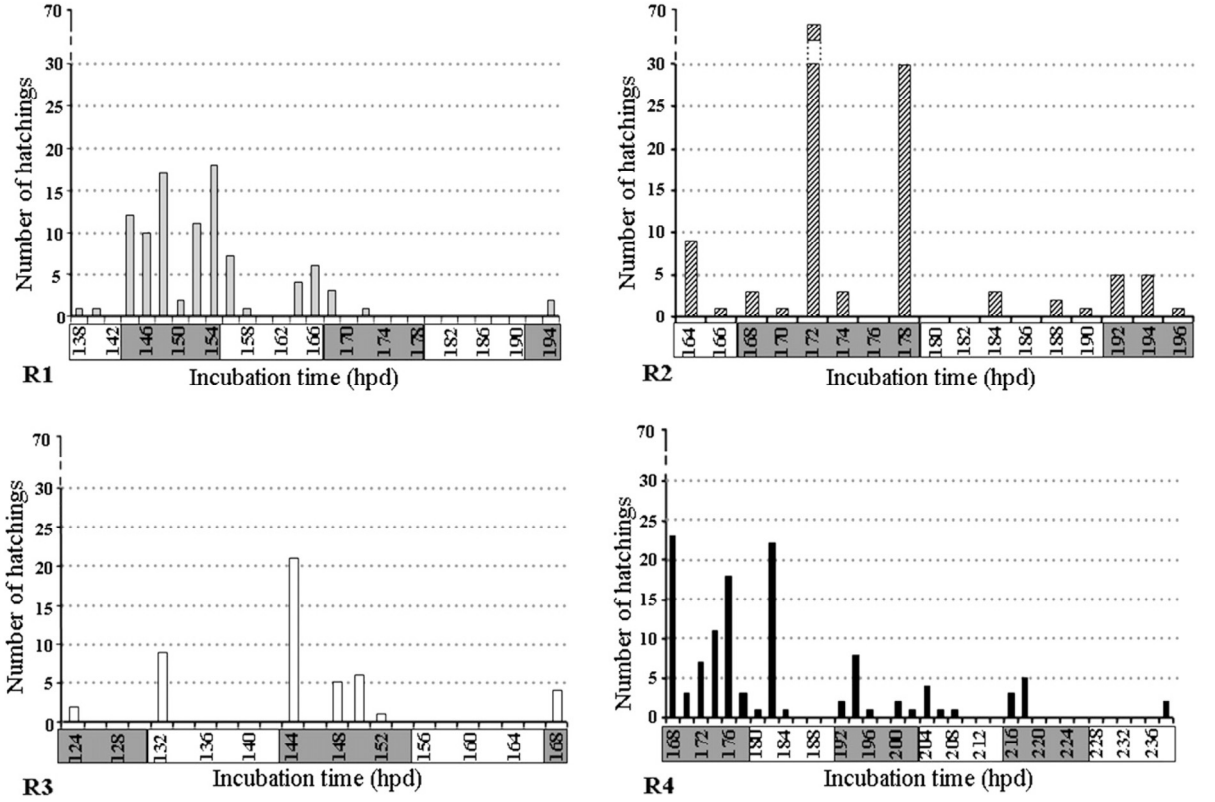

Fig. 2. Number of *Sparicotyle chrysophrii* oncomiracidial hatchings against time in hours post deposition (hpd) for the different replicates indicated as R+"number of replicate". Dark and light periods are shaded in grey and white respectively.

Oncomiracidia swimming on the bottom were rarely able to change to a vertical swimming. Whirling behaviour was more frequently observed at the end of oncomiracidial life. Before death, the ciliated cells lost their activity gradually and, consequently, larvae stopped swimming and remained static on the bottom. Mean swimming speed was 3.97 mm/s (n = 10). During the study of swimming speed, fast (pattern 4 or 6) and slow (pattern 3 or 5) swimmers were analysed separately. The fast swimming speed ranged from 3.37 to 4.56 mm/s (n = 5), and the slow swimming speed ranged from 1.97 to 3.21 mm/s (n = 5).

Similar patterns were observed for survival and swimming times in statistical analyses. A highly significant correlation was found between both variables (r = 0.911, p-value = 0.000). Mean swimming periods lasted approximately 3/4 of the mean survival time. No significant Spearman's correlation was found between incubation times and swimming periods (Fig. 5B). The proportion of individuals capable/not capable of swimming did not show any correlation with incubation time. Significant differences on swimming periods were found between R3 and R4 (M–W: R3–R4, p-value = 0.000). Significant differences were also found between swimming periods for those oncomiracidia emerging during light or darkness (M–W: p-value = 0.007); oncomiracidia emerging during darkness spent more time swimming (Fig. 6). These differences were not significant within each replicate. Moreover, no significant differences were found between oncomiracidia emerging on each light or dark period, although those oncomiracidia emerging during the first dark period post-hatching spent more time swimming (Fig. 6). Mean swimming period for oncomiracidia emerging during one particular dark period was always higher than the mean for those oncomiracidia emerging during the following light period (Fig. 6). No significant differences were found between swimming periods of those oncomiracidia emerging on different light periods throughout the study.

4. Discussion

The main events occurring during the oncomiracidial development of *S. chrysophrii* followed a pattern similar to those described for other monogeneans. The first visible structures are usually hook primordia, later eyespots, and finally cilia and opercula, which usually become visible just one day before hatching (Bondad-Reantaso et al., 1995). Similar to our results, eyespots were first observed at 4 dpd in *Neobenedenia girellae* (see Bondad-Reantaso et al., 1995), and at between 5 and 8 dpd in *Polylabroides multispinosus* (see Roubal and Diggles, 1993), both at 20 °C. Unfortunately, comparative information on the timing of oncomiracidial developmental events is scarce, especially in polyopisthocotyleans and many studies reported that the sequence of timing can vary in relation with temperature (Bondad-Reantaso et al., 1995; Roubal and Diggles, 1993).

Fig. 1. Oncomiracidial development of *Sparicotyle chrysophrii*. A, Recently deposited eggs with a detail of the posterior curved-hooked polar filament. B, Egg after 8 h. C, Eggs after 24 h. D, Eggs after 48 h. E and F, images of eggs after 72 h: E, Whole embryos; F, Details of primordia of hamuli in lateral and dorsal views. G and H, images of eggs after 80 h: G, Whole embryos with primordial eyespots visible; H, Detail of primordia of anterior glands. I and J, images of eggs after 104 h: I, Whole embryos with well defined eyespots and detail of the lateral hooklets and hamulus; J, Detail of lateral cilia. K, Operculated eggs after 120 h. L, Oncomiracidia recently emerged. a–k, Diagrammatic representations of oncomiracidial developmental stages, low letters correspond to the capital letters of the photographs. Abbreviations: ag, anterior glands; e, eyespot; h, hamulus; lc, lateral cilia; lh, lateral hooklets; o, operculum; pe, primordial eyespot; tg, terminal globule. Scale bars represent 50 μm except in figures F, H, J and K where 25 μm are represented.

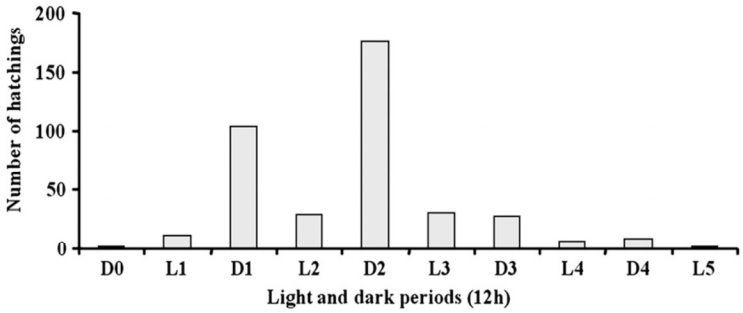

Fig. 3. Total number of *Sparicotyle chrysophrii* hatchings against light and dark periods (12 h). Light and dark periods indicated as "L" and "D" + "number of the period".

The first hatchings within the different replicates in the current study were recorded between 5 and 10 dpd at 20 °C, which agrees with previously published results on the same species, 6 dpd at 22 °C (Euzet and Noisy, 1979). Interestingly, Sitjà-Bobadilla et al. (2006) reported an even longer incubation time for *S. chrysophrii* (on average 14 dpd at 20 °C, LD 12:12). Incubation time herein described is similar to that described for most monogenean species at similar temperatures (mostly below 10 dpd): *Zeuxapta seriolae*, 5 dpd at 21 °C (Tubbs et al., 2005); *N. girellae*, 7 dpd at 20 °C (Bondad-Reantaso et al., 1995); *Benedenia seriolae*, 7.5 dpd at 21 °C (Tubbs et al., 2005); and, *P. multispinosus*, 9.3 dpd at 20 °C (Roubal and Diggles, 1993). However some species, such as *Discocotyle sagittata*, hatch after longer periods (20 dpd at 18 °C; Gannicott and Tinsley, 1998a). Hatchings occurred over a period of 1 to 3 days for each replicate, and over a period of 5 days, for all them together. This period was also very similar to that reported for other monogenean species at similar temperatures: *Z. seriolae* 2 days (Tubbs et al., 2005); *B. seriolae* 4 days (Tubbs et al., 2005); *P. multispinosus* 5 days (Roubal and Diggles, 1993); and *D. sagittata* 3–6 days (Gannicott and Tinsley, 1998a).

Hatching success of *S. chrysophrii* was higher than 87% in 3 of the 4 replicates. This is in accordance with results obtained for other monogenean species with hatching success over 80% (Gannicott and Tinsley, 1998a; Kearn, 1986; Tubbs et al., 2005). Kearn (1986) reported that high fecundity of monogeneans and other parasitic organisms is likely to compensate for the high mortalities of the post-larval stages. Maximum larval emergence for *S. chrysophrii* in each replicate was observed during the first 24 h after the first egg hatched and more than 50% of total egg hatchings also occurred during this period. Similar results were reported for other parasite species such as *B. seriolae* and *Z. seriolae* with maximum hatching peaks at approximately 24 h (Tubbs et al., 2005).

Light changes (light to darkness or darkness to light) usually seemed to be associated with new hatchings in the present study. Moreover, the number of hatchings during darkness was significantly higher than the number of hatchings during light, especially during the first dark period of each replicate. Hatching has been reported in monogeneans in response to stimuli such as chemicals, mechanical disturbance, changes in light intensity (night/day, shadows) (Gannicott and Tinsley, 1997; Kearn, 1986; Whittington et al., 2000). Nocturnal hatching patterns have been reported for *D. sagittata* (see Gannicott and Tinsley, 1997) and *Entobdella hippoglossi* (see Kearn, 1974). In the case of *Entobdella soleae* (Kearn, 1973), eggs hatch soon after dawn, although hatching was finally related to a circadian rhythm (Kearn, 1973). Circadian rhythms may also be involved in the hatching of *S. chrysophrii*.

Maximum longevity recorded for *S. chrysophrii* oncomiracidia was 52 hph (more than two days), a long period in which to find a new host. This value is higher than that recorded for other monogenean species at similar temperatures (Gannicott and Tinsley, 1998b; Thoney, 1986). The longevities of most oncomiracidia do not usually exceed 48 h (Whittington et al., 2000). However, only 10% of all the specimens herein studied lived more than 24 h, and mean survival time was 12.5 (9.5–18.7) hph at 20 °C, similar to that reported for a related microcotylid *Microcotyle sebastis* (see Thoney, 1986).

The average vertical swimming period of the oncomiracidia lasted only about 60% of the survival time. This proportion increased to 69% when considering both vertical and horizontal swimming together. Moreover, those oncomiracidia with especially long survival times also spent longer swimming, until almost the end of their lives.

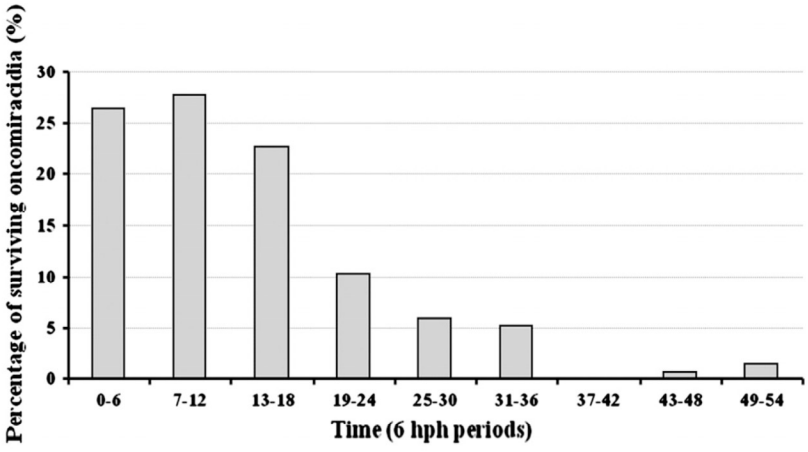

Fig. 4. Percentage of surviving *Sparicotyle chrysophrii* oncomiracidia in relation to time, expressed as 6 h periods post hatching (hph).

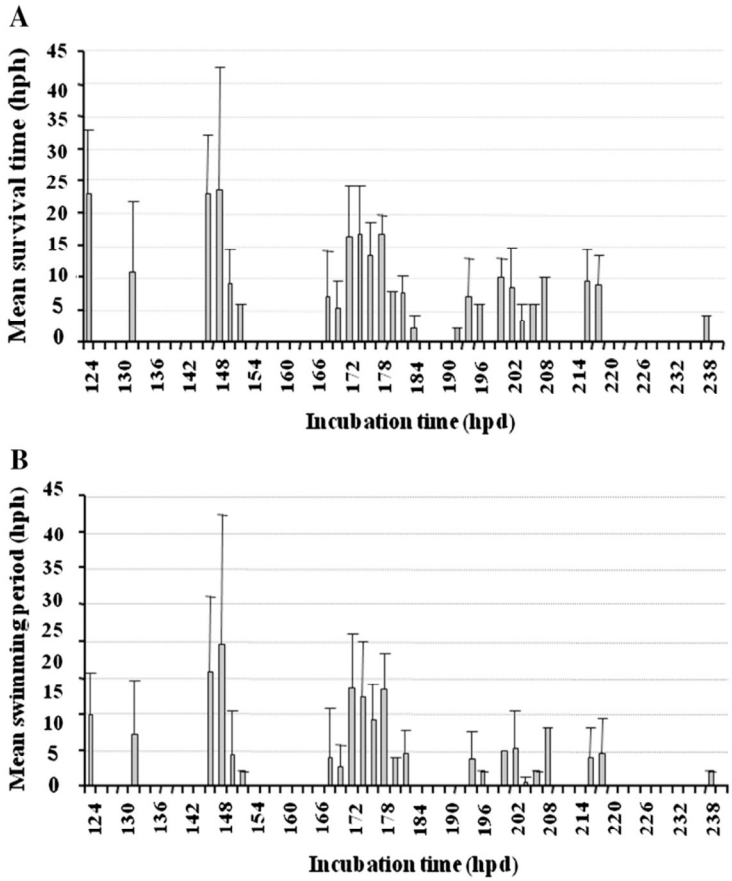

Fig. 5. Mean survival time (A) and mean swimming period (B) of *Sparicotyle chrysophrii* oncomiracidia in hours (hph) against time post deposition (hpd). Bars represent standard deviation (SD).

Similarly, *D. sagittata* oncomiracidia were also found to swim for a large proportion of their life-span (Gannicott and Tinsley, 1998b).

The swimming patterns observed for *S. chrysophrii* in the present study were very similar to those reported for other monogeneans (Gannicott and Tinsley, 1998b; Kearn, 1980; Whittington et al., 2000): firstly a period of vertical swimming, which might lead to contact with hosts in the water column, and a longer second period near the substrate, a "sit and wait" strategy which is less energy consuming. Swimming speed range herein recorded for *S. chrysophrii* also fits within that reported for other species (i.e. 1–5 mm/s; Whittington et al., 2000). Gannicott and Tinsley (1998b) reported that swimming ability, rather than survival, is related to infectivity, as oncomiracidia are able to find new hosts mostly while they can swim in the water column. Kearn (1980) highlighted the importance of the vertical swimming in *E. soleae*, which was probably related to a search pattern for a bottom-dwelling host. If *S. chrysophrii* oncomiracidia are especially infectious while vertically swimming during the first 4–6 h after emerging, this is a short time-span in which to find a new

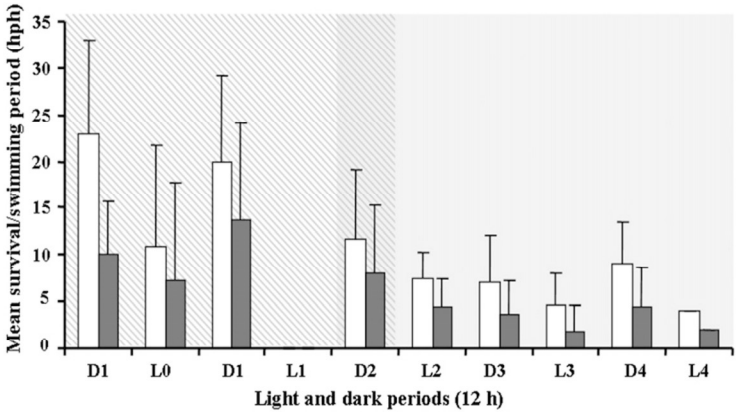

Fig. 6. Mean survival and swimming times against light and dark periods (12 h). Light and dark periods indicated as "L" and "D" + "number of the period". Survival represented by white bars and swimming represented by dark grey bars. The right oblique-lined background represents replicate 3 and the uniform grey background represents replicate 4.

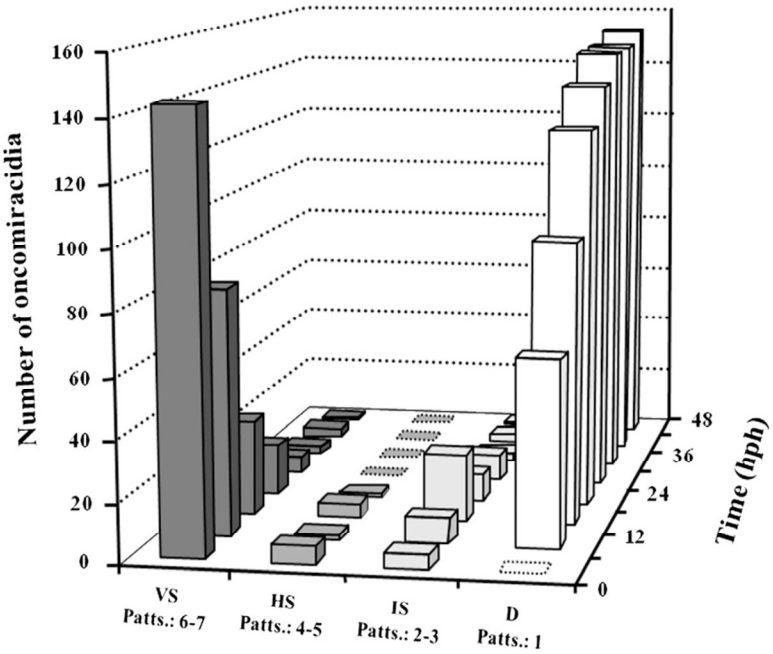

Fig. 7. Number of oncomiracidia with different swimming patterns (referred to as Patts.) against time post hatching (hph). VS, vertical swimming, including patterns 5 and 6; HS, horizontal swimming, patterns 3 and 4; IS: irregular swimming or partial movement, patterns 1 and 2; D, dead oncomiracidia, pattern 0. For explanation of patterns see Materials and methods.

host. This infective period would be artificially prolonged in sea cage conditions as eggs hatch far from the sea bottom and oncomiracidia, both static or in horizontal swimming, will stay for longer in the water column. Due to their small size, larval sedimentation will be slow and the infection chances will be increased.

The coordination between parasite development and fish habits has been reported in other species as a useful strategy to increase location of hosts by parasites. As swimming speeds of oncomiracidia and hosts differ considerably, coordination between the behaviour of host and parasite becomes essential for successful transmission of oncomiracidia (Kearn, 1986). Hatching rhythms in some monogeneans have been related to the behaviour of the host. Macdonald (1975) discovered that various species of *Diclidophora* emerged rhythmically, that the rhythms differed from one species to another and appeared to be adapted to specific host behaviour patterns. In *D. sagittata*, the egg hatching rhythm is apparently coordinated with the resting habits of the trout host. The higher frequency of *S. chrysophrii* hatchings during the dark periods could aid host finding and attachment of the larvae. On the one hand sparids, in general, are known to group for foraging during dusk (Watt-Pringle, 2009), thereby increasing opportunities for parasite transmission. On the other hand, hatchings would also coincide with the nocturnal resting of gilthead seabream (Bégout and Lagardère, 1995). Gill activity is likely to be less in inactive hosts, with less chance of dislodgement of larvae by gill ventilating currents. According to Whittington and Kearn (1988), egg grouping and the presence of hooked egg appendages contribute to maintain the eggs of *Diclidophora luscae* near to the bottom-dwelling host. The filamented eggs of *S. chrysophrii* are similar to those of many other polyopisthocotyleans, as *Diclidophora* spp. (Yamaguti, 1963) and would have the same role. Survival and swimming times also seemed to be higher in those oncomiracidia emerging during dark periods.

Mooney et al. (2008) warned about the unreliability of using in vitro observations to predict events in monogenean life-cycles. Environmental factors (especially temperature) and host condition can modify parasite reproduction patterns (Tinsley, 2004; Whittington and Chisholm, 2008), which can alter life-cycle timing. Kearn (1986) reported that the duration of development of most monogenean eggs shortens at higher temperatures. Temperature also affects hatching success and an optimum temperature for hatching apparently exists for each parasite species (Gannicott and Tinsley, 1998a; Ogawa, 1988; Tubbs et al., 2005). In fact, *S. chrysophrii* showed an apparently longer incubation time at 20 °C (Sitjà-Bobadilla et al., 2006; present study) than at 22 °C (Euzet and Noisy, 1979). The effect of temperature on vitality has also been studied in other monogeneans, longevity and swimming periods being longer at very low temperatures (i.e. 6 °C–7 °C, see Kearn, 1974 and Gannicott and Tinsley, 1998b). Effects of temperatures on larval stages could have implications for seasonal changes in parasite transmission (Gannicott and Tinsley, 1998b). The first annual infections by *S. chrysophrii* are usually detected at the end of Spring, when the temperature of Mediterranean water increases from about 14 °C to 18 °C (Hofrichter et al., 2004). Temperature increase could be a trigger for *S. chrysophrii* infections. Further in vitro studies mimicking different environmental conditions combined with in vivo tests are thus recommended.

Knowledge of the general biological features of parasites such as *S. chrysophrii*, which severely affect gilthead seabream, is especially relevant due to the importance of this species in current Mediterranean fish culture. The available information about the life-cycle of *S. chrysophrii* is useful in order to design prophylactic and therapeutic measures against the parasite in gilthead seabream cultures. Once the disease is diagnosed, usual treatments (formalin, peroxide, freshwater; see Noga, 2000) are able to kill juveniles and adults of *S. chrysophrii* but not the eggs (Sitjà-Bobadilla et al., 2006). According to our results, two treatment applications, one against the worms and the other against the oncomiracidia emerging from the surviving eggs, would be highly efficient and would allow long periods without monogenean infection. In a first application, the fish should be treated with an anthelmintic (see Sitjà-Bobadilla et al., 2006) which would eliminate most of the worms, leaving living eggs. As hatching of *S. chrysophrii* can continue for up to 10 days and as some oncomiracidia can live for up to two and a half days, the maximum period in which eggs and oncomiracidia can continue being infective is approximately 13 days (see also Sitjà-Bobadilla et al., 2006). Thus, a second

application against larvae should be applied at least 15–20 days after the first treatment to ensure the elimination of all possible new oncomiracidia emerged from the eggs still in water, nets or fish. The life cycle (egg to egg) in *S. chrysophrii* takes from 26 to 36 days (Repullés-Albelda et al., 2011). If any egg had survived, no more worm treatments would be needed before about 50 days: 36 days, worm maturation + 10 days, maximum egg incubation time (according to present study) + 3 days, maximum oncomiracidial survival.

Currently, some Spanish Mediterranean fish farms manage to partially control most of the *S. chrysophrii* infections with about 3 net exchanges or chemical preventive treatments per year (April/May–August/September–December/January). However, not all the eggs are entangled in nets. Some of the eggs remain in water and others are entangled in the fish gills. These eggs will continue the infection and from 2 to 3 reproduction cycles would occur during the period within net exchanges (see Repullés-Albelda et al., 2011). Net exchanging is nevertheless recommended, because although this practice is expensive and not performed by all producers it has been observed to be useful as palliative.

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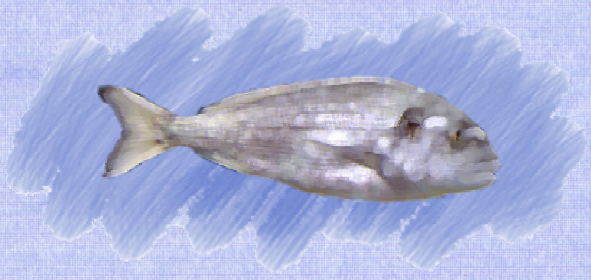
CHAPTER 8.

Post-larval development of the microcotylid monogenean *Sparicotyle chrysophrii* (Van Beneden et Hesse, 1863): comparison with species of Microcotylidae and Heteraxinidae.



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Post-larval development of the microcotylid monogenean *Sparicotyle chrysophrii* (Van Beneden and Hesse, 1863): Comparison with species of Microcotylidae and Heteraxinidae

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ABSTRACT

The chronology of post-larval development in *S. chrysophrii*, a polyopisthocotylean monogenean parasite of the gilthead seabream (*Sparus aurata* L.), was experimentally studied. It is compared with other species within the Microcotylidae and the Heteraxinidae, including an analysis of the changes in attachment and the growth rate. Gilthead seabreams infected by larvae of *S. chrysophrii* were killed periodically in order to collect the different developmental stages. Parasite total body length, haptor length, largest clamp width, and total number of clamps were recorded. Specimens of *S. chrysophrii* in culture conditions at 20 °C became gravid after 26–30 days, with 37 pairs of clamps. The *S. chrysophrii* growth curve appears to be sigmoid with 3 growth periods (slow–fast–slow). The haptor of *S. chrysophrii* grows linearly with total body length, but the main contribution to total body length growth is that of the non-haptoral body. The relationship between number of clamps and total body length during development can be fitted to an exponential curve for all the reviewed species, i.e.: *Microcotyle spinicirrus*, *Microcotyle donavini*, *Microcotyle gotoi*, *Microcotyle sebastis*, *Microcotyle hiatulae*, *Polylabroides multispinosus*, *Bivagina tai*, *Heteraxinoides xanthophilis*, *Heteraxine heterocerca*, and *Zeuxapta seriolae*. The sequence of events was common for all of the species compared: terminal lappet is lost when about 15% of clamps were developed; primordia of testes at approximately 30% of clamps developed, and maturity (as first egg appearance) at about 65% of clamps developed.

1. Introduction

Sparicotyle chrysophrii Van Beneden & Hesse, 1863 is a microcotylid (Monogenea, Polyopisthocotylea) infecting the gills of the gilthead seabream *Sparus aurata* (Linnaeus, 1758) in the Mediterranean region [1]. This species causes severe epizootics in high density cultures of gilthead seabreams mostly due to host re-infection. Epizootic episodes have been mainly reported in Spain and Greece [2–4]. Outbreaks occur more frequently during spring and summer [4–6] although recent studies reported the highest prevalences and abundances during winter [7]. These apparently ambiguous observations can be explained by the effects of the environmental conditions on hosts and parasites. During summer, monogenean life-cycles are usually faster and favour transmission and re-infection [8]. However, during winter, although parasite life-cycles are presumably slower, the gilthead seabreams are particularly immunosuppressed making them especially susceptible to parasites (see [9]). Currently, no effective treatment exists to eradicate these parasites and the disease recurs. Knowledge of life cycle chronology of *S. chrysophrii* is of major importance in order to understand the parasite dynamics in culture. Furthermore experimental treatments have been observed to be differently effective against adults, juveniles and eggs, [10] consequently it is important to know the duration of each parasite developmental stage.

Pathological effects provoked by polyopisthocotyleans are mainly related with blood loss due to attachment and feeding and with a reduction of gill breathing surface due to the parasite presence [8]. Polyopisthocotyleans change their morphology drastically during development, especially increasing the complexity of the haptor. As a consequence, damage provoked by these changing haptors also must vary during development. Furthermore, as parasites grow they consume more blood and, at the same time, their haptors affect a higher gill surface. In this way, the study of parasite developmental stages can provide information about severity of effects on fish in each moment. The first detailed studies on development of Microcotylidae species date from the 1940s (*Microcotyle spinicirrus* [11] *Microcotyle donavini* [12]) and the 1950s (*Microcotyle gotoi* [13]). Euzet [14] reported descriptions of the first post-larval stages of *S. chrysophrii* (therein named as *Microcotyle chrysophrii*) after describing the morphology of the oncomiracidum [15]. Later, several studies

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on the development of other microcotylids have been published: *Microcotyle sebastis* [16]; *Microcotyle hiatulae* [17]; *Polylabroides multispinosus* [18]; *Bivagina tai* [19]. Only the studies on *M. sebastis* and *P. multispinosus* reported the chronology of development.

The main aim of this study was to provide new information on the morphological changes of *S. chrysophrii* in time, especially those of the haptor, finding possible growth patterns. The second objective was to elucidate common growth patterns within species of Microcotylidae and Heteraxinidae, with a close phylogenetic relationship [20]. Finally, as it has been previously stated, this parasitosis is a current uncontrolled problem in Mediterranean culture. For this reason, an applied objective is to obtain information to establish the best possible timing for treatment applications.

2. Material and methods

Seventy-six *S. chrysophrii*-infected gilthead seabreams, 14.5–17.0 cm in total length, were collected from sea-cages of a fishfarm off Murcia, south eastern Spain (37.59 N–1.07 W) in the summer of 2008. These experimental fish were transported alive and maintained for two weeks in the facilities of the Central Service for the Support to Experimental Research ("Servei Central de Suport a la Investigació i Experimental", –SCSIE–, University of Valencia). The experimental fish were arranged separately in four groups of 19 individuals/each and distributed in four isolated tanks (250 l volume) at 20 °C. In order to get "parasite-free" fish, the seabreams were removed from the "infected tanks" and dewormed with a standard protocol of formalin (1 h, 250 ppm., bath) and fresh water (5 min. bath) based on [21]. After the treatment, fish were temporarily reared in four 250 l parasite-free tanks, while the "infected tanks" were preserved with their original water. Eight of the treated fish were checked to ensure that they were uninfected. At the same time, 70 additional infected fish were killed by cervical decapitation and examined in order to estimate the parasite prevalence and intensity in the facilities where fish came from. These fish came from the same sea-cages as the experimental fish, and were collected and transported at the same time. Moreover, in order to increase the experimental infection, 1000 extra eggs were collected from parasites of these additional fish. The extra eggs were maintained and observed in an oxygenated Petri dish at 20 °C. After 3 days in the parasite-free tanks, the experimental fish were returned to the four "infected tanks" and the 1000 extra eggs were added to the water (250 eggs per tank). "Time 0" of the experiment was assumed as the moment when we restocked the fish in the "infected tanks" (the 11th of July 2008 at 12:00 a.m.). Our preliminary in vitro tests revealed that period to hatch for *S. chrysophrii* at 20 °C was 6 days (agreeing with Euzet and Noisy [1] observations at 22 °C). Consequently, as extra eggs had been maintained out of the tank for 3 days, hatchings could have really occurred between 0 and 3 days post-infection (dpi). From this moment, four fish, one from each tank, were killed and analysed, first after 3 dpi and then every two days for 21 dpi. At 21 dpi, main developmental events had occurred, and fish were then sampled every 5 days until 36 dpi, when a second-generation of larvae was found to be attached to the gills. The remaining fish (N = 16) were sampled at 51 dpi, 15 days later, collecting adults and second generation post-larvae.

The four fresh right-gill arches were isolated in different Petri dishes with seawater and analysed immediately after dissection. Each first lamella was observed with a Leica MZ AP0 (8×–80×) stereomicroscope using transmitted light, paying special attention to the attached parasites and its movements. Parasites were collected, recording the number per arch. Differences between numbers of parasites per arch were statistically tested with the ANOVA one-way analysis. Some living parasites (N = 140) were killed with hot saline, fixed in 70% ethanol, stained with iron acetocarmine and mounted on permanent slides with Canada balsam. All morphometrical data, except for those of sclerotised elements of the smallest post-larvae, were obtained from ethanol-fixed specimens using a Leica DMR (100×–1000×) light microscope. The sclerites of post-larvae with between 0 and 10 pairs of clamps were measured from 27 small specimens mounted on temporary slides with glycerine jelly after being killed with hot saline. Measurements are given in microns. The developmental stage of each parasite was recorded, paying special attention to haptor, gut and genitalia changes. Previous descriptions of *S. chrysophrii* development stages reported by Euzet [14] were taken into account in order to categorise and name the stages in the current study. The morphology of clamp-bearing stages has not been completely described in detail in the present paper, as exhaustive descriptions and drawings were made by Euzet [14] and Euzet and Noisy [1]. Hooked early specimens without clamps (corresponding to the "oncomiracidium peu de temps après sa fixation" stage in Euzet [14]) have been named as Hooked type 1, 2 and 3 (H1, H2 and H3), based on total length of individuals, hook features and distribution, and pharynx and gut arrangement. In this classification H1 is the earliest post-larva. Clamp-bearing post-larvae have been named according to the Clamp Pair Number in each stage (CPN#). For example, CPN1 is a post-larva with the first pair of clamps ("larve au stade 1" in Euzet [14]).

The relationships between morphological variables of *S. chrysophrii* during development were analysed and the growth rate calculated. Variables studied were: clamp pair number, total body length, partial body lengths (i.e. haptor length and non-haptoral body length) and largest clamp width. In this paper, parasite growth has mainly been considered as an increase in clamp pair number since it has been reported to be a better indicator of development rather than body length (see Thoney [16,22]). The resulting information was compared with the available data of other microcotylid species using measurements of different individuals obtained from bibliographic information (from numerical data in the text and from drawings when numbers were not indicated). The available data on heteraxinids was also studied, in order to compare our results with those from closely related asymmetrical polyopisthocotyleans. The species compared included seven microcotylids, i.e. *M. spinicirrus* [11], *M. donavini* [12], *M. gotoi* [13], *M. sebastis* [16], *M. hiatulae* [17], *B. tai* [19] and *P. multispinosus* [18]; and 3 heteraxinids, i.e. *Heteraxine heterocerca* [23], *Heteraxinoides xanthophilis* [22] and *Z. seriolae* [24]. The haptor of the heteraxinid species is asymmetrical, and available data mostly refer to the long side. Therefore, only clamp growth on this side was compared. Main developmental events of these species were compared with those of *S. chrysophrii*, both from the current study and from bibliographic data [4,15]. Statistical tests were performed using SPSS® 15.0 (SPSS, Inc., Norusis, 2006). All variables were transformed to natural logarithms before statistical test application. Ecological terms follow [25].

3. Results

Total prevalence and mean intensity before deworming was 73% and 4 ± 3 respectively (n = 70). Prevalence during the experiment was 78% and mean intensity was 12 ± 13, with a maximum abundance of 46 (n = 76). Total number of parasites collected was 582, 36 of them being adult. The prevalence at the final sampling of the experiment (n = 16, at 51 dpi) was 75%, mean intensity was 3 ± 4, and maximum abundance was 9, including adults and second-generation larvae. The percentage of parasites per each right gill arch was similar: Arch I, 26.6%; Arch II, 28.0%; Arch III, 25.4%; and Arch IV; 19.9%. Significant differences between parasite abundance in each gill arch were not found.

3.1. Morphology

Main morphological changes of the earliest developmental stages are showed in Table 1. The 3 hooked and non-clamp-bearing post-larvae (H1, H2 and H3, see Fig. 1a–c) were detected from 3 to 11

Table 1
Main morphological changes of early postlarvae (H1, H2 and H3) of *Sparicotyle chrysophrii*. Data about oncomiracidia was obtained from [14]. Measurements are given in μm. Dpi means days post-infection.

	dpi	Total size (length × width)	Haptor width	Hamulus length; posterior hook length	Primordia of buccal suckers	Pharynx length; position in body	Anterior glands	Caecum wall	Dark spots number; feature; length × width
Oncomiracidia	_	250 × 75	Narrower or equal to body width	40; 40	Undefined	29; medial position	Well-defined	Undefined	Several; Scattered granules in some specimens; –
H1	3–5	120–130 × 34–41	Narrower or equal to body width	43–52; 40–45	Undefined	18–23; progressively anterior	Well-defined	Undefined	4; Compact; (10 × 6) each
H2	5–7	130–70 × 37–49	Approximately equal to body width	43–52; 42–45	Visible in some specimens	18–23; progressively anterior	Slightly undefined	Undefined	4; Compact; (10 × 6) each
H3	5–11	170–220 × 43–56	Wider than body width	45–55; 42–46	Visible in most specimens	18–23; progressively anterior	Progressively indiscernible	Visible in some specimens	4; Compact; (10 × 6) each

dpi (see Fig. 2a). H1 stages are very similar to oncomiracidia, however none of the attached specimens had cilia or eyespots. Haptoral posterior hooklets and hamuli in H1 (Fig. 1a) almost have a definitive size, except for the width of the handles of the posterior hooklets, which still thicken and firmly fix to their blade. At the beginning, posterior hooklets and hamuli of H1 stage occupy most of the haptor (Fig. 1a). As worms grow, all posterior hooks are progressively displaced backwards from the haptor centre, staying in a narrow terminal lappet (Fig. 1a–c). The terminal lappet becomes narrower during development and posterior hooks and hamuli become progressively closer and overlap. The lateral hooklets remain in the anterior part of the haptor. Parasites with hooks and clamps were found from 7 to 21 dpi (Fig. 2). The first pair of clamps appears in the haptor just anterior to the terminal lappet, where the most posterior pair of lateral hooklets is (CPN1; 7–15 dpi) (Fig. 1a,d). When the first pairs of clamps become visible, lateral hooklets still persist. As early pairs of clamps develop, lateral hooklets were observed to progressively displace laterally to the haptor edge and remain there until the surrounding tissue tears. The rest of the clamps develop gradually in the haptor towards the anterior end. The oldest specimens with a terminal lappet were CPN5 and CPN6, which appeared from 15 to 21 dpi (Fig. 2). The terminal lappet remained in 6 of 7 CPN5 specimens (Fig. 1f), and only a small number of CPN6 were hooked (3 of 12). Parasites with clamps and no hooks were observed from 15 dpi until the end of the experiment (Fig. 1f,g). The stage with the maximum number of clamps (CPN72) was found in the last fish sampling, 51 dpi.

The pharynx develops and slightly grows during the early stages (Fig. 1b–f). At the following stages the pharynx becomes larger and closer to the anterior end. At CPN4 the pharynx (20–25 length × 19–22 width) is half the length of that in adults. Four elliptical dark brownish spots, similar in size, were always observed at the gut level, posterior to the pharynx, from H1 to CPN1 (3–15 dpi; Figs. 1b,c and 2a). The spots are formed by compact masses of dark yellowish granules. The posteriormost dark spot was the only located within the haptor and the two anterior ones were always joined. These 4 spots could be observed associated with the caecum wall in H3 and CPN1, when it became visible. From CPN2 (9–15 dpi) the spots are more scattered and lighter, progressively increasing in number. Dark spots are always associated with the gut. The gut wall becomes more defined progressively, first being saccular, and becoming bifurcated from CPN13 (17 dpi), when the oesophagus and caeca are also completely defined. The genital atrium (Fig. 1 g) is first seen as a primordium in CPN18 (19 dpi) and is completely defined in CPN22. Parasites are protandrous. First primordia of testes are found in CPN18 to CPN20 (19 to 21 dpi; see Table 2). The germarium is first observed in CPN25 (21 to 26 dpi) and grows in length and width while the oocytes become progressively larger and mature. The first intrauterine eggs were detected at 26 dpi (CPN37; see Table 2) and from 36 dpi, most of the parasites were able to produce eggs (see Fig. 2). The first post-larvae from a second generation of parasites were found on 36 dpi. Post-larvae were observed to be progressively more sedentary as they grew. Stages up to CPN12 (hook-bearing without clamps and hook-clamp-bearing) were able to move by leech-like locomotion, combining both haptor and anterior glands. Adults were found to be sedentary and, once settled, the haptor became the main attachment structure.

3.2. Growth

Parasite growth curves, represented by number of clamps and mean body length, were sigmoid-shaped (Fig. 3) i.e.: i) early slow growth period, approximately 18 dpi (up to 300–400 long specimens); ii) middle fast growth period, about 33 dpi (300/400–3000 long); and iii) late slow growth period, until adults reach the maximum number of clamps (3000–5200 long). Coefficients of determination (R^2) of the logistic and exponential curve estimation regressions were 0.807 and 0.694, respectively, for clamp pair number, and 0.984 and 0.943, respectively, for mean body length against time. The relation between error and curve fit showed a randomised distribution of the points around 0. The mean clamp pair addition rate per day was between 1 and 2, 0–1 during the first period, 2–3 during the second and 1–2 during the third.

High correlations were found between body length and pair clamp number: $R^2 = 0.968$. High linear correlations were also found between total body length and both mean haptor length and mean non-haptoral body length ($R^2 = 0.98$ with slope 0.8090 and $R^2 = 0.97$ with slope 0.3405, respectively). Least-squares regression supported linear relationships of largest clamp width with total body length ($R^2 = 0.870$) and pair clamp number ($R^2 = 0.950$) and, subsequently, with haptor length ($R^2 = 0.940$). The residual plot showed random distribution to be around 0. The largest clamp width increased from 30 wide to a maximum of 80–100 wide, in specimens with about 70 pairs of clamps. The increase of the largest clamp width during parasite life after the last clamp was added was not studied in detail.

3.3. Comparative review of growth rates in microcotylids and heteraxinids

The relationship between number of clamps and total body length was exponential for all of the species of Microcotylidae and Heteraxinidae reviewed (Fig. 4). Four groups of species could be distinguished according to the slope of the regression line when logarithmically transformed: i.e. i) between 0.0217 and 0.0229 (*S. chrysophrii*, *P. multispinosus* and *B. tai*), ii) between 0.0280 and 0.0311 (*M. sebastis*, *M. spinicirrus*, and *M. hiatulae*), iii) between 0.0405 and 0.0439 (*H. xanthophilis* and *M. donavini*), iv) between 0.0517 and 0.0523, which increases relatively in length more than twice that of the first group (*H. heterocerca* and

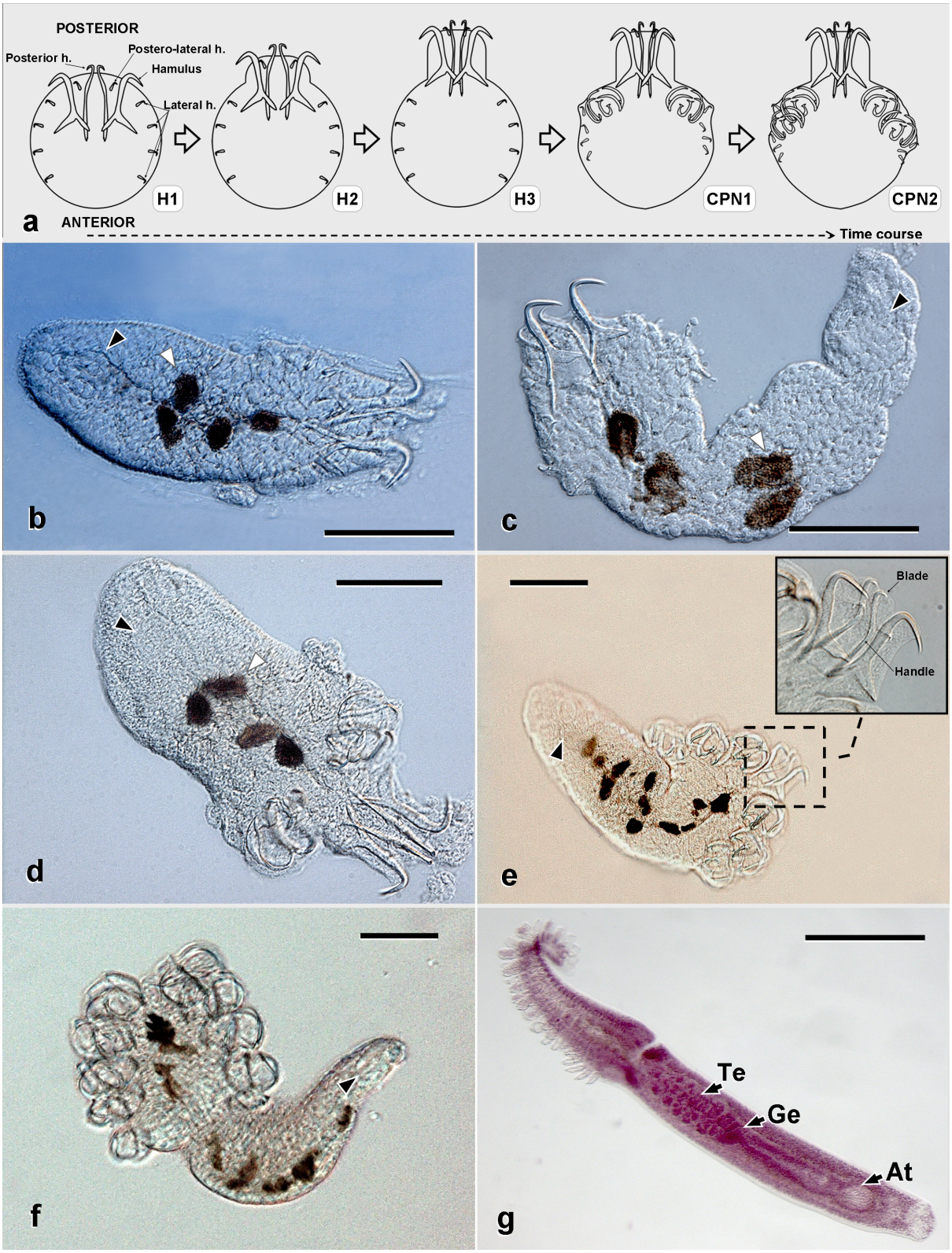

Fig. 1. Post-larvae of *Sparicotyle chrysophrii*. (a) Morphological changes of larval haptor during development; h. means hooklet. (b, c & d) Post-larvae H2, H3 and CPN1 respectively (H1could not be photographed). (e) CPN3 specimen with a detail of larval hooks. (f) CPN5 specimen without larval hooks. (g) Post-larvae specimen without eggs. White arrowheads point to dark spots and black arrowheads to the pharynx. At, atrium; Ge, germarium primordium; and Te, testes. Scale bars represent 50µm except in figure g where 1000µm are represented.

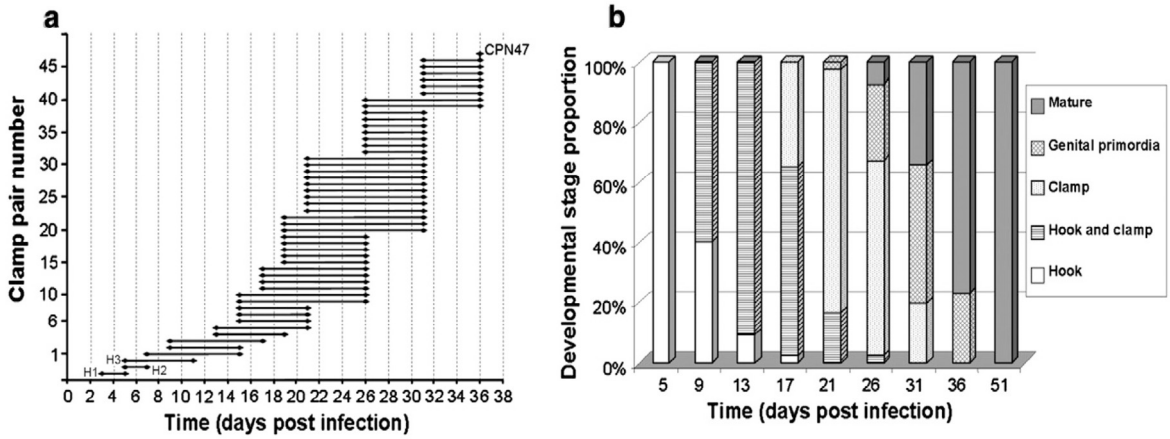

Fig. 2. Chronology of the development of *Sparicotyle chrysophrii* in *Sparus aurata* at 20 °C. (a) Chronology of the findings of developmental stages according to their clamp pair numbers. (b) Proportions of developmental stages in time.

M. gotoi). *Z. seriolae* from the Mediterranean, with a slope of 0.0324 (unpublished results) would be included within the second group of species.

4. Discussion

4.1. Morphological comments

Studies on monogeneans are mainly focussed on the structures related to functional processes relevant to fish damage: attachment, mobility, nutrition and reproduction. These studies usually stress the analysis of the haptor, anterior cephalic glands (sometimes referred to as "adhesion glands" in oncomiracidia, see [26]), digestive tract, and their development [8]. This paper provides new information on the morphology, development, and chronology changes for *S. chrysophrii*, adding data to the previous detailed morphological descriptions of oncomiracidium [15], post-larvae [14] and adult [1] of this species. In contrast to other studies on Microcotylidae species [14,16–18], including *S. chrysophrii*, which show a replacement of the lateral hooklets by the early four pairs of clamps, this study shows that these hooklets remain after the complete development of the early clamps. These tiny hooklets could have gone unnoticed in other studies, as they are often difficult to be detected, especially when worms grow and become thicker or when clamps cover them up. Lateral hooklets fall off later, after a progressive lateral displacement out of the haptor when clamps grow. Hamuli and posterior hooklets of the larval haptor of *S. chrysophrii* remain approximately the same size from the swimming oncomiracidium to their loss, in CPN5-CPN6 post-larvae. Therefore, the earliest post-larvae are able to attach to gills with hooks of the same length, despite the size increase during development. In contrast, the anterior glands, the other attachment structures of the oncomiracidia and the first post-larval stages of *S. chrysophrii* (H1, H2 and H3) were progressively less evident during development, and almost undetectable in the first stages with clamps (up to CPN4). This result coincides with Euzet's observations [15] as the first stage with clamps was depicted without anterior glands. Adhesive secretions in the haptor and the anterior end have seldom been reported in adults [8]. Although the haptor is the main attachment organ of monogeneans, the movement of early developmental stages is also achieved by the anterior gland's adhesion properties [26,8]. Oncomiracidia use the anterior glands to settle on the host and, later, to migrate with the help of the haptor until they find their final location. Early hook-and-clamp-bearing stages are able to move by leech-like locomotion using haptoral hooks and anterior glands. Adults, only attached by the haptor, become sedentary. The help provided by buccal suckers is probably enough for adult worms to attach the mouth while feeding [8,27].

The dark spots in non-clamp-bearing post-larval specimens of *S. chrysophrii* were interpreted by Euzet ([14], reported as "trois taches brunâtres") as blood within the primordia of the digestive saccular tract. This statement implies that these post-larvae are already feeding on blood as adult polyopisthocotyleans are known to do [8,28–32]. However information about early post-larval stage feeding is scarce. The early post-larval pharynx is not located completely anterior until CPN1 stages, when it also becomes more defined, thus its complete functionality should be studied. Moreover, adult monogeneans have been reported to feed discontinuously and egest food waste through the mouth [8,33]. Variations in number and aspect of the post-larval dark spots could be expected if they were blood or food waste However in the current study, we documented that these structures always had a similar morphology, colour, number (four), and arrangement in all the early post-larvae (H1, H2, H3 and CPN1), perhaps due to the slight growth of the specimens in these stages. Some *S. chrysophrii* oncomiracidia, which are not supposed to eat, showed dispersed small dark granules in the posterior half of body (personal observation; see example in Fig. 1a of [10]). The appearance of these granules is very similar to that of the yolk in adult vitelline follicles and also to that of those granules found inside of the dark spots of the early post-larval stages. Therefore, we believe that the dark spot granules, at least during the H1–H3 stages, could be, among other things, yolk. In fact, vitelline follicles have been reported to be interspersed with digestive caeca. Haematin was not observed in early post-larval specimens however, chemical analysis should be performed to elucidate the real nature of these dark spots.

4.2. Growth

The *S. chrysophrii* growth curve looked sigmoid, and three sections with different growth rhythms could be distinguished (slow–fast–slow). This growth pattern is partially shared with other microcotylids. Growth differences could be explained by the lack of information on some parts of their chronological register. In the case of *M. sebastis* [16] growth adjusted to a logarithmic growth curve (fast–slow). The early period of slow growth of *M. sebastis* would not be reported in that study. The first sampling of *S. chrysophrii* was performed after 3 dpi while the first sampling of Thoney's experiment [16] was performed after 7 dpi, with findings of specimens from 2 to 10 pairs of clamps. In the case of *P. multispinosus* [18] two periods of different clamp addition rates (slow: 1 pair per day–fast: 2–3 pairs per day) were described for the development. The first slow growth period seems to be shorter in *P. multispinosus*, which could be explained by the fact that the oncomiracidium of this species hatches with one pair of clamps already developed. The third growth period described for *S. chrysophrii* (slow) was

Table 2
Main developmental events of different Heteraxinidae and Microcotylidae species related with clamp pair number (CPN), total length and timing. Information about short and long haptor sides in asymmetric worms represented as "short side data/long side data".

Developmental event		*Sparicotyle chrysophrii* (present study)	*Bivagina tai* [19]	*Microcotyle donavini* [12]	*Microcotyle gotoi* [13]	*Microcotyle hiatulae* [17]	*Microcotyle sebastis*[a] [16,36]	*Microcotyle spinicirrus* [11]	*Polylabroides multispinosus*[b] [18]	*Heteraxine heterocerca* [23]	*Heteraxinoides xanthophilis*[c] [22]	*Zeuxapta. seriolae*[d] [24,36]
Terminal lappet fall	CPN	5–6	6–7	>12	2–3	5	3–5	13	12 (9–15)	7–8/10	–/5–6	–/–
	Length (μm)	313–450	540	812	250–300	580	551–650	1000	400–800	1500–2700	822	<1100
	Time (days)	15–21	–	–	–	–	7–9		6–9	–		13
Primordia of testes	CPN	18–20	15–20	–	–	9–10	10–15	13	22 (17–31)	8–9/12–15	–/7	–/–
	Length (μm)	950–1000	800–1000	–	–	768–818	927–1250	1000	900	2000	915	1200–1300
	Time (days)	19–26	–	–	–	–	18	–	10–14	–		16
Germarium primordium	CPN	25	15–20	26	–	12–14	14–19	–	30 (24–37)	8–92/5–27	–/13–15	–/–
	Length (μm)	1310	800–1000	3063	–	923–1033	1181–1547	–	1200	4000–5000	1225–1460	1300–3500
	Time (days)	21–26	–	–	–	–	23	–	14–18	–		16–20
Maturity (egg formation)	CPN	37	40	–	–	15–20	19–30	–	35–45	8–9/25	–/17	–/–
	Length (μm)	1960	2050	–	–	1091–1406	1547–2582	–	1400	6000	1585	>3600
	Time (days)	26–31	–	–	–	–	27	–	20	–		25
Maximum size reported	CPN	72[e]	50–65 38–60[f]	45–55	33–35	16–28	36	99	55–60	9/27–30	29/39	32/40
	Length (μm)	4500–5200	2700–4000 540–7900[f]	6000	6000	2100–5100	3300 5500[f]	13000	980–2250	9000	3482–5685	4100–8500
	Time (days)	51	–	–	–	–	–	–	–	–	–	–
	N clamps at maturation/ Max. N clamps	0.50	0.61	–	–	0.7	0.60	–	0.75	0.66	0.42–0.50	0.57
	Max. length/Max CPN	61–71	61–80; 225–131[f]	109–133	171–182	182–318	92; 153[f]	131	37–41	1000/300–333	196/146	265/212
	Host	*Sparus aurata*	*Pagrus major*	*Labrus bergylta*	*Hexagrammos octogrammus*	*Tautoga onitis*	*Sebastes melanops*	*Aplodinotus grunniens*	*Acanthopagrus australis*	*Seriola quinqueradiata*	*Leiostomus xanthurus*	*Seriola lalandi*

[a] Data obtained from curve B in Fig. 6 of [16].
[b] Data of maximum body size obtained from [41].
[c] Data obtained from line A in Fig. 2 of [22].
[d] Data of maximum size obtained from [36].
[e] Data coinciding with [1].
[f] Data obtained from [36].

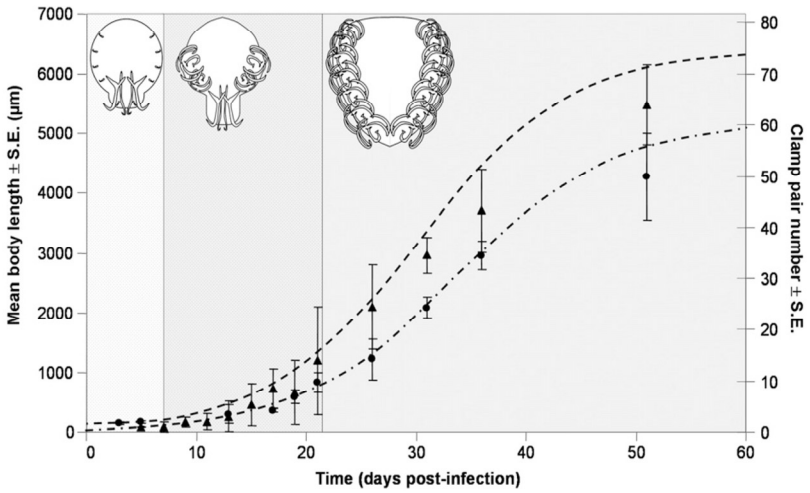

Fig. 3. Sigmoid growth curves of *Sparicotyle chrysophrii* represented by body length and clamp pair number against time. Full dots represent mean body length, logistic curve as dashed and dotted discontinuous line; full triangles represent clamp pair number, logistic curve as dashed discontinuous line. Bars represent SD. Coloured areas represent different developmental stages: hook-bearing-without-clamps, hook-and-clamp-bearing and clamp-bearing stages.

not reported in *P. multispinosus*. However, a third slower clamp addition rate (1.5 clamp pairs/day) can be obtained when using the data reported for the two oldest specimens in [18]. Similar results were found for the case of the heteraxinid *Z. seriolae*, as Tubbs et al. [24] reported exponential curves (slow–fast) for the increase of parasite length over time. More data on this developmental period would be needed to find a third section of growth rhythm, as these experiments stopped when specimens were gravid.

Changes in growth rates during development are usual in many aquatic free-living organisms like fishes, molluscs and crustaceans [34], animals with significant biological changes during their development, many of them including metamorphosis and ecological changes. In the microcotylid and heteraxinid species, the transition period between the early, slow growth to faster growth approximately coincides with the terminal lappet loss. As oncomiracidial life is very short, proper settlement is critical during early parasite life. Main parasite investments are probably destined to get a minimum number of clamps to grasp the secondary lamellae and properly attach in the most suitable region to live, before hooks become useless. This critical period also exists in some sessile molluscs, which need to properly fix on a substrate to continue developing [35]. Later, during the second period (fast) parasites grow faster until maturity. At this period, polyopisthocotyleans change from mobile to almost sedentary, and once stable and properly fixed in the optimum gill location, they can invest more resources in growth. Furthermore, the greater facility of larger worms to feed on different areas could also allow for a more profitable use of resources (and, at the same time, spread damage). Small mobile parasites feed in a relatively small area, which they have to reach by moving the whole body, while sessile larger-sized parasites can elongate their bodies to find distant adequate feeding points [8]. The third period (slow) would be prolonged from maturity until reaching the maximum number of clamps. After maturity, an important part of the investments are dedicated to reproduction, and this could cause a growth slowdown when more resources are used for egg production. Finally, the senescence effect may also contribute to growth slowdown.

Lengths of both the haptor and non haptoral body of *S. chrysophrii* increased linearly with total length. The slope of non-haptoral body growth with total body length is 2.35 times that of the haptor. Therefore, in this species, the haptor contributes approximately 2/7 to the total body growth. An even slightly higher haptor/body growth ratio (2/5) was reported by Remley [11] for *M. spinicirrus*. Thoney [22] reported that this ratio did not change for *H. xanthophilis* infecting hosts of different sizes. Although the ratio is maintained, the same author suggested that variations of size and distance of the host secondary lamellae directly affect parasite haptor growth. Therefore, effects of host ontogenetic changes in parasite growth should also be considered, especially if the monogenean has long life span (unknown for

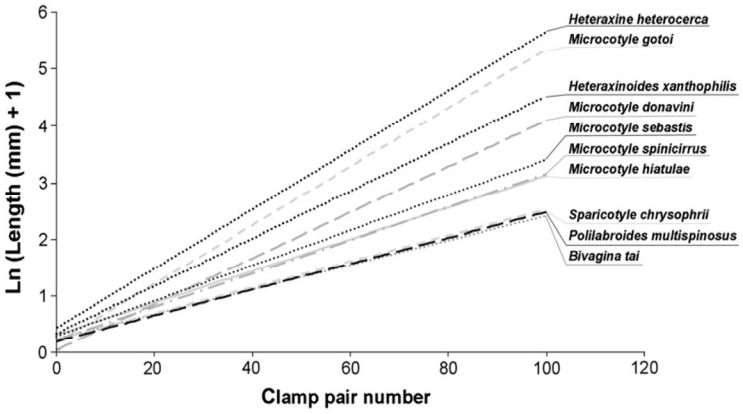

Fig. 4. Length of Heteraxinidae and Microcotylidae species as a function of clamp pair number. Heteraxinid species data belong to the long side of the haptor. Bibliographic references in Table 2.

most of monogeneans, including *S. chrysophrii*) and for those host species with fast development.

4.3. Comparative review of growth rates in microcotylids and heteraxinids

The relationships between the number of clamps and body length of all species could be depicted by exponential curves (log-transformed in Fig. 4). The same type of relationship is observed both in the microcotylids and the heteraxinids. The slopes of these relationships seemed to be ordered from the higher to the lower ratios between maximum total length and maximum number of clamps reported for each species (see Fig. 4). Clamp addition is then a key determinant in body growth. Results indicate that most of the relative body length increase occurs during the clamp addition period and the final number of clamps seems to be a good predictor of body growth. The only analysed species that did not accomplish this ordering was *B. tai*, according to measurements obtained from Yamaguti [36]. However Yamaguti [36] is a taxonomic and systematic study, and results fitted better when using maximum measurements of *B. tai* from Ogawa [19], an experimental study of development.

Mean growth rate differs among the species with available data: 1.4 clamp pairs/day and 105.5 μm/day in *S. chrysophrii* (present study); 0.8 clamp pairs/day and 150.8 μm/day in *M. sebastis* [16]; and 1.7 clamp pairs/day and 73.9 μm/day in *P. multispinosus* [18]. These differences could be explained by the definitive total length and number of clamps of each species. Maximum total length of *S. chrysophrii* is similar to that of *M. sebastis*, but as the maximum number of clamps of *S. chrysophrii* is twice the number of *M. sebastis*, the mean clamp addition per day is also double. *S. chrysophrii* has similar maximum number of clamps as *P. multispinosus*, but is twice as long, and increases approximately double its length for every pair of clamps added. Further studies must be done in order to analyse body length increase after the last clamp develops.

Common patterns between species were found for the timing of some developmental events of all the reviewed species, despite their different maximum number of clamps. Whittington et al. [26] reported that terminal lappet loss occurs when 2 to 14 pairs of clamps have been developed. In most of the species reviewed here, the terminal lappet usually falls off when 10% to 20% of the total number of clamps has been developed (excepting *M. gotoi* and *M. donavini*) (see references in Table 2). Regarding the development of the genitalia, Thoney [16] reported a common developmental pattern for two *Microcotyle* species, *M. sebastis* and *M. mormyri*, which also become gravid in similar developmental stages (60%–70% of clamps developed). Roubal and Diggles [18] found similar results regarding the time of sexual maturity of other species such as *M. hiatulae*, *P. multispinosus*, and *B. tai*. Attainment of maturity for parasites reviewed herein is found when between 50% and 75% of the total number of clamps have been developed (most of them at between 60% and 70% of clamps developed).

4.4. Pathogenetic and therapeutic considerations

The distinct post-larval morphologies and habits point to different pathogenetic effects on the host during development that must be taken into account in order to design new treatments and to establish priority prophylactic actions. Mechanical damage associated with hook piercing is known to be more serious than that associated with clamp grasping [8] as, although both actions provoke epithelial lesions, hooks also perforate blood vessels causing haemorrhages. In the current experiment with *S. chrysophrii*, hooks were found in parasites up to 21 dpi when the terminal lappet fell off. This would be the first period of infection with intrusive injuries. Parasites with clamps could be found from 7 dpi and, therefore, a second period of about 14 days exists with combined intrusive and compression action. Gradual clamp addition during growth extends gill damage and decreases the gas interchange surface [8,37–39]. During the last and longest period of infection, after terminal lappet loss, there are no hook-intrusive lesions. Anaemia is then presumably mainly produced by blood feeding.

Knowledge of developmental key events (i.e. attachment, maturation, egg release; see Fig. 2b) could help to establish adequate intervals to apply anthelmintic treatments. *S. chrysophrii* was observed to become gravid between 26 and 36 dpi, when a second generation of larval specimens was found. Moreover, time to hatch for *S. chrysophrii* eggs at 22 °C is 6–14 days [1,10]. In this way, a two-dose treatment, separated by more than 14 days (perhaps 20 days, to include possible late hatchings), could be optimal for a lasting deworming. This treatment would eliminate worms in the first dose and the post-larvae from the eggs remaining in the environment in the second dose. However, for more accurate treatment design, detailed information about time to egg hatching, and oncomiracidia survival of *S. chrysophrii* must be known. In culture conditions, infections are desynchronised as specimens of all developmental stages cohabit at the same time and different kinds of injuries are produced simultaneously [40]. Effective treatments in culture tend to synchronise the infection. When the treatment is applied most of the worms die and infections start almost simultaneously from a new infection wave contributed by the unaffected eggs in the environment. Experimental conditions, [40] as well as environmental conditions [25,33], could modify the results obtained in this study, however present data can be used as useful orientation in order to predict the infection progression and determine the adequate anthelmintic applications.

Acknowledgements

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Conclusions

As a result of this study the following conclusions were drawn:

1. The parasite fauna of *Seriola dumerili* in the western Mediterranean Sea is higher in species richness than previously thought. Seventeen out of the twenty-six parasite species recovered from the greater amberjacks of western Mediterranean fish are new registers: two new species for science (*Paradeontacylix balearicus* and *P. ibericus*), one new host and locality record (*Parabrachiella seriolae*), eight new host records (*Aphanurus* sp., *Capillaria* sp., *Camallanus* sp., *Cardiocephaloides* sp., *Cucullanus* sp., *Floriceps saccatus*, *Galactosomum lacteum*, *Lecithocladium* sp.) and six new locality records (*Caligus aesopus*, *Caligus* sp., *Neometanematobothrioides periorbitalis*, *Prosorhynchus facilis*, *Hysterothylacium seriolae*, Tetraphyllidean larva); and nine of them had been previously reported. According to their prevalence in fish from Majorca, most of these species are accidental (13). The rest of the species are mostly common (9) and a few of them are rare (2). Common species are found in all the localities studied.

2. The parasite community of juvenile *S. dumerili* is mostly composed by heteroxenous parasites, mainly trematodes, which are transmitted *via* food chain. This fish is the definitive host of most of them as it is a species at a high trophic level. It is only the intermediate host of two widespread and unspecific brain metacercariae species and two metacestodes. Most of the species found in *S. dumerili* are strict specialists or specialists of Carangidae. Therefore, phylogenetic relatedness seems to be crucial for parasite-community composition.

3. Seasonal infection patterns are observed in five out of the nine common species described in *S. dumerili* from Majorca: *Bucephalus gorgon*, *Hemiurus communis*, *Paradeontacylix. balearicus* (eggs), *Stephanostomum. ditrematis* and *Zeuxapta seriolae.* Prevalences apparently increase from autumn to winter in *B. gorgon* and in *H. communis*, and from spring to summer in *P. balearicus* (eggs), *S. ditrematis* and *Z. seriolae*.

4. Among the parasites reported from western Mediterranean Sea, blood flukes and monogeneans are the most dangerous species in culture conditions as they have already been involved in *S. dumerili* outbreaks in the Mediterranean. Metacercariae of heterophyid and strigeid species, didymozoids, caligids and gnathiids, are considered potentially harmful. They belong to taxa which include species causing severe infections although have not been associated with *S. dumerili* outbreaks. The rest of species are considered harmless or less hazardous because of their mild effects (e.g. bucephalids and hemiurids) or their low prevalence in this region (cestodes and nematodes).

5. Two new species of *Paradeontacylix* from *S. dumerili* are described: *P. balearicus* from the Balearic Islands, and *P. ibericus* from the Iberian Peninsula. Each species can be distinguished morphologically from the other species of the genus by its size and shape. Discriminant analyses separate these species from those infecting *S. dumerili* in Japan (*P. grandispinus* and *P. kampachi*). However, morphological differences between *P. grandispinus* and *P. balearicus* are not statistically significant.

6. The cladistic tree of the genus *Paradeontacylix* is in accordance with the phylogenetic tree based on molecular data. *Paradeontacylix* spp. split into two well supported clades, separating (*P. ibericus*+*P. kampachi*) from (*P. godfreyi*+(*P. balearicus*+*P. grandispinus*)); *P. odhneri* sequence was not available for molecular analyses although in cladistic tree this species diverges early. The lowest sequence differences are found between *P. grandispinus* and *P. balearicus* (0.2%, 2.5%, 6.3% for 28S, ITS2 and COI respectively) and, to a slightly smaller extent, between *P. kampachi* and *P. ibericus* (0.2%, 4.7%, 7%). *P. godfreyi* show the highest percentage of sequence divergence among all species, with values as high as 12.5% (ITS2) and 16.0% (COI).

7. Clustering of Japanese and Mediterranean species together highlights their morphological and molecular similarity over their geographical separation. These findings suggest that both groups of blood fluke species or their ancestors existed before both fish populations (Japanese and Mediterranean) were separated.

8. *Z. seriolae* infection levels observed in fish off Majorca from 2005 to 2007 are the highest recorded in wild *S. dumerili* to date, and similar or higher than those reported in epizootics of this parasite in fish culture. A recurrent and seasonal pattern of infection by *Z. seriolae* has been observed in the populations of *S. dumerili* off Majorca, with substantially higher parasite loads during the warm season (April to June). This is consistent, in spite of the significant trend of decrease in abundance from 2005 to 2007, and significantly correlated with the increases in mean and maximum seawater temperatures.

9. Parasitological data obtained from three additional localities in the western Mediterranean (off Alicante, Corsica and Sardinia) generally coincide with the seasonal pattern of abundance of *Z. seriolae* observed in Majorca, except for the low parasite loads in the population sampled off Sardinia. Geographical variation of the distribution and abundance of *Z. seriolae* on *S. dumerili* in the Mediterranean requires further exploration.

10. The high infection levels of *Z. seriolae* recorded do not significantly affect Fulton's condition factor. However, three lines of evidence indicate a possible parasite-induced mortality of

juvenile *S. dumerili*: (*i*) the significant negative association between abundance of *Z. seriolae* and fish length; (*ii*) the association between the increases in parasite abundance during the warm weather months and the sharp increases in monogenean aggregation levels and; (*iii*) the strong negative correlation between the levels of aggregation of *Z. seriolae* and mean fish length which indicates that heavily infected individuals are rapidly removed. Parasite mortality due to host immunity and seasonality in parasite transmission may have also contributed to the observed patterns in monogenean abundance and spatial aggregation.

11. Eggs of *Sparicotyle chrysophrii*, at 20ºC and *in vitro*, hatch after 5 to 10 days post deposition and hatching success is high (87.3 %). Most of the hatchings occur during darkness periods (> 75%), especially during the first and second nights after the first hatching. Nocturnal hatchings could be associated with parasite-host coordination as gilthead seabreams rest during night decreasing their gill activity and avoiding the parasite washing. Moreover this fish also group for foraging during dusk, increasing local density and easing the parasite transmission. Oncomiracidial survival time does not usually exceed 24h (<10 %) although larvae can live more than 2 days (52 h) at 20ºC. After emerging, most of the oncomiracidia (93%) swim vertically, although after 12 h this percentage descends to 15%. Thus, the period to actively find a new host is short and does not usually exceed 12 h.

12. Some new morphological observations on the development of the first post-larval stages of *S. chrysophrii* are recorded. The most relevant are: (*i*) lateral hooklets remain some time after clamp development, they are laterally displaced and finally fall off with surrounding tissue; (*ii*) pharynx early develops in a provisional posterior position which could affect its functionality. (*iii*) the hook size does not change from the development of the first pairs of clamps to the hook loss and, therefore, post-larvae of different sizes attach with hooks of the same size; (*iv*) four granular dark spots associated with the digestive tract are invariably visible in recently emerged specimens (hooks-and-no-clamp-bearing stages). This dark spots, usually interpreted as food waste, could also include yolk.

13. Three periods with different growth rates are distinguished in the development of *S. chrysophrii*. The first period (slow) finishes with the terminal lappet loss, after the development of 5 pairs of clamps. The second period (fast) lasts until specimen maturation. The last one (slow) is observed from maturation to the development of all the clamps (from 36 to 70 pairs). In accordance with these periods, common timings are observed for the terminal lappet loss and the attainment of maturity of all the microcotylids and heteraxinids compared (i.e. *Microcotyle spinicirrus*, *Microcotyle donavini*, *Microcotyle gotoi*, *Microcotyle sebastis*, *Microcotyle*

hiatulae, *Polylabroides multispinosus*, *Bivagina tai*, *Heteraxinoides xanthophilis*, *Heteraxine heterocerca* and *Z. seriolae*). The terminal lappet usually falls off when 10-20% of the clamps have been developed and attainment of maturity occurs when 60-70% of them have done it.

14. Pathological effects caused by attachment of *S. chrysophrii* change during its development. First, hooks pierce gill epithelium (during the first 21 days since emerging), and thereafter, clamps compress the gill filaments (from the seventh day). Therefore, exist a transition period of 14 days with combined action. Gradual clamp addition extends gill damage and decreases the breathing surface. Moreover, as parasites grow, blood ingestion increases and anaemia is more likely to occur.

15. Two treatment applications against *S. chrysophrii*, one against the worms on gills and the other against the oncomiracidia emerging from the surviving eggs (separated by 15 to 20 days), would be highly efficient and would allow for long periods without monogenean infection in cultures. Further treatments against worms could be postponed for at least 50 days: 36 days (worm maturation) + 10 days (maximum egg incubation time) + 3 days (maximum oncomiracidial survival).

Appendix

Table 10.1. Check-list of metazoan parasites of *Seriola dumerili*. Abbreviations: Atlantic Ocean (A), China Sea (CS), Indian Ocean (I), Mediterranean Sea (M), Pacific Ocean (P), North (N), South (S), East (E) West (W), Non-specified (Ns). Area is defined as follows: first [Centre/North /South]; second [East /West]; third [Location].

PARASITE	AREA	REFERENCE
CILIOPHORA		
Family Holophryidae Perty, 1852		
Cryptocaryon irritans Brown, 1951	WM	Rigos *et al.*, 2001; De la Gándara *et al.*, 2004
MYXOZOA		
Family Kudoidae Meglitsch, 1960		
Kudoa amamiensis Egusa et Nakajima, 1978	P	Sugiyama *et al.*, 1999; Ogawa, 2005; Burger *et al.*, 2008.
Kudoa insolita Shulman et Kovaleva, 1979	A	Kovaleva *et al.*, 1979 (in Swearer & Robertson, 1999)
Family Myxobolidae Thélohan, 1892		
Myxobolus acanthogobii Hoshina, 1952	P	Yokoyama *et al.*, 2004; Ogawa, 2005
Syn. *Myxobolus buri* Egusa, 1985		
Myxobolus sp.	WM	Grau *et al.*, 1999
ZYGOMYCOTA		
Family Microsporida incertae sedis		
Microsporidium seriolae Egusa, 1982	P	Ogawa, 2005; Yokoyama *et al.*, 2011
MONOGENEA		
Monopistocotylea		
Family Capsalidae Baird, 1853		
Benedenia seriolae (Yamaguti, 1934) Meserve, 1938	NWP	Egusa, 1983; Ogawa & Yokoyama, 1998; Cook *et al.*, 2001; Ernst *et al.*, , 2001; Ernst *et al.*, , 2002; Whittington, *et al.*, 2001a,b
Syn. *Epibdella seriolae* (Yamaguti, 1934)	Australia	Whittington & Chisholm, 2008
Neobenedenia 'girellae' ** (Hargis, 1955)	NWP	Williams & Bunkley-Williams, 1996*; Bondad-Reantaso, *et al.*,1995; Ogawa *et al.*, 1995; Ogawa & Yokoyama, 1998; Kinami *et al.*, 2005; Hirayama *et al.*, 2009; Ohno *et al.*, 2009; Hirazawa *et al.*, 2010; Whittington, 2012
	Australia	Deveney *et al.*, 2001
Neobenedenia 'melleni' ** (MacCallum, 1927) Yamaguti, 1963	NWP	Ogawa *et al.*, 1995
Syn. *Epibdella melleni* (MacCallum, 1927)	SCS	Wang *et al.*, 2004

*Williams & Bunkley-Williams (1996) reported that "*Seriola dumerili* has also been called *S. lalandi*". Consequently, some of the species assigned to *S. dumerili* in this publication could also be parasites of *S. lalandi*. **Whittington (2004 & 2005) has hypothesized that *Neobenedenia girellae* and *N. melleni* could each represent a complex of morphologically indistinguishable species.

PARASITE	AREA	REFERENCE
Polyopisthocotylea		
Family Discocotylidae Price, 1936		
Vallisia striata Parona et Perugia, 1890	CM	Palombi, 1949
Family Gastrocotylidae Price, 1943		
Gastrocotylinea sp.	CWA	Kritsky *et al.*, 2011
Pseudaxinoides vietnamensis Lebedev, Parukhin et Roitman, 1970	SCS	Parukhin, 1976; Lebedev, 1970; Lebedev, 1986 (in: Zhang *et al.*, 2003
Family Heteraxinidae Unnithan, 1957		
Allencotyla mcintoshi Price, 1962	NWA	Williams & Bunkley-Williams, 1996*
	WM	Montero, 2001; Montero *et al.*, 2001a-c; 2003a; Nielsen *et al.*, 2003; Repullés *et al.*, 2005
Heteraxine heterocerca Goto 1894	P	Ogawa & Fukudome, 1994; Ogawa & Yokohama, 1998
	WM	Grau *et al.*, 1999
Heteraxine sp. Yamaguti, 1938	WM	Di Cave *et al.*, 2004
Zeuxapta seriolae (Meserve 1938) Price, 1962	P	Ogawa & Fukudome, 1994; Ogawa & Yokohama, 1998
Syns. *Axine seriolae* Meserve, 1938; *Microcotyle seriolae* Yamaguti, 1940; *Z. zyxivaginata* Unnithan, 1957; *Zeuxapta japonica* Yamaguti, 1963; *Z. seriolae australica* Lebedev, 1968	SCS	Zhang *et al.*, 2003
	WM	Giannetto *et al.*, 1998; Montero & Raga, 2000; Montero, 2001; Montero *et al.*, 2001a-c; Grau *et al.*, 2003; Montero *et al.*, 2004; Repullés *et al.*, 2005; Lia *et al.*, 2007; Genç *et al.*, 2007; **Current study**
Zeuxapta sp.	WM	De Liberato *et al.*, 2000
Family Microcotylidae Taschenberg, 1879		
Aspinatrium kahala Yamaguti, 1968	P	Yamaguti, 1968; Williams & Bunkley-Williams, 1996*
Tonkinaxine homocerca Lebedev, Parukhin & Roitman, 1970	SCS	Parukhin, 1976; Lebedev, 1970; Lebedev *et al.*, 1970
TREMATODA		
Family Acanthocolpidae Lühe, 1906		
Acanthocolpus liodorus Luhe, 1906	WM	Grau *et al.* 1999
Stephanostominae sp.	WM	Montero, 2001; Montero *et al.*, 2001b
Stephanostomum cesticillum Molin, 1858	WM	Bartoli & Bray, 2001

*Williams & Bunkley-Williams (1996) reported that "*Seriola dumerili* has also been called *S. lalandi*". Consequently, some of the species assigned to *S. dumerili* in this publication could also be parasites of *S. lalandi*. **Whittington (2004 & 2005) has hypothesized that *Neobenedenia girellae* and *N. melleni* could each represent a complex of morphologically indistinguishable species.

PARASITE	AREA	REFERENCE
Stephanostomum ditrematis (Yamaguti, 1939) Manter, 1947 Syns. *Echinostephanus ditrematis* (Yamaguti, 1939) and *Stephanostomum seriolae* Yamaguti, 1970	NWA	Cordero *et al.*, 1980; Gijón-Botella *et al.*, 1992;Williams & Bunkley-Williams, 1996*
	SCS	Parukhin, 1966; 1976; Shen, 1990a; Huanf, 1994
	CP	Yamaguti, 1970; Williams & Bunkley-Williams, 1996*; Bray & Cribb, 2003
	WM	Montero, 2001; Montero *et al.*, 2001b; **Current study**
Stephanostomum euzeti Bartoli et Bray, 2004	WM	Bartoli & Bray, 2004; **Current study**
Stephanostomum filiforme Linton, 1940	WM	Bartoli & Bray, 2004; **Current study**
Stephanostomum hispidum Yamaguti, 1934	P	Manter, 1940; Williams & Bunkley-Williams, 1996*
Stephanostomum orientalis Srivastava, 1939	SCS	Parukhin 1966; 1976
Stephanostomum petimba Yamaguti, 1970	WM	Bartoli & Bray, 2004; **Current study**
Stephanostomum pristis Deslongchamps, 1824	WM	Grau *et al.* 1999
Tormopsolus hawaiiensis Yamaguti, 1970	CP	Yamaguti, 1970; Williams & Bunkley-Williams, 1996*; Bray & Cribb, 2001
Tormopsolus orientalis Yamaguti, 1934 Syn. *Tormopsolus medius* Reimer, 1983	CWA	Yamaguti, 1934; Nahhas & Cable, 1964; Nahhas & Carlson, 1994; Williams & Bunkley-Williams, 1996*; Bray & Cribb, 2001
	WM	Bartoli *et al.*, 2004; **Current study**.
Family Aporocotylidae Odhner, 1912 *Paradeontacylix balearicus* Repullés-Albelda, Montero, Holzer, Ogawa, Hutson et Raga, 2008	WM	**Current study** (Repullés-Albelda *et al.*, 2008)
Paradeontacylix grandispinus Ogawa et Egusa, 1986	NWP	Ogawa *et al.*, 1993; Ogawa & Fukudome, 1994; Williams & Bunkley-Williams, 1996*; Smith, 1997b; Ogawa & Yokoyama, 1998
Paradeontacylix ibericus Repullés-Albelda, Montero, Holzer, Ogawa, Hutson et Raga, 2008	WM	**Current study** (Repullés-Albelda *et al.*, 2008)

*Williams & Bunkley-Williams (1996) reported that "*Seriola dumerili* has also been called *S. lalandi*". Consequently, some of the species assigned to *S. dumerili* in this publication could also be parasites of *S. lalandi*. **Whittington (2004 & 2005) has hypothesized that *Neobenedenia girellae* and *N. melleni* could each represent a complex of morphologically indistinguishable species.

PARASITE	AREA	REFERENCE
Paradeontacylix kampachi Ogawa et Egusa, 1986	NWP	Ogawa *et al.*, 1993; Ogawa & Fukudome, 1994; Williams & Bunkley-Williams, 1996*; Smith 1997b; Ogawa & Yokoyama, 1998
	WA	Williams & Bunkley-Williams, 1996*
	WM	Montero, 2001; Montero *et al.*, 2001b; Montero *et al.*, 2003b
Paradeontacylix sanguinicoloides McIntosh, 1934	NWA	Williams & Bunkley-Williams, 1996*
Paradeontacylix cf. *kampachi*	WAustralia	Hutson *et al.*, 2011
Paradeontacylix sp.	WM	Grau *et al.*, 1999; Montero *et al.*, 1999; Di Cave *et al.*, 2004
	WAustralia	Hutson *et al.*, 2011
Sanguinicolidae sp.	WM	Smith, 1997b
Family Bucephalidae Poche, 1907		
Bucephalus gorgon (Linton, 1905) Eckmann, 1932	NWA	Williams & Bunkley-Williams, 1996*
Syns. *Gasterostomum gorgon* Linton, 1905; *Nannoenterum gorgon* (Linton, 1905) Linton, 1940	CWA	Manter, 1940
	CWA	Corkum, 1968; Manter, 1940
	EM	Fischthal, 1982; Fischthal *et al.*, 1982
	WM	Montero, 2001; Montero *et al.*, 2001b; Bartoli *et al.*, 2005; **Current study**
Bucephalus paraheterotentaculatus Velasquez, 1959	SCS	Parukhin, 1966 Parukhin, 1976
Bucephalus polymorphus Von Baer, 1827	WM	Grau *et al.*, 1999
Bucephalus margaritae Ozaki & Ishibashi, 1934	NWA	Williams & Bunkley-Williams, 1996*
Syn. Bucephalus varicus Manter, 1940	CWA	Manter, 1940; Nahhas & Cable, 1964
Prosorhynchus crucibulum Rudolphi, 1819	NWA	Williams & Bunkley-Williams, 1996*
	WM	Grau *et al.*, 1999
Prosorhynchus facilis (Ozaki, 1924) Eckmann, 1932	NWA	Eckmann, 1932 (in Yamaguti, 1971);
	SCS	Gu & Shen, 1976; Shen, 1990a
	ECS	Shih *et al.*, 2004
	WM	**Current study**
Prosorhynchus kahala Yamaguti, 1970	CP	Yamaguti, 1970; Williams & Bunkley-Williams, 1996*
	WA	Williams & Bunkley-Williams, 1996*
Prosorhynchoides sp.	Persian Gulf	Al Kawari *et al.*, 1996
Syn. *Bucephalopsis sp.*	WM	Grau *et al.*, 1999

*Williams & Bunkley-Williams (1996) reported that "*Seriola dumerili* has also been called *S. lalandi*". Consequently, some of the species assigned to *S. dumerili* in this publication could also be parasites of *S. lalandi*. **Whittington (2004 & 2005) has hypothesized that *Neobenedenia girellae* and *N. melleni* could each represent a complex of morphologically indistinguishable species.

PARASITE	AREA	REFERENCE
Rhipidocotyle bartolii Bray et Justine, *2011* Syn. *Bucephalopsis longicirrus* Nagaty, 1937	WM	Bartoli *et al.*, 2005
Rhipidocotyle sp.	WM	Montero, 2001; Montero *et al.*, 2001b
Family Didymozoidae (Monticelli, 1888)		
Didymozoid sp.	WM	Montero, 2001; Montero *et al.*, 2001b
Koellikeria micropterygis Richardi, 1902	M	Williams & Bunkley-Williams, 1996*
Metanematobothrium seriolae Shen, 1990	SCS	Ku & Shen (1965); Shen, 1990a
Nematobothrium scombri (Taschenberg, 1879)	WM	Grau *et al.*, 1999
Neometanematobothrioides periorbitalis Yamaguti, 1970	P	Yamaguti, 1970; Pozdnyakov, 1996; Williams & Bunkley-Williams, 1996*
	WA	Williams & Bunkley-Williams, 1996*
	WM	**Current study**
Neometanematobothrioides sp.	WM	Montero, 2001; Montero *et al.*, 2001b
Patellokoellikeria seriolae Yamaguti, 1970	CP	Yamaguti, 1970; Pozdnyakov, 1996 Shen, 1990b
	SCS	
Tergestia acanthocephala (Stossich, 1887)	WM	Bartoli *et al.*, 2003
Tergestia laticollis Rudolphi, 1819	WM	Montero, 2001; Montero *et al.*, 2001b
Wedlia bipartita (Wedl, 1855) *Syn. Koellikeria bipartita* (Wedl, 1855) Ishii, 1935	NEA	Williams & Bunkley-Williams, 1996*
	M	Williams & Bunkley-Williams, 1996*
	WM	Grau *et al.*, 1999
Family Hemiuridae Looss, 1899		
Aphanurus sp.	WM	**Current study**
Ectenurus lepidus Looss, 1907	CWP	Nahhas & Cable, 1964
	EM	Fischthal, 1982
	Ns	Williams & Bunkley-Williams, 1996*
Ectenurus sp.	Persian Gulf	Al Kawari *et al.*, 1996
Hemiurus communis Odhner, 1905	WM	Grau *et al.*, 1999; **Current study**
Lecithochririum jaffense Fischthal, 1982	EM	Fischthal, 1982
	Ns	Bray, 1991
Lecithochririum microstomum Chandler, 1935	CWP	Nahhas & Cable, 1964
	NWA	Williams & Bunkley-Williams, 1996*

*Williams & Bunkley-Williams (1996) reported that "*Seriola dumerili* has also been called *S. lalandi*". Consequently, some of the species assigned to *S. dumerili* in this publication could also be parasites of *S. lalandi*. **Whittington (2004 & 2005) has hypothesized that *Neobenedenia girellae* and *N. melleni* could each represent a complex of morphologically indistinguishable species.

PARASITE	AREA	REFERENCE
Lecithochririum parvum Manter, 1947	CWP	Nahhas & Cable, 1964
Syn. Brachyphallus parvus (Manter, 1947)	CWA	Williams & Bunkley-Williams, 1996*
Lecithocladium sp.	WM	**Current study**
Parahemiurus merus Linton, 1910	CWP	Nahhas & Cable, 1964
	NWA	Bray, 1990; Williams & Bunkley-Williams, 1996*
	M	Bray, 1990
	EM	Fischthal, 1982
Family Heterophydae Odhner 1914		
Galactosomum sp.	WM	**Current study**
Family Lecithasteridae Odhner 1905		
Aponurus sp.	WM	Grau *et al.*, 1999
Family Lepocreadiidae Odhner 1905		
Lepocreadium pegorchis (Stossich, 1901) Stossich, 1904	EM	Fischthal, 1982
Family Sclerodistomidae Odhner, 1927		
Sclerodistomum italicum Stossich, 1893	CEA Senegal	Fischthal & Thomas, 1972
Family Strigeidae Railliet, 1919		
Cardiocephaloides sp.	WM	**Current study**
CESTODA (larval forms)		
Family Bothriocephalidae Blanchard, 1849		
Bothriocephalus sp. (larval form)	NWA	Williams & Bunkley-Williams, 1996*
Family Lacistorhynchidae Guiart, 1937		
Dasyrhynchus giganteus (Diesing, 1850)	NWA	Williams & Bunkley-Williams, 1996*
Dasyrhynchus variouncinatus (Pintner, 1913)	Ns	Bates, 1990
Syn. *Halysiorhynchus variouncinatus* Pintner, 1913		
Floriceps sp.	WM	Montero, 2001; Montero *et al.*, 2001b
Floriceps saccatus Cuvier, 1817	WM	**Current study**
Protogrillotia zerbiae (Palm, 1995)	NWA	Williams & Bunkley-Williams, 1996*
Syn. *Pseudogrillotia zerbiae* Palm, 1995	A	Palm, 1995
Family Pterobothriidae Pintner, 1931		
Family Tentaculariidae Poche, 1926		
Heteronybelinia estigmena Dollfus, 1960	NW Africa	Palm & Walter, 2000
Nybelinia punctatissima Dollfus, 1996	Ns	Bates, 1990
	SEA	Williams & Bunkley-Williams, 1996*

*Williams & Bunkley-Williams (1996) reported that "*Seriola dumerili* has also been called *S. lalandi*". Consequently, some of the species assigned to *S. dumerili* in this publication could also be parasites of *S. lalandi*. **Whittington (2004 & 2005) has hypothesized that *Neobenedenia girellae* and *N. melleni* could each represent a complex of morphologically indistinguishable species.

PARASITE	AREA	REFERENCE
Nybelinia sp.	SCS	Lebedev 1970
Tetraphyllidean larvae	NWA	Williams & Bunkley-Williams, 1996*
	WM	**Current study**
NEMATODA		
Family Ascarididae Baird, 1853		
Porrocaecum **sp.**	SCS	Parukhin, 1964; 1966; Lebedev, 1970; Parukhin, 1976
Family Anisakidae Skrjabin et Karokhin, 1945		
Anisakis physeteris Baylis, 1923	WM	Montero, 2001; Montero *et al.*, 2001b
Anisakis sp.	NWA	Yoshinaga *et al.*, 2006
	SCS	Lebedev 1970
	CS	Yoshinaga *et al.*, 2006
Contracaecum sp. larva	SCS	Parukhin 1964; Lebedev 1970; Parukhin, 1976
Family Cucullanidae Cobbold, 1864		
Cucullanus sp.	WM	**Current study**
Family Camallanidae Railliet et Henry, 1915		
Camallanus sp.	WM	**Current study**
Family Raphidascarididae Hartwich, 1954		
Hysterothylacium seriolae Yamaguti, 1941	P	Yamaguti 1941; Deardorff & Overstreet, 1981; Williams & Bunkley-Williams, 1996*
	WM	**Current study**
Hysterothylacium sp.	NWA	Williams & Bunkley-Williams, 1996*
Family Trichuridae Railliet, 1915		
Capillaria sp.		**Current study**
Family Philometridae Baylis et Daubney, 1926		
Philometra globiceps (Rudolphi, 1819) Railliet, 1916	WM	Grau *et al.*, 1999
Philometra sp.	WM	Montero, 2001; Montero *et al.*, 2001b; Moravec *et al.*, 2003;
ACANTHOCEPHALA		
Family Rhadinorhynchidae Travassos, 1923		
Gorgorhynchoides elongatus Cable et Lideroth, 1963	NWA	Williams & Bunkley-Williams, 1996*

*Williams & Bunkley-Williams (1996) reported that "*Seriola dumerili* has also been called *S. lalandi*". Consequently, some of the species assigned to *S. dumerili* in this publication could also be parasites of *S. lalandi*. **Whittington (2004 & 2005) has hypothesized that *Neobenedenia girellae* and *N. melleni* could each represent a complex of morphologically indistinguishable species.

PARASITE	AREA	REFERENCE
COPEPODA		
Family Caligidae Burmeister, 1835		
Caligus aesopus Wilson, 1921	P	Lin & Ho, 2007
	WM	**Current study**
Caligus curtus OF Müller, 1785	WM	Grau *et al.*, 1999
Caligus diaphanus von Nordmann, 1832	WM	Montero, 2001; Montero *et al.*, 2001b
Caligus lalandei Barnard 1948	SEA	Williams & Bunkley-Williams, 1996*
Caligus sp.	NWA	Ogawa & Yokoyama, 1998
	WM	**Current study**
Lepeophtheirus sp.	M	Raibaut *et al.*, 1998
	SCM	Benmansour & Ben-Hassine, 2009
Family Lernaeopodidae Milne Edwards, 1840		
Brachiella thynni Cuvier, 1830	NWA	Williams & Bunkley-Williams, 1996*
Eobrachiella elegans (Richiardi, 1880)	M	Raibaut *et al.*, 1998
	SCM	Benmansour & Ben-Hassine, 2009
	Ns	Williams & Bunkley-Williams, 1996*
Parabrachiella seriolae (Yamaguti et Yamasu, 1960)	WM	**Current study**
Family Lernanthropidae Kabata, 1979		
Lernanthropus giganteus Krøyer, 1863	NWA	Williams & Bunkley-Williams, 1996*
Lernanthropus micropterygis Richiardi, 1884	M	Williams & Bunkley-Williams, 1996*;Raibaut *et al.*, 1998
Family Pandaridae Milne Edwards, 1840		
Nesippus crypturus Heller, 1865	P	Williams & Bunkley-Williams, 1996*
Syns.: *Nesippus costatus* Wilson CB, 1924; *Nesippus gracilis* Wilson CB, 1935; *Nesippus occultus* Wilson CB, 1924		
Family Pennellidae Burmeister, 1835		
Pennella filosa (Linaeus, 1758)	EM	Tuncer *et al.*, 2010
Syns.: *Lernaea cirrhosa* La Martiniere, 1787; *Pennatula filosa* Linnaeus, 1758; *Pennella crassicornis* Steenstrup et Lütken, 1861; *Pennella germonia* Leigh-Sharpe, 1931;*Pennella germonia fagei* Poisson & Razet, 1954;*Pennella histiophori* Thomson GM, 1890; *Pennella orthagorisci* Wright EP, 1870; *Pennella plumosa* DeKay, 1844; *Pennella pustulosa* Baird, 1847; *Pennella remorae* Murray, 1856; *Pennella rubra* Brian, 1906; all them in Boxshall (2012)		
Pennella instructa Wilson CB, 1917	EM	Öktener, 2009b
Syn. Pennella zeylanica Kirtisinghe, 1932		
Lernaeenicus longiventris Wilson CB, 1917	NWA	Williams & Bunkley-Williams, 1996*;

*Williams & Bunkley-Williams (1996) reported that "*Seriola dumerili* has also been called *S. lalandi*". Consequently, some of the species assigned to *S. dumerili* in this publication could also be parasites of *S. lalandi*. **Whittington (2004 & 2005) has hypothesized that *Neobenedenia girellae* and *N. melleni* could each represent a complex of morphologically indistinguishable species.

PARASITE	AREA	REFERENCE
Family Philichthyidae Vogt, 1877		
Colobomatus lichiae (Richiardi, 1880) Syns. *Philichthys lichiae* Richiardi, 1877; *Polyrrhynchus lichiae* (Richiardi, 1877); *Richiardia lichiae* Bassett-Smith, 1899; all them in Boxshall (2012)	P	Williams & Bunkley-Williams, 1996*
	M	Williams & Bunkley-Williams, 1996*; Raibaut *et al.*, 1998
ISOPODA		
Family Gnathiidae Leach, 1814		
Gnathia vorax Lucas, 1849	WM	Grau *et al.*, 1999; **Current study**
ANNELIDA		
Family Piscicolidae Johnston, 1865		
Limnotrachelobdella okae Moore, 1924	NWP	Nagasawa & Hirai, 2009

*Williams & Bunkley-Williams (1996) reported that "*Seriola dumerili* has also been called *S. lalandi*". Consequently, some of the species assigned to *S. dumerili* in this publication could also be parasites of *S. lalandi*. **Whittington (2004 & 2005) has hypothesized that *Neobenedenia girellae* and *N. melleni* could each represent a complex of morphologically indistinguishable species.

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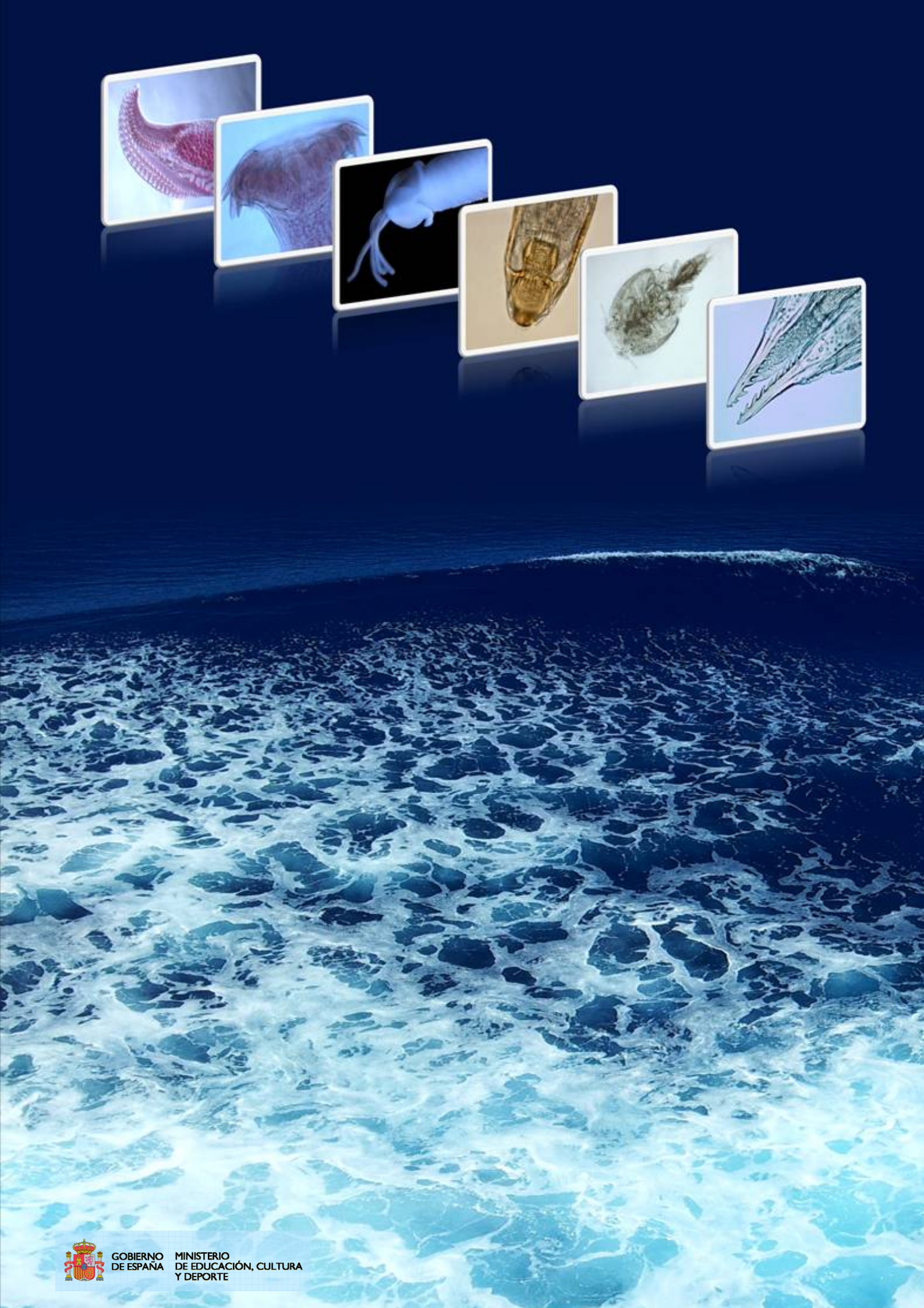

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