HELMINTHS FROM THE DIGESTIVE TRACT OF THE STRIPED DOLPHIN *(STENELLA COERULEOALBA)* IN THE WESTERN MEDITERRANEAN:

INFECTION PATTERNS WITHIN A LONG-TERM SCALE

Tesis doctoral por:
Paula Mateu Puncel
Directores:
Fco. Javier Aznar Avendaño
Mercedes Fernández Martínez
Valencia, mayo 2015

PROGRAMA DE DOCTORADO
EN BIODIVERSIDAD 3001

VNIVERSITAT DE VALÈNCIA
Facultat de Ciències Biològiques

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Directores

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CERTIFICAN que Dª **Paula Mateu Puncel** ha realizado bajo nuestra dirección, y con el mayor aprovechamiento, el trabajo de investigación recogido en esta memoria y que lleva por título **"Helminths from the digestive tract of the striped dolphin (*Stenella coeruleoalba*) in the western Mediterranean: infection patterns within a long-term scale"**, para optar al grado de Doctora en Ciencias Biológicas.

Y para que así conste, en cumplimiento de la legislación vigente, expedimos el presente certificado en Valencia a 11 de mayo de 2015.

Firmado: Fco. Javier Aznar Avendaño

Firmado: Mercedes Fernández Martínez

A Diego y Ana

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SUMMARY

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Cetaceans harbour a relatively diverse and highly specific fauna of trophically-transmitted metazoan parasites including digeneans, cestodes, nematodes and acanthocephalans (Aznar *et al*., 2001; Raga *et al*., 2009). Due to the origin of host-parasite associations (see Section 1.1.2) and the phylogenetic distance between cetaceans and other marine vertebrates, most helminth species found in cetaceans are specific to them. Therefore, the composition and species richness of helminth faunas from any cetacean species in any locality (i.e., the helminth component community) is expected to be restricted by two factors. First, the local diversity of helminth species would largely result from an interplay between regional history and local ecology (Holmes *et al*., 1990). Second, cetaceans would behave as an isolated group for parasitic exchange, which would not share helminth species with other marine vertebrates and, therefore, the local community of cetacean species would be the most relevant factor setting the upper limits of diversity and composition of their helminth faunas (Aznar *et al*., 1998). At the level of individual hosts (infracommunity level), species richness and composition would primarily depend on the probability of encounter between hosts and parasites. It has been suggested that the infective stages of helminths in the marine realm are highly "diluted" (Hoberg, 1996, 2005; Raga *et al*., 2009; Valente *et al*., 2009; Santoro *et al*., 2010), thus the likelihood of contact between cetaceans and infected prey would be low. This could bring about two consequences. First, helminth infracommunities should be depauperate compared with the richness observed at component community; second, infracommunities should constitute random subsets of the pool of species of the component community. These predictions about the structure and composition of helminth communities of cetaceans have been tested only in a few species (Raga *et al*., 2009) and, therefore, much more data are required from other systems to confirm them.

On the other hand, the peculiarities associated with trophically-transmitted parasites also make them ideal candidates to monitor not only trophic interactions, but also long-term changes in the ecosystem. However, the use of helminths as biological indicators of global change is far from understood and requires further investigation. To our knowledge, long-term (i.e., decadal) parasitological studies have never been carried out in the case of cetaceans due to the difficulties to obtain appropriate host sample sizes. Certainly, there are surveys collating parasitological data for long periods (e.g., Carvalho *et al*., 2010), but data have never analyzed using an explicit framework to analyze temporal changes.

A key factor to interpret factors that drive structure and temporal dynamics of helminth communities is a good knowledge of life cycles. However, very little is known about the life-cycles of helminths from cetaceans due to the obvious difficulties associated with working on hosts living in the marine realm, especially those inhabiting oceanic waters (Aznar *et al*., 2001; Raga *et al*., 2009). In general, there are major gaps about the identity of intermediate and paratenic hosts (if any) of the taxa infecting cetaceans. In fact, the complete life-cycle of trophically-transmitted cetacean helminths has only been elucidated for a few species, i.e. *Anisakis* spp.

The striped dolphin, *Stenella coeruleoalba*, is the most abundant cetacean in central Spanish Mediterranean waters (Gómez de Segura *et al*., 2006). Parasitological research on this species is scarce and based on small host sample sizes. The marine mammal stranding network of the Marine Zoology Unit, University of Valencia, has been collecting data from this species from western Mediterranean waters since the late 1980s. Animals were found stranded dead or alive along the coasts of the Valencian Community, although in 1990 additional samples were available from Catalonia and Murcia. This has provided an invaluable set of long-term parasitological data which makes the striped dolphin a good candidate to investigate the factors that determine the structure and composition of helminth communities from the digestive tract, to explore long-term changes in their structure and to elucidate the life cycles of its helminth species.

The following objectives have been addressed:

1. To describe the helminth fauna infecting the digestive tract of the striped dolphin, *Stenella coeruleoalba*, from the western Mediterranean.

2. To investigate the factors which determine the structure and composition, including specificity, of the helminth communities.

3. To explore long-term changes in the structure of helminth communities.

4. To search cetacean helminth larvae down the trophic web aiming to elucidate life-cycles and routes of infection.

The intestinal helminth community of the striped dolphin in western Mediterranean waters was investigated based on a sample of 52 animals stranded along the coasts of Catalonia, Valencian Community and Murcia in 1990. The community was composed of three species of tetrabothriid cestode, namely, *Tetrabothrius forsteri*, *Trigonocotyle globicephalae* and *Strobilocephalus triangularis*, and immature individuals of the acanthocephalan *Bolbosoma vasculosum*. All these species are specific to cetaceans but *B. vasculosum* probably reproduces in other cetaceans, perhaps sympatric fin whales. Infection levels were low except for *T. forsteri*. At infracommunity level, co-occurrence of helminth species exhibited just a slight excess of positive associations between species. Overall, these data could be interpreted as evidence that some helminth species (most likely tetrabothriid species) use the same intermediate/paratenic hosts, or simply as evidence that some species tend to co-occur simply because one of them (*T. forsteri*) is highly prevalent. Significant effects of host body length, age or sex on the abundance of any of the helminth species, or on infracommunity descriptors (total abundance, species richness and Brillouin's diversity index) were not detected. Note, however, that the host sample was mostly composed of adult animals and, therefore, ontogenetic effects on infection levels cannot be ruled out (see also below). Overall, the observation of depauperate, largely unpredictable helminth infracommunities agrees with the hypothesis that large and vagile oceanic predators have few contacts with infective stages of parasites.

Two species of the family Brachycladiidae, namely, *Brachycladium atlanticum* and *Oschmarinella rochebruni*, were found infecting the hepato-pancreatic ducts of striped dolphins (n= 103). A parasitological survey of four additional sympatric cetacean species, i.e., Risso's dolphin (n= 18), bottlenose dolphin (n= 14), common dolphin (n= 8) and long-finned pilot whale (n= 5) indicated that *O. rochebruni* was restricted to the striped dolphin, whereas *B. atlanticum* was found in both striped dolphins and common dolphins. Infection parameters were not significantly different between both dolphin species. In the striped dolphin, host individual exerted significant effects upon the morphology and fecundity of *B. atlanticum* but these effects did not result from density-dependence (i.e., crowding effects). However, a question that would deserve further investigation is to ascertain the proportion of worms from the same individual dolphin that is genetically identical. Digeneans multiply asexually within the first intermediate (mollusc) host and, therefore, worms might be recruited as clonal packets through the food web up to the cetacean definitive host. If so, intrahost variability in individual cetaceans might be lower than interhost variability, such as we observed. Interhost variability of morphological traits related to host individual had previously been reported in other brachycladiids. Our study therefore support the claim by other authors that diagnosis at the species level based on morphometry or morphometrical ratios might be prone to error. Individuals of *B. atlanticum* collected from common dolphins had a significantly smaller body size and fecundity than those from striped dolphins. Our analyses also suggested that the effects upon fecundity were a mere consequence of having a smaller body size. *Brachycladium atlanticum* is therefore able to reproduce in common dolphins but its fitness is arguably lower than that in the striped dolphin. In summary, specificity of *O. rochebruni* for striped dolphins could be defined by a contact filter, whereas that of *B. atlanticum* could result from a combination of both contact and compatibility filters.

The gastric helminth fauna of the striped dolphin in western Mediterranean waters was composed of one digenean species, *Pholeter gastrophilus*, and one nematode species, *Anisakis pegreffii*. All stomach chambers were infected with *P. gastrophilus*, but the bulk of fibrotic nodules was found in the fundic stomach. The number of nodules of *P. gastrophilus* did not correlate significantly with the length or age of the dolphins, and there were not significant differences between sexes. The identity of *A. pegreffii* was ascertained based on molecular analysis of both larval and adult individuals. Most anisakid individuals were collected in the pyloric stomach, and some in the forestomach; no worms were detected within the fundic stomach. *Anisakis pegreffii* showed low infection levels in Mediterranean striped dolphins compared with infection of *Anisakis* spp. in other cetaceans. As noted in Chapter 4, the probability of transmission is especially low for trophically-transmitted helmiths in the oceanic realm because infective stages are highly "diluted" (Valente *et al*., 2009 and Santoro *et al*., 2010 for marine turtles; Hoberg, 1996, 2005 for marine birds; and Raga *et al*., 2009 for marine mammals). Mateu *et al*. (2015) reported a prevalence of 1.4% of *A. pegreffii* in *N. elongatus* (see Chapter 8), and the present study could support the idea that, in western Mediterranean waters, striped dolphins become infected with *A. pegreffii* by the ingestion of, *inter alia*, parasitized myctophid species.

During the period 1990-2010, two events could have impacted recruitment of trophically-transmitted helminths of striped dolphins. First, the western Mediterranean population of striped dolphin suffered two outbreaks of mortality (in 1990 and 2007) caused by the dolphin morbillivirus (Raga et al. 2008). Although the total number of individuals killed could not be calculated, Aguilar and Raga (1993) suggested that several thousands individuals could have died. Second, there is correlational evidence that overfishing of sardine, *Sardina pilchardus*, one of the putative main prey species of striped dolphins in the area, may have caused a significant dietary shift towards demersal prey, particularly hake, *Merluccius merluccius* (Gómez-Campos *et al*., 2011). Therefore, we investigated the long-term dynamics of the intestinal helminth community of striped dolphin based on a sample of 128 animals collected during the period 1990-2010. Two sets of data were defined: (i) dolphins which died from DMV outbreaks during 1990 and 2007 (Epizootic sample) (n= 66), and (ii) dolphins which stranded from unknown causes during other years (Non-epizootic sample) (n= 62). In addition, the BIO-ENV procedure of PRIMER (Clarke and Gorley, 2006) was used to explore whether there was a significant relationship between the structure of the intestinal helminth community and the diet of the striped dolphin. This analysis was carried out based on a sample of 71 dolphins from which both parasitological and dietary data were available. Out of the 128 dolphins examined, only 4 were uninfected. The very same 4 helminth species, i.e. *Tetrabothrius forsteri*, *Trigonocotyle globicephalae*, *Strobilocephalus triangularis* and *Bolbosoma vasculosum* were found in this enlarged sample of striped dolphin (see Chapter 4). This strongly suggests that specificity likely prevents the contact and/or establishment of other helminths (Mateu *et al*., 2011), i.e., there is a 'pool exhaustion' of all potential local immigrants to the community. A PERMANOVA analysis indicated that there were significant differences in the structure of the helminth community between the 'epizootic' samples. In particular, *B. vasculosum* was fairly frequent in 1990 (prevalence: 51.9%) and did not appear in 2007; differences in abundance were highly significant. In contrast, differences in community structure were not significant in the comparison between non-epizootic samples. Furthermore, we did not find a significant relationship between diet and community structure. Thus, the question remains whether we are unable to detect clear long-term changes in the helminth fauna of *S. coeruleoalba* because we analysed only heavily parasitized animals (i.e., a non-random sample) or because there were really no changes to be detected. In any event, it seems clear that infection levels of *B. vasculosum* were significantly higher in 1990. There are no specific changes in diet to blame because the identity of potential paratenic hosts for *B. vasculosum* could not be ascertained through dietary analysis. Whether 1990 was an exceptional year is therefore an open question.

According to the above results, the description of larval stages of helminths infecting striped dolphins and the identification of intermediate and/or paratenic hosts of these parasites became an important task to assist interpretation of patterns in definitive hosts. Thus, we selected species reported as important prey for striped dolphins and other oceanic cetacean species, i.e. mesopelagic fish and cephalopods, and examined them for infective stages of cetacean helminths. In particular, we analyzed 1012 individuals of eight myctophid species (*Ceratoscopelus maderensis*, *Lampanyctus crocodilus*, *Notoscopelus elongatus*, *Benthosema glaciale*, *Myctophum punctatum*, *Lobianchia dofleini*, *Diaphus holti* and *Hygophum benoiti*) and 792 individuals of two cephalopod species (*Alloteuthis media* and *Sepietta oweniana*). Hosts were collected during 2010-2012 from the Gulf of Valencia and Alboran Sea (Spanish Mediterranean), which include localities proposed as Protected Areas of Mediterranean Importance due to the high cetacean diversity and abundance. Five helminth taxa were found in myctophiids, and none in cephalopods. Only the nematodes *Anisakis pegreffii* and *A. physeteris* were identified as larvae of species infecting cetaceans, and were found only in *N. elongatus* and *C. maderensis* with very low prevalence (overall prevalence for *Anisakis*: 8.1% and 0.5%, respectively). Their prevalence in *N. elongatus* was significantly higher than that from the other three myctophid species with n>50 individuals. Our study suggests, for the first time, that myctophids could play a role as paratenic hosts in the oceanic life-cycle of *A. pegreffii* and *A. physeteris* in the western Mediterranean. None of the other larvae identified at least to family level infect cetaceans, but some of them can be transmitted to large predatory fish. The extreme scarcity of such cetacean parasites in this, and previous parasitological surveys of mesopelagic fish and cephalopods is at odds with the key role of these preys in the diet of oceanic cetaceans.

Infective stages of trophically-transmitted helminth circulate through oceanic food webs and, therefore, they can be detected in many fish and cephalopod species even though many of them are not prey of cetaceans. For this reason, we investigated the helminth fauna of *Bathypterois mediterraneus*, a demersal deep-sea that is not consumed by striped dolphins, but (i) it is the most common fish below 1500m in western Mediterranean waters (Carrasson and Matallanas, 2001); (ii) it occurs on the continental slope, where striped dolphins are frequently found (Gómez de Segura *et al*., 2008) and (iii) the most important food items of its diet are benthopelagic planktonic calanoid copepods (Carrasson and Matallanas, 2001) which may act as intermediate and/or paratenic hosts for cetacean parasites. In July 2010, 170 specimens of *B. mediterraneus* were captured from the continental slope in Western Mediterranean waters. Samples were obtained from the continental slope of two different areas, namely, the coast of Catalonia (off Barcelona) and the Balearic Islands in three different bathymetric strata at depths between 1000 and 2200m. The parasite fauna of *B. mediterraneus* included a narrow range of species: *Steringophorus* cf. *dorsolineatum*, *Scolex pleuronectis*, *Hysterothylacium aduncum*, *Anisakis* sp. larva 3 type II and *Sarcotretes* sp. *Steringophorus* cf. *dorsolineatum* and *H. aduncum* were the most predominant parasites. *Hysterothylacium aduncum* showed significant differences in abundance between depths of 2000-2200m with 1000-1400m and 1400-2000m, irrespective of locality, whereas *S.* cf. *dorsolineatum* showed significant differences between the two localities at all depths except for 2000-2200m. We suggest the possible usefulness of these two parasites as geographical indicators for discriminating discrete stocks of *B. mediterraneus* in western Mediterranean waters. Only 4 specimens of *Anisakis* sp. were identified as infective stages of helminths infecting cetaceans.

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RESUMEN

0.1. INTRODUCCIÓN GENERAL

0.1.1. DIVERSIDAD

Los cetáceos albergan una fauna específica de parásitos de transmisión trófica que incluye digeneos, cestodos, nematodos y acantocéfalos (Aznar *et al*., 2001; Raga *et al*., 2009). Dentro de los digeneos se han citado unas 40 especies pertenecientes a cuatro familias (Heterophyidae, Brauninidae, Notocotylidae y Brachycladiidae), la mayoría encontradas en el tracto digestivo de cetáceos, aunque algunas especies también parasitan los senos aéreos y los pulmones (Raga *et al*., 2009). Además, se han citado tres familias de cestodos (Tetrabothriidae, Diphyllobothriidae y Phyllobothriidae) que incluyen unas 30 especies (Aznar *et al*., 2001; Raga *et al*., 2009). Un dato interesante sobre dichos cestodos es el hecho de que no sólo encontramos formas adultas, sino también larvas de distintas especies, lo que sugiere que los cetáceos podrían estar actuando como hospedadores definitivos e intermediarios. Dentro de los nematodos, también encontramos tres familias parasitando cetáceos (Anisakidae, Crassicaudidae y Pseudaliidae), aunque, sin lugar a dudas, la más estudiada es la familia Anisakidae, probablemente por su importancia económica y sanitaria, y el hecho de que es muy común, tanto en cetáceos como en un gran número de peces. Por último, dentro de los acantocéfalos, en cetáceos se han registrado nueve especies de *Bolbosoma* (Raga *et al*., 2009) y dos especies de *Corynosoma* (Sardella *et al*., 2005).

Uno de los problemas principales para el estudio de parásitos de cetáceos es la disponibilidad de muestras, ya que se depende de muestreos oportunistas, lo que generalmente conlleva un tamaño muestral relativamente pequeño. Además, los especímenes de helmintos no suelen estar en buenas condiciones, ya que los hospedadores pueden llevar varios días muertos. Todo ello obstaculiza la descripción, identificación y clasificación de los distintos taxones (Gibson, 2002, 2005), por lo que hay una gran escasez de información sobre distintas áreas relacionadas con la diversidad de helmintos de cetáceos.

0.1.2. EL ORIGEN DE LAS ASOCIACIONES

Durante el Eoceno (hace unos 50 millones de años), los ancestros de los actuales cetáceos colonizaron el medio marino (Berta *et al*., 2006), lo que supuso una barrera ecológica para los parásitos de origen terrestre (Hoberg, 1987; Balbuena y Raga, 1993; Aznar *et al*., 1994, 2001). De hecho, varios autores sugieren que dicha transición desde un medio terrestre a un medio marino supuso la extinción de aquellos parásitos de origen terrestre que no pudieron adaptarse al nuevo entorno (Delyamure, 1955; Aznar *et al*., 2001). Según esta hipótesis, los parásitos observados en la actualidad en los cetáceos serían el resultado de una “asociación por colonización”, es decir, la colonización de nuevas especies hospedadoras por parte de los parásitos que ya existían en el medio marino cuando los ancestros de los cetáceos lo colonizaron (véase Brooks y McLennan, 1991 y referencias incluidas en el mismo). Para que esto ocurra se requieren dos condiciones. En primer lugar, el parásito y el hospedador deben encontrarse, y la probabilidad de encuentro depende de factores ecológicos y/o biogeográficos; en segundo lugar, el parásito debe poder sobrevivir en el nuevo hospedador, es decir, si el parásito y el hospedador se encuentran, el éxito de la colonización dependerá de si ambos son compatibles (a nivel fisiológico e inmunológico) o no. Puesto que la mayoría de parásitos de mamíferos marinos se transmiten por vía trófica, es altamente probable que acaben en especies “correctas” e “incorrectas” de hospedadores (Raga *et al*., 2009). Si el encuentro con hospedadores “incorrectos” es predecible y se repite en el tiempo, puede que haya especies de parásitos capaces de superar el filtro de compatibilidad y acabar incorporando dicho hospedador a su ciclo vital. De hecho, hay estudios filogenéticos que sugieren que digeneos, cestodos, nematodos y acantocéfalos de cetáceos son de origen marino y fueron transferidos a partir de parásitos que infectaban otros grupos de hospedadores como peces, aves marinas y pinnípedos (véase Aznar *et al*., 2001 y referencias incluidas).

0.1.3. CICLOS VITALES

Debido a dificultades obvias relacionadas con el muestreo de cetáceos, especialmente oceánicos, se conoce muy poco de los ciclos vitales de sus helmintos (Aznar *et al*., 2001; Raga *et al*., 2009). A continuación, resumiremos las características principales de los ciclos vitales de los cuatro grandes grupos de helmintos presentes en cetáceos.

Todos los digeneos son parásitos y sus ciclos vitales están normalmente vinculados a los hábitos alimenticios del hospedador definitivo (Bush *et al*., 2001). Aunque existe una gran diversidad de especies y una gran variedad de ciclos vitales, hay algunas características que son comunes a la mayoría de especies (Gibson, 1987; Bush *et al*., 2001). El adulto libera los huevos al medio, donde emerge un miracidio que busca e infecta el primer hospedador intermediario, típicamente un molusco (por lo general un gasterópodo o un bivalvo). Dentro del molusco, el parásito sufre fases de reproducción asexual y, finalmente, una cercaria de vida libre se libera al medio donde, dependiendo de la especie, puede infectar un segundo hospedador intermediario o el hospedador definitivo (Poulin, 1998; Bush *et al*., 2001). Los cetáceos son grandes depredadores, por lo que el ciclo vital de sus digeneos podría requerir un segundo hospedador intermediario, o incluso, un hospedador paraténico (de transporte) para cubrir el “vacío” trófico entre el segundo hospedador intermediario y el definitivo (Combes, 1995).

Todos los cestodos tetrabotrídeos son parásitos con ciclos vitales complejos. Sin embargo, hasta la fecha no se conoce ningún ciclo vital de ninguna de las especies que infectan cetáceos. Se cree que el primer hospedador intermediario es un crustáceo, que consume el huevo del que emerge un procercoide que espera ser ingerido por el segundo hospedador intermediario, probablemente un cefalópodo o un teleósteo. Una vez infectado el segundo hospedador intermediario, la larva se transforma en plerocercoide y queda latente hasta que es ingerida por el hospedador definitivo (Bush *et al*., 2001). Se han sugerido ciclos vitales similares para los cestodos tetrafilídeos, que también incluirían crustáceos, cefalópodos y teleósteos como hospedadores intermediarios y/o paraténicos, y elasmobranquios como hospedadores definitivos (Hoberg, 1987). Sorprendentemente, se han citado cuatro formas larvarias de cestodos tetrafilídeos típicas de cetáceos, dos plerocercoides y dos merocercoides, lo que sugiere que los cetáceos podrían actuar como hospedadores intermediarios para cubrir el "vacío" trófico entre cefalópodos y teleósteos, y elasmobranquios que depredan sobre cetáceos (Aznar *et al*., 2007). En el caso de los cestodos difilobótridos, el primer hospedador intermediario sería un copépodo o un teleósteo (Raga *et al*., 2009), y el hospedador definitivo es un pinnípedo (Lauckner, 1985), aunque algunas especies usan cetáceos.

Los nematodos se encuentran entre las criaturas más diversas de la tierra, pudiéndose encontrar especies de vida libre, parasitando plantas, invertebrados y vertebrados (Bush *et al*., 2001). Se han descrito tres géneros de anisákidos en mamíferos marinos, *Pseudoterranova*, *Anisakis* y *Contracaecum*. El ciclo vital de las especies de estos tres géneros es bastante similar. Generalmente, una larva de vida libre (L2) emerge del huevo y es ingerida por eufausiáceos y copépodos pequeños, en donde muda la larva L3. Si el hospedador definitivo es un cetáceo planctívoro no se requieren más hospedadores intermediarios. Sin embargo, para algunas especies es necesaria la presencia de teleósteos y/o cefalópodos que cubren el "vacío" trófico entre el segundo hospedador intermediario y el hospedador definitivo (Bush *et al*., 2001). En muchas áreas geográficas se conoce la identidad de los hospedadores implicados en especies de *Anisakis*. Por ejemplo, en el estuario de San Lorenzo (Canadá), el eufausiáceo *Thysanoessa raschii* se considera el principal hospedador intermediario, y los teleósteos *Mallotus villosus* y *Clupea harengus* actúan como hospedadores paraténicos, siendo los cetáceos, especialmente las belugas, *Delphinapterus leucas*, los hospedadores definitivos de *A. simplex* s.l. (Hays *et al*., 1998a, b). El ciclo vital de las especies de la familia Crassicaudidae es bastante desconocido y, por lo que sabemos, no se han registrado, hasta el momento, fases larvarias. Dentro de la familia Pseudaliidae hay siete géneros, generalmente asociados al tracto respiratorio de odontocetos (Lehnert *et al*., 2010, y referencias incluidas). Probablemente, la mayoría de las especies se transmiten por vía trófica, excepto las del género *Halocercus*, para las que se ha sugerido también una transmisión transplacentaria (Dailey, 2005). Por lo que conocemos, sólo hay un estudio en el que se han identificado larvas de especies de la familia Pseudaliidae. Lehnert *et al*. (2010) identificaron, usando técnicas moleculares, larvas de *Pseudalius inflexus* y *Parafilaroides gymnurus* en varias especies de peces, principalmente en el pez gallo, *Limanda limanda,* y la solla, *Pleuronectes platessa* en aguas alemanas.

Respecto a acantocéfalos, se han encontrado adultos de especies de la familia Polymorphidae en anfibios, reptiles, aves marinas, peces y mamíferos. El acantor permanece en el huevo hasta que es ingerido por el primer hospedador intermediario, que en el caso de las especies de *Bolbosoma* parece ser un copépodo o un eufausiáceo (Bush *et al*., 2001). Una vez infectado se convierte en cistacanto y permanece a la espera de ser ingerido por un segundo hospedador intermediario o por el hospedador definitivo. Aunque se han citado distintas especies del género *Bolbosoma* en hospedadores paraténicos (Costa *et al*., 2000; Klimpel *et al*., 2006), la identidad de los hospedadores intermediarios generalmente se desconoce. Una excepción es *Bolbosoma balaenae*, para la cual se ha identificado el eufausiáceo *Nyctiphanes couchii* como hospedador intermediario (Gregori *et al*., 2012). En las especies marinas del género *Corynosoma*, parece ser que los anfípodos actúan como hospedadores intermediarios, los teleósteos como paraténicos y los mamíferos marinos como hospedadores definitivos principales (Aznar *et al*., 2006).

0.1.4. ESTRUCTURA DE LA COMUNIDAD PARÁSITA

Debido al origen de las asociaciones parásito-hospedador (véase el Apartado 0.1.2), las especies helmínticas encontradas en cetáceos son generalmente específicas de este grupo de hospedadores. Por ello, tanto la composición como la riqueza de especies están restringida por dos factores, a saber, la diversidad local de especies de helmintos y la comunidad local de especies de cetáceos (Dailey and Perrin, 1973; Forrester *et al*., 1980; Balbuena and Raga, 1993; Hoberg *et al*., 1993; Gibson *et al*., 1998; Aguilar-Aguilar *et al*., 2001; Andrade *et al*., 2001; Fernández *et al*., 2003, 2004).

A nivel de hospedador individual (infracomunidad), la riqueza y composición de especies de helmintos depende, en primer término, de la probabilidad de encuentro entre parásito y hospedador. Varios estudios sugieren que en el medio marino los estados infectivos están altamente “diluidos” (Hoberg, 1996, 2005; Raga *et al*., 2009; Valente *et al*., 2009; Santoro *et al*., 2010), por lo que la probabilidad de encuentro es bastante baja. Esta característica tiene dos consecuencias principales. Primero, la infracomunidad helmíntica debería ser depauperada si la comparamos con la riqueza observada en la comunidad componente y, segundo, las infracomunidades deberían constituir subconjuntos aleatorios de las especies que componen la comunidad. De hecho, existen varios estudios que han constatado la baja riqueza de especies de helmintos en cetáceos (Balbuena and Raga, 1993; Dans *et al*., 1999; Fernández *et al*., 2003). Sin embargo, la cuestión de la predecibilidad de las infracomunidades ha sido examinada en sólo dos estudios. Las infracomunidades de helmintos intestinales del calderón común (*Globicephala melas*), una especie oceánica, parecen ser altamente impredecibles (Balbuena and Raga, 1993), mientras que para la franciscana (*Pontoporia blainvillei*), una especie costera y sedentaria, es altamente predecible (Aznar *et al*., 1994, 1995). Por lo tanto, la probabilidad de infección no parece depender sólo del grado de "dilución" de los estados infectivos, sino también del hábitat y la movilidad de los hospedadores.

0.1.5. LA IMPORTANCIA DE ESTUDIOS A LARGO PLAZO

El estudio del efecto de procesos naturales o antropogénicos exige no sólo estudios puntuales, sino también series de datos a largo plazo. Un claro ejemplo de esta necesidad es la detección y evaluación de los efectos del cambio climático (Collins *et al*., 2013). Sin embargo, la mayoría de estudios se realizan a escalas temporales pequeñas, por la que tan sólo proporcionan una 'instantánea' de los procesos ecológicos. Existen estudios parasitológicos a largo plazo tanto en hábitats de agua dulce (Esch *et al*., 1988; Kennedy, 2001; Kennedy *et al*., 2002) como terrestres (Cattadori *et al*., 2005; Hudson *et al*., 2006), que subrayan la importancia de una correcta elección de la escala temporal de cualquier estudio. En cambio, debido a la limitación del muestreo oportunista de cetáceos, no existe ningún estudio que investigue cambios de sus faunas parásitas a largo plazo.

0.1.6. PARÁSITOS COMO MARCADORES BIOLÓGICOS

0.1.6.1. Antecedentes

Los parásitos son excelentes marcadores biológicos de sus hospedadores debido a su ubicuidad ecológica y dependencia estricta del hospedador (McKenzie y Abaunza, 2005; Whiteman y Parker, 2005). Además, los parásitos de transmisión trófica también pueden indicar interacciones en las redes tróficas, ya que sus ciclos vitales incluyen estados de vida libre y hospedadores intermediarios, paraténicos y definitivos, que están vinculados a través de interacciones hospedador-presa. De esta manera, los cambios en los niveles de infección de los parásitos de transmisión trófica pueden indicar cambios significativos en las condiciones abióticas, tamaño poblacional, uso del hábitat o interacciones tróficas de sus hospedadores (Marcogliese y Cone, 1997; Marcogliese, 2004). Estas peculiaridades hacen de los parásitos candidatos ideales tanto para el estudio de interacciones tróficas como de cambios a largo plazo. Existen muchos estudios que usan a los parásitos como marcadores biológicos de contaminación (ver MacKenzie *et al*., 1995; MacKenzie, 1999; Sures *et al*., 1999; Lewis *et al*., 2003; Malek *et al*., 2007), o estudios poblacionales y de discriminación de stocks (Bower y Margolis, 1991; Williams *et al*., 1992; Balbuena *et al*., 1995; MacKenzie y Abaunza, 1998; Timi, 2007). Algunos estudios han empleado parásitos también para detectar variaciones asociadas con el cambio climático (ver Mackenzie, 1987; Khan and Chandra, 2006).

0.1.6.2. Parásitos como indicadores de dieta y marcadores de cambios en la red trófica

La utilidad de los helmintos de transmisión trófica como indicadores radica en el hecho de que permanecen en el hospedador durante meses e incluso años después de la infección (Polis y Hurd, 1996; Lafferty *et al*., 2008). Por ello, proporcionan una ventaja sobre estudios de dieta basados en contenido estomacal, que sólo proporcionan una 'instantánea' de la última ingesta antes de la muerte del hospedador. En este contexto, se considera que los parásitos representan una buena fuente de datos que complementa otros métodos, como podrían ser el contenido estomacal o el análisis de isótopos estables (Marcogliese, 2002, 2004). Sin embargo, hay que tener en cuenta que la interpretación de posibles cambios a largo plazo requiere un profundo conocimiento de los ciclos vitales de los distintos taxones helmínticos y de la dinámica poblacional de sus hospedadores.

0.1.6.3. Parásitos como marcadores biológicos de cetáceos

La mayoría de estudios parasitológicos de cetáceos se centra en la descripción de la fauna parásita (véase Zam *et al*., 1971; Dailey y Stroud, 1978; Raga y Carbonell, 1985; Balbuena y Raga, 1993; Cerioni y Mariniello, 1996; Gibson *et al*., 1998; Aguilar-Aguilar *et al*., 2001; Rosas *et al*., 2002; Fernández *et al*., 2003, 2004). Además, algunos autores han usado distintas especies parásitas para estudios de comportamiento (Balbuena y Raga, 1991), estructura social (Balbuena y Raga, 1993; Balbuena *et al*., 1995), filogeografía (Kaliszewska *et al*., 2005), discriminación de stocks (Aznar *et al*., 1995) y estado de salud (Aznar *et al*., 1994, 2005) de sus hospedadores. Sin embargo, los estudios parasitológicos aún no han alcanzado su máximo potencial. Dentro de las redes tróficas, los cetáceos son depredadores tope, lo que significa que normalmente actúan como hospedadores definitivos para muchas de las especies helmínticas. Si se detectan cambios a largo plazo en la composición o estructura de la comunidad helmíntica sería razonable pensar que ha habido cambios en la dieta y/o en la red trófica. Sin embargo, hasta donde conocemos, no hay estudios basados en un tamaño muestral lo suficientemente grande y con una serie temporal lo suficientemente larga como para investigar dicha posibilidad.

0.1.7. CETÁCEOS Y SUS HELMINTOS EN EL MEDITERRÁNEO OCCIDENTAL

Actualmente existen unas 83 especies de cetáceos divididas en dos subórdenes, odontocetos y misticetos, que virtualmente habitan todos los ambientes marinos, desde los polos (como las belugas, *Delphinapterus leucas*, en aguas del Ártico) hasta aguas tropicales (como el tucuxi, *Sotalia fluviatilis*, en Sudamérica) (Borobia *et al*., 1991; Silva y Best, 1996). De los dos subórdenes, los odontocetos representan el grupo más numeroso, con 10 familias, 40 géneros y al menos 70 especies, mientras que en los misticetos encontramos 4 familias, 6 géneros y 13 especies.

Actualmente se conocen siete especies de cetáceos con poblaciones residentes en el Mar Mediterráneo: el delfín común, *Delphinus delphis*, el delfín listado, *Stenella coeruleoalba*, el delfín mular, *Tursiops truncatus*, el calderón gris, *Grampus griseus*, el calderón negro, *Globicephala melas*, el cachalote, *Physeter macrocephalus* y el zifio de Cuvier, *Ziphius cavirostris* (Notarbartolo di Sciara, 2002).

En aguas del Mediterráneo occidental se han citado al menos 21 taxones de helmintos de cetáceos, incluyendo cinco digeneos, ocho cestodos, siete nematodos y un acantocéfalo (Raga, 1985; Raga y Carbonell, 1985; Raga *et al*., 1992; Raga y Balbuena, 1993; Fernández *et al*., 2003, 2004; Aznar *et al*., 2006, 2007; Quiñones *et al*., 2013). Sin embargo, no podemos olvidar que, debido a los problemas relacionados con el muestreo de cetáceos, puede que haya otras especies parásitas aún por describir.

0.1.7.1. El delfín listado, *Stenella coeruleoalba*

El delfín listado, *Stenella coeruleoalba*, es una especie cosmopolita que podemos encontrar en aguas templadas y tropicales de todo el mundo (Archer, 2009). En las costas españolas del Mediterráneo, Gómez de Segura *et al*. (2006) concluyeron que el delfín listado es la especie de cetáceo más abundante. Sin embargo, la población del Mediterráneo está considerada como “Vulnerable” en la Lista Roja de Especies Amenazadas de la IUCN. Por ello, no es sorprendente que existan numerosos estudios acerca de distintos aspectos de su biología reproductiva (Aguilar, 1991; Calzada *et al*., 1996, 1997), distribución y uso del hábitat (Reilly, 1990; Cañadas *et al*., 2002; Azzellino *et al*., 2008), hábitos alimenticios (Blanco *et al*., 1995; Hassani *et al*., 1997) y estado de salud (Van Bressem *et al*., 1999, 2003; González *et al*., 2002; Muñoz *et al*., 2006; Raga *et al*., 2008).

El delfín listado se considera un depredador oportunista que se alimenta de teleósteos, cefalópodos y crustáceos. Existen muchos estudios acerca de la dieta del delfín litado basados en contenido estomacal en distintas áreas geográficas, como las costas de Japón, el Océano Atlántico y el Mar Mediterráneo (Miyazaki *et al*., 1973; Desportes, 1985; Pulcini *et al*., 1992; Sekiguchi *et al.*, 1992; Würtz y Marrale, 1993; Blanco *et al*., 1995; Hassani *et al*., 1997; Meotti y Podestà, 1997; Ringelstein *et al*., 2006; Spitz *et al*., 2006; Öztürk *et al*., 2007; Santos *et al*., 2008). Otros estudios de dieta se basan en isótopos estables (Das *et al*., 2000; Gómez-Campos *et al*., 2011; Meissner *et al*., 2012). En el Mediterráneo, la dieta del delfín listado consiste en teleósteos y cefalópodos mesopelágicos (Würtz y Marrale, 1993; Blanco *et al*., 1995; Meotti y Podestà, 1997).

En 1990, el delfín listado del Mediterráneo occidental sufrió una mortandad masiva causada por un morbillivirus. Aunque el número total de bajas no se pudo calcular, se estima que miles de individuos murieron a causa de encefalitis y/o neumonía (Domingo *et al*., 1992; Duignan *et al*., 1992). Un censo realizado entre 2001-2003 sugirió que la población en el Golfo de Valencia pareció haberse recuperado, con números cercanos a los estimados antes de la mortandad (Gómez de Segura *et al*., 2006). Sin embargo, en 2007 hubo un segundo brote epidémico del virus (Raga *et al*., 2008), probablemente favorecido por la alta densidad poblacional.

0.1.7.2. Fauna helmíntica del delfín listado

La fauna parásita del delfín listado se ha estudiado en distintas áreas geográficas, como el Atlántico (Zam *et al*., 1971; Abollo *et al*., 1998; Gibson *et al*., 1998), el Pacífico (Dailey y Stroud, 1978; Dailey y Walker, 1978) y el Mediterráneo (Raga y Carbonell, 1985; Raga 1986). Dichos estudios se basan en un tamaño muestral pequeño (n ≤ 15) y se centran en determinar la identidad de los distintos taxones parásitos. Hasta la fecha, se han identificado varias especies de nematodos (*Anisakis* spp., *Crassicauda* sp., *Skrjabinalius guevarai* y *Sterunus ovatus*), digeneos (*Pholeter gastrophilus*, *Oschmarinella rochebruni* y *Brachycladium atlanticum*), cestodos (*Monorygma grimaldii*, *Phyllobothrium delphini*, *Tetrabothrius forsteri*, *Strobilocephalus triangularis* y *Trigonocotyle* sp.) y un acantocéfalo (*Bolbosoma vasculosum*) (Raga y Carbonell, 1985: Fernández *et al*., 1991; Manfredi *et al*., 1992; Agustí *et al*., 1999).

Aunque se conoce la identidad de la fauna helmíntica del delfín listado, hay otros muchos aspectos ecológicos que aún se desconocen. Existe escasa información sobre la composición faunística basada en un tamaño muestral grande. Además, también hay una falta de información cuantitativa desde la perspectiva de la comunidad helmíntica, que ayudaría a entender el papel de los factores ecológicos y filogenéticos en la dinámica de las faunas parásitas (Poulin, 1995). En este sentido, existen algunos estudios sobre las comunidades de parásitos de cetáceos que sugieren el papel fundamental de los factores filogenéticos para explicar la diversidad y composición de las comunidades (Balbuena y Raga, 1993; Aznar *et al*., 1994). Sin embargo, un aspecto relativamente inexplorado en cetáceos es el de los efectos que las variables asociadas al hospedador, p.e., especie, longitud total, edad y sexo, sobre las comunidades parásitas (Balbuena y Raga, 1993; Balbuena *et al*., 1994). Además, es necesario llevar a cabo estudios a largo plazo para investigar la dinámica de las relaciones parásito-hospedador, especialmente si se dan perturbaciones en el sistema, tanto naturales (como las epizootias virales) como antropogénicas. Esto se discute en la siguiente sección.

0.1.7.3. Impactos a largo plazo en las poblaciones del delfín listado del Mediterráneo

Durante las pasadas décadas, la población del delfín listado en aguas del Mediterráneo occidental sufrió impactos de carácter antropogénico y natural. Hay evidencias de que la sobrepesca de la sardina (*Sardina pilchardus*), supuestamente una de las principales presas del delfín listado en el área, podría haber causado un cambio importante en su dieta, incrementando el número de presas demersales, especialmente la merluza, *Merluccius merluccius* (Gómez-Campos *et al*., 2011). Una dieta basada en la merluza proporciona menos valor energético que una dieta basada en la sardina, por lo que se especula con la posibilidad de que dicho cambio pudiera causar efectos perjudiciales a largo plazo, particularmente para aquellos individuos con una demanda nutricional más elevada, como podrían ser individuos jóvenes o hembras preñadas y lactantes (Gómez-Campos *et al*., 2011).

Durante la primera mortandad masiva de 1990 se detectaron niveles anormalmente altos de compuestos organoclorados. Existen evidencias de que dichos niveles podrían haber tenido efectos inmunodepresores, lo cual podría haber incrementado la susceptibilidad de los delfines a la infección con el morbillivirus (Aguilar y Borrell, 1994). Curiosamente, Aguilar y Raga (1993) también sugirieron que las temperaturas del agua durante el invierno anterior a la primera epizootia fueron anormalmente altas, lo que podría haber dado lugar a una disminución de la productividad, con la consiguiente reducción de alimento disponible y falta de capacidad por parte de los delfines para hacer frente al morbillivirus. Las olas de calor y otro tipo de condiciones meteorológicas extremas parecen ser más comunes en las últimas décadas, y la pregunta es si están relacionadas o no con el cambio climático (Coumou y Rahmstorf, 2012). En el Mediterráneo occidental existe una clara evidencia al aumento de la temperatura y la salinidad de la superficie del mar (Vargas-Yáñez *et al*., 2010), lo que podría afectar no sólo a las poblaciones de grandes depredadores, como el delfín listado, sino también a la función de todo el ecosistema (Bianchi, 2007).

Por tanto, sería interesante explorar si dichos impactos, tanto naturales como antropogénicos, se han visto reflejados en cambios a largo plazo en la fauna helmíntica de cetáceos.

0.2. JUSTIFICACIÓN Y OBJETIVOS

La presente tesis doctoral tiene tres propósitos generales. Primero, proporcionar información sobre la diversidad específica y estructura de la comunidad de la fauna helmíntica del tracto digestivo del delfín listado (*Stenella coeruleoalba*) en las aguas del Mediterráneo occidental. Segundo, investigar si, tanto factores naturales como antropogénicos han tenido un efecto a largo plazo sobre la estructura de dichas comunidades helmínticas. Tercero, investigar los ciclos vitales de los helmintos de cetáceos, usando esta información para interpretar patrones ecológicos encontrados en el hospedador definitivo.

Los objetivos específicos son los siguientes:

1. Describir la fauna helmíntica del aparato digestivo del delfín listado, *Stenella coeruleoalba*, en aguas del Mediterráneo occidental.

2. Investigar los factores que determinan la estructura y composición de las comunidades helmínticas.

3. Explorar cambios a largo plazo en la estructura de las comunidades helmínticas.

4. Examinar la presencia de larvas de los distintos taxones parásitos de cetáceos, tratando de elucidar ciclos vitales y rutas de infección.

0.3. MATERIALES Y MÉTODOS

En este resumen, los materiales y métodos se describen por separado para cada uno de los capítulos.

0.4. COMUNIDAD PARÁSITA INTESTINAL DEL DELFÍN LISTADO (*STENELLA COERULEOALBA*) EN AGUAS DEL MEDITERRÁNEO OCCIDENTAL: EFECTOS DE LA LONGITUD, EDAD Y SEXO DEL HOSPEDADOR.

0.4.1. INTRODUCCIÓN

La parasitofauna del delfín listado, *Stenella coeruleoalba*, se ha descrito en distintas regiones geográficas, incluyendo el Atlántico norte (Zam *et al*., 1971; Abollo *et al*., 1998; Gibson *et al*., 1998), el Pacífico norte (Dailey y Stroud, 1978; Dailey y Walker, 1978) y el Mar Mediterráneo (Raga y Carbonell, 1985; Raga, 1986; Abril *et al*., 1991). Sin embargo, estos estudios están basados en tamaños muestrales pequeños ($n \leq 15$) y se limitan a proporcionar información sobre la identidad de los taxones parásitos encontrados. Por lo tanto, existe una escasez de información cuantitativa desde una perspectiva de la comunidad parasitaria, lo que ayudaría a entender el papel de la filogenia y los factores ecológicos sobre la dinámica de las faunas parásitas en el delfín listado. Por otra parte, el efecto del hospedador (longitud, edad o sexo) sobre la estructura de las comunidades parásitas ha sido poco estudiado (Balbuena y Raga, 1993; Balbuena *et al*., 1994).

La población del delfín listado del Mediterráneo sufrió en 1990 una epizootia causada por el morbillivirus de cetáceos (CeMV) que produjo la muerte de miles de individuos (Aguilar y Raga, 1993) por encefalitis y/o neumonía (Domingo *et al*., 1992; Duignan *et al*., 1992). Dado que todos los individuos varados presentaban una causa de muerte conocida y afectó a un gran número de individuos de todas las edades y sexos en un corto periodo de tiempo, esta mortandad masiva proporcionó una oportunidad única para (1) describir la fauna intestinal del delfín listado basada en un gran tamaño muestral, (2) estudiar, por primera vez, parámetros de infracomunidad y (3) explorar los efectos de la longitud, edad y sexo del delfín en la estructura de la comunidad parásita intestinal.

0.4.2. MATERIALES Y MÉTODOS

Se analizó un total de 52 ejemplares de delfín listado varados en las costas de la Comunidad Valenciana (España) durante la epizootia de 1990 (28 machos y 24 hembras). Antes de la necropsia se determinó el sexo de los delfines y se tomaron medidas estandarizadas (ver Geraci y Lounsbury, 2005). Durante la necropsia, los intestinos se recogieron y congelaron a -20°C para su posterior examen. Para el análisis parasitológico, los intestinos se dividieron en 20 secciones de la misma longitud. Cada sección se lavó por separado sobre un tamiz con una luz de malla de 0,02mm y se recogieron los restos sólidos en una placa de Petri. Los parásitos encontrados se lavaron en solución salina, se identificaron, se contaron y fueron fijados y conservados en etanol al 70%.

Para la identificación de los parásitos, los cestodos se tiñeron con acetocarmín (Georgiev *et al*., 1986), se deshidrataron y se montaron en preparaciones permanentes con bálsamo de Canadá. Posteriormente, algunos individuos se dibujaron con la ayuda de un tubo de dibujo y se identificaron según Hoberg (1987a, 1989, 1990) y Nikolov *et al*. (2010). Los acantocéfalos se transparentaron en lactofenol, se montaron en preparaciones temporales para poder ser dibujados y se identificaron según Petrochenko (1958) y Costa *et al*. (2000). Se depositaron especímenes de referencia en el Museo de Historia Natural de Londres (Reino Unido) con número de acceso: *Tetrabothrius forsteri*, NHMUK 2013.5.31.6-7 y *Strobilocephalus triangularis*, NHMUK 2013.5.31.8-9.

Se investigó si la abundancia de las distintas especies parásitas y las distintas variables de infracomunidad (abundancia total, riqueza e índice de diversidad de Brillouin) covariaban significativamente con la longitud y/o edad del hospedador mediante una correlación de Spearman y se compararon diferencias entre machos y hembras usando un test de Mann-Whitney. Además, se llevó a cabo un test de varianza de Schluter y el test de Fisher para señalar asociaciones significativas entre especies parásitas. Por último, se investigaron los posibles efectos de la longitud, edad y/o sexo de los delfines sobre la estructura de las infracomunidades parásitas con un PERMANCOVA.

0.4.3. RESULTADOS

Todos los intestinos examinados contenían, al menos, una especie parásita. Se recogieron un total de 2.517 helmintos pertenecientes a 4 especies: adultos de 3 especies de cestodos de la familia Tetrabothriidae e individuos inmaduros de acantocéfalos pertenecientes a la familia Polymorphidae. La especie más frecuente y abundante fue *Tetrabothrius forsteri* (prevalencia: 96,2%; intensidad media [95% IC]: 47,4 [34,4-68,2]), que se encontró a lo largo de todo el intestino (con la mayor parte de la población en el duodeno y disminuyendo hacia la parte posterior del intestino). *Trigonocotyle globicephalae* se encontró en bajas intensidades (9,6%; 1,8 [1,0-2,2]) y también en la primera mitad del intestino. Al contrario, *Strobilocephalus triangularis* (23,1%; 5,3 [2,9-10,2]) se encontró exclusivamente en la última sección (recto), a excepción de un único individuo localizado en la sección 18. Además se encontraron individuos inmaduros de *Bolbosoma vasculosum* sin una preferencia aparente por ninguna sección en particular del intestino (51,9%; 2,7 [2,0-3,4]). El 80% de los delfines analizados en este estudio estaban infectados por 1-2 especies y la abundancia de los parásitos fue muy baja (<60 individuos). Los resultados para los descriptores de infracomunidad mostraron comunidades depauperadas (abundancia total (95% IC) [rango]: 48,4 (36,1-68,9) [1-294]; riqueza de especies (95% IC) [rango]: 1,81 (1,59-2,04) [1-4]; índice de Brillouin (95% IC) [rango]: 0,172 (0,123-0,233) [0-0,817]).

No se detectaron diferencias significativas ni en las abundancias ni en los descriptores de infracomunidad calculados (abundancia total, riqueza e índice de Brillouin) entre delfines de distinto sexo. Asimismo, tampoco se hallaron correlaciones significativas de los descriptores de infracomunidad con la longitud ni con la edad de los delfines. El test de Schluter indicó que, en esencia, las infracomunidades estaban compuestas por combinaciones aleatorias de especies.

0.4.4. DISCUSIÓN

Las especies de parásitos encontradas en este estudio son exclusivas de cetáceos, principalmente las de la familia Delphinidae que habitan aguas oceánicas. Además, los patrones de especificidad coinciden con los de otros cetáceos oceánicos en los que la fauna intestinal está dominada por cestodos de la familia Tetrabothriidae y especies del género *Bolbosoma*.

Trigonocotyle globicephalae siempre ha sido asociado a delfínidos teutófagos, habiéndose citado en el calderón gris, *Grampus griseus*, en las costas de Japón, en el calderón negro, *Globicephala melas*, en aguas del Atlántico norte y en el calderón tropical, *G. macrorhynchus*, (Yamaguti, 1959; Kikuchi *et al*., 1988; Balbuena y Raga, 1993). Asimismo, en el Mar Mediterráneo se ha registrado típicamente en el calderón común y en el calderón gris (Raga y Balbuena, 1993; Fernández *et al*., 2003) Por lo tanto, el presente estudio representa la primera cita de *T. globicephalae* en una especie del género *Stenella*.

Strobilocephalus triangularis también se ha encontrado en aguas del Atlántico y Pacífico, principalmente en delfínidos como *Cephalorhynchus commersonii*, *Delphinus delphis*, *Hyperoodon ampullatus*, *Lagenodelphis hosei*, *Lagenorhynchus acutus*, *Mesoplodon bidens*, *Stenella attenuata*, *S. longirostris* y *S. bredanensis* (Baer, 1932; Delyamure, 1955; Dailey y Otto, 1982; Raga, 1985; Berón-Vera *et al*., 2001). En el Mediterráneo, tan sólo se ha citado en aguas occidentales infectando *Stenella coeruleoalba* (Raga y Carbonell, 1985) y *Tursiups truncatus* (Quiñones *et al*., 2013).

Tetrabothrius forsteri ha sido citado en varias especies de delfínidos en aguas del Atlántico y el Pacífico, como por ejemplo, en *L. hosei*, *L. acutus*, *Steno bredanensis*, *S. attenuata*, *S. longirostris*, y *T. truncatus* (Forrester y Robertson, 1975; Dailey y Otto, 1982; VanWaerebeek *et al*., 1990; Mignucci-Giannoni *et al*., 1998; Olson y Caira, 1999). En aguas del Mediterráneo, existen citas en *S. coeruleoalba*, *D. delphis*, *T. truncatus* y

Mesoplodon bidens (Raga y Carbonell, 1985; Raga y Balbuena, 1987; Quiñones *et al*., 2013).

Todos los individuos de *B. vasculosum* encontrados eran inmaduros, por lo que sugerimos que este acantocéfalo es capaz de establecerse, pero no de madurar y reproducirse en el delfín listado. Esta observación es común a la mayoría de registros de *B. vasculosum* a nivel mundial. Además de las citas de cistacantos registrados en peces (Bakay y Zubchenko, 1984; Panebianco y Sebastio, 1988; Klimpel *et al*., 2006; Costa *et al*., 2000; García *et al*., 2008), se han encontrado individuos juveniles de *B. vasculosum* en odontocetos, principalmente oceánicos, en aguas del Pacífico (Pendergraph, 1971; Zam *et al*., 1971), Atlántico (Dailey y Perrin, 1973; Mignucci-Giannoni *et al*., 1998; Costa *et al*., 2000; Aguilar-Aguilar *et al*., 2001) y del Mediterráneo (Raga y Carbonell, 1985; Fernández *et al*., 2004). Hay tres estudios en los que se citan individuos adultos de *B. vasculosum*. Williams y Bunkley-Williams (1996) encontraron un especimen en la sierra, *Scomberomorus cavalla*, de Puerto Rico; Harada (1935) encontró tres individuos en un atún rojo, *Thunnus thynnus*, de Japón, y Yamaguti (1963) citó adultos de *B. vasculosum* en el delfín común, *D. delphis* y en el zifio de Sowerby, *M. bidens*. Sin embargo, la ausencia de registros adicionales en estas especies tan estudiadas hace que la identidad del hospedador definitivo principal de *B. vasculosum* esté aún por determinar.

A nivel de infracomunidad, la riqueza de especies depende en la probabilidad de encuentro entre parásito y hospedador. Nuestros resultados coinciden con la hipótesis de que en el medio marino las fases infectivas de los parásitos de transmisión trófica están altamente “diluidas” (ver Valente *et al*., 2009 y Santoro *et al*., 2010, para tortugas marinas; Hoberg, 1996, 2005, para aves marinas, y Raga *et al*., 2009, para mamíferos marinos). Cabe destacar que *T. forsteri* se encontró en 50 de los 52 delfines analizados, por lo que se podría especular con que dicha “dilución” no se aplica a esta especie, al menos en el área de estudio.

En cetáceos hay un sólo estudio en el que se investigaron los efectos del hospedador sobre los parámetros de la infracomunidad. En el calderón negro, Balbuena y Raga (1993) encontraron una relación positiva entre la edad del hospedador con la diversidad de especies y la abundancia de las infracomunidades intestinales. Estos autores sugirieron que dichos resultados podrían deberse a que los individuos de mayor edad son de mayor tamaño, por lo que necesitan la ingesta de un mayor número de presas para satisfacer su demanda metabólica. Dicho incremento en el número de presas significa, a su vez, un incremento en la probabilidad de infección. Nuestros resultados no muestran efectos del hospedador ni en las abundancias ni en los descriptores de infracomunidad. Sin embargo, si comparamos los datos, observamos que los de Balbuena y Raga (1993) incluyen un mayor número de individuos juveniles que los nuestros, por lo que un efecto de la edad y longitud del hospedador sobre la fauna intestinal no puede descartarse, y nos hacen sugerir la necesidad de investigaciones futuras sobre el delfín listado que incluyan una mayor cantidad de hospedadores de menor tamaño y edad (véase el Apartado 0.7).

0.5. ESPECIFICIDAD DE *OSCHMARINELLA ROCHEBRUNI* Y *BRACHYCLADIUM ATLANTICUM* (DIGENEA: BRACHYCLADIIDAE) EN CINCO ESPECIES DE CETÁCEOS DEL MEDITERRÁNEO OCCIDENTAL.

0.5.1. INTRODUCCIÓN

La mayoría de las especies (35 de las 42 especies descritas) de digeneos de la familia Brachycladiidae (antes Campulidae) se encuentran en cetáceos de todo el mundo mientras que el resto se pueden encontrar en pinnípedos y en la nutria marina (Aznar *et al*., 2001; Dailey, 2007). La información sobre la biología de los braquicládidos es muy limitada. Se desconoce, por ejemplo, la identidad de los hospedadores intermediarios y paraténicos (Fernández, 1996; Barnett *et al*., 2008) y los datos sobre los patrones de especificidad son escasos. Sin embargo, todo indica que dichos patrones son muy variables, por lo que encontramos especies que parasitan especies simpátricas de mamíferos marinos, mientras que otras están restringidas a una sola especie hospedadora. Por ejemplo, *Orthosplanchnus arcticus* se ha citado en seis especies de pinnípedos y dos especies de cetáceos en aguas árticas y subárticas (Adams, 1988; Raga y Balbuena, 1993), y *Brachycladium goliath* (=*Lecithodesmus goliath*) se ha encontrado en cinco especies de ballenas en el Pacífico Norte. Sin embargo, un estudio parasitológico de más de 300 cetáceos pertenecientes a 13 especies varados en las costas del Reino Unido (Gibson *et al*., 1998) reveló que *Campula oblonga* se encuentra casi exclusivamente en la marsopa común, *Phocoena phocoena*, y numerosos estudios han sugerido que *Synthesium pontoporiae* está restringido a la franciscana, *Pontoporia blainvillei*, en aguas del Atlántico Sur (Andrade *et al*., 2001; Melo *et al*., 2006; Berón-Vera *et al*., 2008; Marigo *et al*., 2008, y referencias en los mismos).

Las razones por las que aparecen diferencias tan marcadas en la especificidad de distintas especies parásitas se desconocen. Sin embargo, este aspecto puede investigarse mediante el examen de los filtros de encuentro y compatibilidad sugeridos por Euzet y Combes (1980). En el caso de los braquicládidos sabemos que los cetáceos se infectan mediante el consumo de presas que albergan el parásito (probablemente peces y/o cefalópodos, véase Fernández *et al*., 1998b). Por tanto, podemos estudiar los hábitos alimenticios de los hospedadores para determinar la probabilidad de contacto entre parásito y hospedador (filtro de encuentro). Por otro lado, como los braquicládidos se transmiten por vía trófica, no sería sorprendente encontrarlos en especies hospedadoras con dietas similares (p.e., cetáceos, pinnípedos o aves marinas), por lo que diferencias en el crecimiento y/o reproducción del parásito podrían indicar diferencias en compatibilidad entre las distintas especies hospedadoras (filtro de compatibilidad).

En este estudio se utilizan los filtros de encuentro y compatibilidad para determinar, por primera vez, los patrones de especificidad de los braquicládidos hepatopancreáticos en el delfín listado y otros cetáceos del Mediterráneo occidental. Primero se determinarán los niveles de infección para, posteriormente, comparar la eficacia biológica de las especies parásitas en las distintas especies hospedadoras.

0.5.2. MATERIALES Y MÉTODOS

Este estudio se basa en datos de 148 cetáceos varados en las costas de la Comunidad Valenciana (España) entre 1982 y 2008. Los conductos hepatopancreáticos de 103 ejemplares del delfín listado, *Stenella coeruleoalba*, 18 de calderón gris, *Grampus griseus*, 14 de delfín mular, *Tursiops truncatus*, 8 de delfín común, *Delphinus delphis*, y 5 de calderón común, *Globicephala melas*, se analizaron a nivel parasitológico.

Para confirmar que los individuos de *Brachycladium atlanticum* encontrados en distintas especies hospedadoras pertenecían a la misma especie se procedió al análisis molecular. Para ello se extrajo ADN ribosomal mediante el protocolo de fenol-cloroformo (Holzer *et al*., 2004) y se comparó el espaciador transcrito interno 2 (ITS2) (ver Nolan y Cribb, 2005).

Para determinar la compatibilidad de *Brachycladium atlanticum* con las distintas especies hospedadoras se hizo un estudio morfológico y se estimó el número de huevos en 106 especímenes adultos recogidos en el delfín listado y 17 del delfín común. Además, se llevó a cabo un análisis de componentes principales (ACP) basado en una matriz de varianza-covarianza de 7 variables morfométricas de *B. atlanticum* para visualizar diferencias en la alometría estática multivariada entre especies hospedadoras.

0.5.3. RESULTADOS

Se encontraron dos especies de la familia Brachycladiidae, *Brachycladium atlanticum* y *Oschmarinella rochebruni*, en los conductos hepatopancreáticos de los cetáceos analizados. *Oschmarinella rochebruni* se encontró exclusivamente en el delfín listado, mientras que el análisis molecular confirmó que los individuos de *B. atlanticum* encontrados en el delfín listado y en el delfín común pertenecían a la misma especie. En el resto de especies de cetáceos no se encontraron digeneos.

Aunque el análisis estadístico demostró una alta variabilidad morfológica entre los individuos parásitos encontrados en la misma especie hospedadora (el delfín listado), el análisis de componentes principales confirmó la existencia de diferencias de tamaño entre los individuos de *B. atlanticum* encontrados en el delfín listado y en el delfín común. Los valores morfológicos mostraron que los individuos de *B. atlanticum* del delfín común eran aproximadamente un 50% más pequeños que los del delfín listado, no así el tamaño de los huevos, cuyo tamaño no difirió significativamente entre especies hospedadoras. Aun así, la fecundidad en individuos de *B. atlanticum* del delfín común también se vio afectada, ya que el número medio de huevos se encontraba significativamente por debajo del número medio estimado para los individuos encontrados en el delfín listado.

0.5.4. DISCUSIÓN

Los datos indican que dos especies de la familia Brachycladiidae infectan los conductos hepatopancreáticos de los cetáceos presentes en aguas del Mediterráneo occidental. El delfín listado parece ser el hospedador definitivo de *O. rochebruni*. Sin embargo, estudios en otras localidades demuestran que *O. rochebruni* no está restringido a dicha especie hospedadora, ya que se ha encontrado en otras especies de delfínidos. En el Pacífico norte, por ejemplo, se ha citado en el delfín común, el delfín de hocico largo, *Stenella longirostris*, y el delfín manchado tropical, *Stenella attenuata*, pero no en el delfín listado (Dailey y Perrin, 1973; Dailey y Otto, 1982). Asímismo, en el Atlántico norte se ha encontrado en el delfín común pero no en el delfín listado (Gibson *et al*., 1998).

En este estudio, *Brachycladium atlanticum* se encontró en el delfín listado y delfín común, sin mostrar diferencias significativas ni en la prevalencia ni en la abundancia media entre especies. Hasta la fecha, *B. atlanticum* sólo se había citado en el delfín listado en aguas de las Islas Canarias (Abril *et al*., 1991), por lo que este estudio representa la primera cita de *B. atlanticum* en el delfín común.

La restricción de *O. rochebruni* y *B. atlanticum* a 1-2 especies hospedadoras podría ser el resultado de diferencias en la probabilidad de contacto (filtro de encuentro) entre dichos parásitos y las diferentes especies hospedadoras y la capacidad de estos braquicládidos de madurar y reproducirse (filtro de compatibilidad) en el hospedador correspondiente. Analizando los hábitos alimenticios de las cinco especies de cetáceos analizados observamos que las principales presas del delfín listado en el área de estudio son peces y cefalópodos mesopelágicos (Blanco *et al*., 1995, 2009; Bosch de Castro, 2014) y que el delfín común también depreda sobre peces mesopelágicos (Pusineri *et al*., 2007 y referencias incluidas). Sin embargo, el delfín mular se alimenta principalmente de peces neríticos (Blanco *et al*., 2001), mientras que el calderón gris y el calderón común son mayoritariamente teutófagos, alimentándose de cefalópodos mesopelágicos (Blanco *et al*., 2006; Blanco, com. pers.). Por ello, sugerimos que tanto *O. rochebruni* como *B. atlanticum* podrían infectar el delfín listado y el delfín común mediante la ingesta de peces y cefalópodos mesopelágicos infectados con las fases larvarias, y que rara vez contactan con las otras especies de cetáceos.

Los resultados también muestran una alta variabilidad en la morfología de *B. atlanticum* en el delfín listado. Dado que el rango de intensidad en el delfín listado va de 1 a 69 individuos, y que no hubo relación entre morfología o fecundidad y tamaño infrapoblacional, consideramos que no hay evidencias de que dicha variabilidad sea debida a efectos de hacinamiento o "crowding". Asimismo, en el delfín común infectado tan sólo se encontraron 19 individuos, por lo que tampoco cabría esperar que la disminución del tamaño de *B. atlanticum* en esta especie se debiera a dicho efecto.

Fernández *et al*. (1995) destacaron que el tamaño del huevo es un carácter altamente conservado en *Synthesium tursionis* (Brachycladiidae), independientemente del tamaño del parásito o del hospedador. En el presente estudio, los individuos de *B. atlanticum* recolectados del delfín común parecen ser más pequeños que los del delfín listado y presentan un menor número de huevos. Sin embargo, nuestros resultados coinciden con Fernández *et al*. (1995), ya que el tamaño del huevo es el único carácter morfológico que parece no variar significativamente en ambas especies hospedadoras.

En conclusión, en aguas del Mediterráneo occidental parece que el hospedador definitivo de *O. rochebruni* es el delfín listado. Además, *B. atlanticum* puede encontrarse no sólo en el delfín listado sino también en el delfín común, aunque éste último parece ser una especie hospedadora menos adecuada para dicho digeneo.

0.6. COMUNIDAD PARÁSITA GÁSTRICA DEL DELFÍN LISTADO (*STENELLA COERULEOALBA*): EFECTOS DE LA LONGITUD, EDAD Y SEXO DEL HOSPEDADOR.

0.6.1. INTRODUCCIÓN

Se han citado al menos 12 taxones de metazoos parásitos de transmisión trófica en el delfín listado: 3 digeneos, 5 cestodos, 3 nematodos y 1 acantocéfalo (Raga, 1985; Raga y Carbonell, 1985; Raga *et al*., 1992; Raga y Balbuena, 1993; Aznar *et al*., 2006, 2007; Mateu *et al*., 2011, 2014). Tan sólo dos de estas especies se han encontrado infectando el estómago: el digeneo *Pholeter gastrophilus* y nematodos del género *Anisakis*. A nivel global, *Pholeter gastrophilus* se ha citado en al menos 17 especies de cetáceos (Aznar *et al*., 1992; Raga, 1994), incluyendo especies costeras (Dollfus, 1974; Van Waerebeek *et al*., 1993; Aznar *et al*., 1994; Gibson *et al*., 1998; Berón-Vera *et al*., 2001) y oceánicas (Aznar *et al*., 1992; Raga y Balbuena, 1993; Fernández *et al*., 2003).

Todos los estudios indican que los individuos de *P. gastrophilus* se encuentran dentro de quistes en el sistema digestivo de cetáceos (Raga y Carbonell, 1985; Fernández *et al*., 1991; Manfredi *et al*., 1992; Gibson *et al*., 1998), ya que el hospedador forma nódulos alrededor del parásito en respuesta a la infección (Woodard *et al*., 1969; Migaki *et al*., 1971; Howard *et al*., 1983).

Según Mattiucci y Nascetti (2006), actualmente hay 9 especies que pertenecen al género *Anisakis* (*A. pegreffii, A. simplex sensu stricto, A. berlandi, A. typica, A. ziphidarum, Anisakis* sp.*, A. physeteris, A. brevispiculata* and *A. paggiae*). Desafortunadamente, las larvas de anisákidos son indistinguibles morfológicamente, por lo que ha sido necesario aplicar técnicas moleculares durante las últimas décadas para reorganizar la taxonomía de anisákidos y describir nuevas especies (Paggi *et al*., 1998; Mattiucci *et al*., 1997, 2005, 2009). En aguas del Mediterráneo occidental, diversos estudios han citado la presencia de especies de *Anisakis* en el delfín listado (Raga y Carbonell, 1985; Fernández *et al*., 1991; Manfredi *et al*., 1992). Sin embargo, la identidad específica de estos individuos no se conoce.

Durante el verano de 1990, los delfines listados del Mediterráneo occidental sufrieron una mortandad masiva causada por un morbillivirus que acabó con la vida de miles de ejemplares. Este brote proporcionó una oportunidad única para estudiar un gran número de individuos en un corto periodo de tiempo y con una causa conocida de la muerte. El objetivo de este estudio es describir la parasitofauna gástrica del delfín listado en dicha región y explorar los efectos de la longitud y edad del hospedador sobre los parámetros de infección.

0.6.2. MATERIALES Y MÉTODOS

Se analizó un total de 47 ejemplares de delfín listado varados en las costas de Cataluña y la Comunidad Valenciana (España) entre agosto de 1990 y marzo de 1991. Antes de la necropsia se determinó el sexo de los delfines y se tomaron medidas estandarizadas (véase Geraci y Lounsbury, 2005) (véase Apéndice 3 para más detalles). Los dientes se enviaron al Departamento de Biología Animal de la Universidad de Barcelona para determinar la edad de los individuos (véase Calzada *et al*., 1994 para más detalles). Durante la necropsia se recogieron los estómagos y se congelaron a -20°C para su posterior examen. En lo referente a la recolección y procesado de los parásitos, se utilizó el mismo procedimiento descrito en el apartado 0.4.2. Además, se examinó detenidamente la pared estomacal y se contaron los quistes característicos que se forman en respuesta a la infección por *Pholeter gastrophilus*. Los individuos de *Anisakis* difíciles de identificar morfológicamente se analizaron a nivel molecular. Se realizó el análisis molecular de seis especímenes obtenidos del delfín listado (5 larvas y un adulto).

Se extrajo ADN mitocondrial usando el protocolo previamente descrito por Valentini *et al*. (2006) y Mattiucci *et al*. (2009, 2014) y se comparó el citocromo c oxidasa II (*cox*2) para su identificación (ver Nadler y Hudspeth, 2000).

0.6.3. RESULTADOS

Se detectó la presencia de *Pholeter gastrophilus* en 40 de los 47 estómagos analizados, con una prevalencia del 85,1%. Además, se encontraron 46 individuos de anisákidos infectando siete de los 47 estómagos analizados. La mayoría fueron recogidos en el estómago pilórico (n= 40), mientras que el resto se encontraron en el estómago mecánico. No se detectaron correlaciones significativas entre la longitud total del hospedador o su edad y la abundancia de ninguna de las especies helmínticas, ni diferencias significativas entre sexos ($p > 0.05$ en todos los casos). Todas las secuencias obtenidas del análisis molecular fueron idénticas, permitiendo la identificación de los seis individuos analizados como *A. pegreffii*. La secuencia está disponible en GenBank con número de acceso: KR149283.

0.6.4. DISCUSIÓN

Pholeter gastrophilus se ha citado en distintas especies de odontocetos en aguas del océano Atlántico, Pacífico y mar Mediterráneo (Zam *et al*., 1971; Dollfus, 1974; Raga y Carbonell, 1985; Aznar *et al*., 1992, 1994; Van Waerebeek *et al*., 1993; Raga y Balbuena, 1993; Raga, 1994; Gibson *et al*., 1998; Berón-Vera *et al*., 2001; Fernández *et al*., 2003). Los altos valores de infección encontrados en este estudio contrastan con la baja prevalencia en el delfín oscuro (*Lagenorhynchus obscurus*) de Perú (Van Waerebeek *et al*., 1993), o en el delfín mular, *Tursiops truncatus*, en aguas de Florida (Zam *et al*., 1971). Sin embargo, hay otros estudios que también han citado prevalencias superiores al 50%, como Aznar *et al*. (2006) en aguas del océano Atlántico y el Mediterráneo.

En aguas del Mediterráneo, *A. pegreffii* es considerada la más común de todas las especies de *Anisakis* (Mattiucci y Nascetti, 2008), siendo el delfín listado, *Stenella coeruleoalba*, el delfín común, *Delphinus delphis*, y el delfín mular, *T. truncatus*, sus principales hospedadores definitivos (Mattiucci *et al*., 1997, 2004, 2014; Mattiucci y Nascetti, 2008). Se han citado larvas en distintas especies de peces demersales y pelágicos, tanto en el Mediterráneo occidental como el oriental (Farjallah *et al*., 2008; Mattiucci y Nascetti, 2008; Pekmezci *et al*., 2014). Mateu *et al*. (2015) (Capítulo 8) citan dos especies de anisákidos (*Anisakis physeteris* y *A. pegreffii*) en distintas especies de mictófidos capturadas en aguas del Mediterráneo occidental y sugieren que la especie *Notoscopelus elongatus* podrían jugar un papel en el ciclo vital de dichos anisákidos. Sin embargo, la baja prevalencia encontrada en este estudio contrasta con otros estudios en las costas de Chile (Torres *et al*., 1992), Brasil (Carvalho *et al*., 2010) o incluso en las costas españolas del Atlántico (Abollo *et al*., 1998), en donde la prevalencia es mayor.

Gómez-Campos *et al*. (2011) sugieren que el delfín listado cambia de dieta con la edad; sin embargo, nosotros no detectamos diferencias significativas en la abundancia de *A. pegreffii* con la longitud total del hospedador ni su edad. Dichos resultados sugieren que el cambio de dieta no afecta al consumo del hospedador intermediario, o, en el caso de *A. pegreffii*, en que la señal estadística es baja debido a los reducidos niveles de infección.

0.7. CAMBIOS A LARGO PLAZO EN LA COMUNIDAD PARÁSITA INTESTINAL DEL DELFÍN LISTADO (*STENELLA COERULEOALBA*) EN AGUAS DEL MEDITERRÁNEO OCCIDENTAL.

0.7.1. INTRODUCCIÓN

Durante las últimas décadas ha aumentado la preocupación sobre la estabilidad a largo plazo de las poblaciones de mamíferos marinos (Tynan y DeMaster, 1997; Learmonth *et al*., 2006). Hay evidencias de que la sobrepesca ha causado cambios en la distribución de distintas especies de mamíferos marinos (Bearzi *et al*., 2003, 2004, 2006, 2008; Cañadas y Hammond, 2008), lo cual podría conllevar, además, un agotamiento de las poblaciones por malnutrición (Gulland *et al*., 2005; Rosen y Trites, 2005; Rosen, 2009). Además, se sabe que hay contaminantes que se acumulan en los mamíferos marinos (Tanabe *et al*., 1994; Watanabe *et al*., 2002; Wafo *et al*., 2005; McHugh *et al*., 2007; Lailson-Brito *et al*., 2010) y podrían favorecer las enfermedades y los eventos de mortalidad masiva (Gulland y Hall, 2007; Van Bressem *et al*., 2001). Por último, también hay evidencias de que el cambio climático también está afectando la distribución de la poblaciones (MacLeod *et al*., 2005; Azzellino *et al*., 2008b; Salvadeo *et al*., 2010), puediendo afectar las relaciones depredador-presa de los mamíferos marinos (McLean *et al*., 2001; Walther *et al*., 2002).

La población del delfín listado, *Stenella coeruleoalba*, del Mediterráneo occidental no es una excepción. En las últimas décadas, esta población ha sufrido diversos impactos ambientales asociados al cambio climático global, tanto de origen natural como antropogénico, que podrían alterar de forma irreversible la estabilidad del ecosistema (Gambaiani *et al*., 2009; Coll *et al*., 2010). Gómez-Campos *et al*. (2011) sugieren que la sobrepesca de la sardina, *Sardina pilchardus*, una de las principales presas del delfín listado en dicha localidad, ha causado un cambio en la dieta hacia presas más demersales como la merluza, *Merluccius merluccius*. Dicho cambio de dieta conllevaría una ingestión menor de energía, por lo que podría generar problemas a largo plazo en el equilibrio energético de la población, afectando principalmente a aquellos individuos con exigencias nutricionales más elevadas, como individuos jóvenes o hembras preñadas o lactantes. Además, durante las últimas décadas la población mediterránea del delfín listado ha sufrido dos mortandades masivas en 1990 y 2007 (Aguilar y Raga, 1993; Raga *et al*., 2008) causadas por el morbillivirus de los delfines (DMV). La primera epizootia empezó en el verano de 1990 en aguas españolas, extendiéndose hacia Italia y Grecia en 1991 y 1992, respectivamente. Diversos autores sugirieron varias características que pudieron intensificar los efectos del DMV. Aguilar y Borrell (1994) encontraron altos niveles de compuestos organoclorados asociados con efectos inmunosupresores en los individuos afectados por el DMV, lo que pudo aumentar la probabilidad de infección entre delfines. Por otro lado, Aguilar y Raga (1993) sugirieron que las altas temperaturas del agua registradas durante el invierno previo a la primera mortandad también pudieron haber tenido consecuencias directas en la productividad, lo que pudo haber reducido el alimento disponible, y por tanto, la capacidad de los delfines de combatir al DMV. Las olas de calor y las condiciones meteorológicas extremas son cada vez más frecuentes, lo que típicamente se ha asociado al cambio climático (Coumou y Rahmstorf, 2012). En el Mediterráneo occidental hay claras evidencias de un incremento en la temperatura y la salinidad del agua (Vargas-Yáñez *et al*., 2010), lo que podría tener graves consecuencias, no sólo en las poblaciones de los grandes depredadores, como el delfín listado, sino también en el funcionamiento de todo el ecosistema (Bianchi, 2007). El objetivo de este trabajo es usar un gran tamaño muestral (128 indivíduos) varados durante los últimos 20 años (1990-2010) para analizar, por primera vez, tendencias a largo plazo en la abundancia y composición de la comunidad de helmintos intestinales en el delfín listado. Además, examinaremos si hay una relación significativa entre la dieta y la carga parásita.

0.7.2. MATERIALES Y MÉTODOS

Se analizó un total de 128 ejemplares de delfín listado varados en las costas de la Comunidad Valenciana (España) entre 1990 y 2010. Tanto para el análisis de los intestinos como para la correcta identificación de los parásitos se siguió la misma metodología que en el apartado 0.4.2. de este resumen. Se depositaron especímenes de referencia en el Museo de Historia Natural de Londres (Reino Unido) con número de acceso: *Tetrabothrius forsteri* (NHMUK 2013.5.31.1-2), *Trigonocotyle globicephalae* (NHMUK 2013.5.31.3) y *Strobilocephalus triangularis* (NHMUK 2013.5.31.4-5).

Se dispone de datos de dieta basados en el análisis del contenido estomacal de 71 de los 128 individuos analizados (Blanco *et al*., 1995; Míguez, 2009; Bosch de Castro, 2014; C. Blanco datos no publicados). Para la identificación de presas se recogieron los otolitos y los picos de cefalópodos del contenido estomacal de cada delfín. Las presas se agruparon en seis categorías ecológicas: (1) peces neríticos demersales, (2) peces neríticos pelágicos, (3) peces demersales de talud, (4) mictófidos y otros peces mesopelágicos, (5) cefalópodos neríticos y (6) cefalópodos oceánicos. Asímismo, se dividió la muestra de delfines en dos grupos: (i) aquellos individuos afectados por el morbilivirus ('muestras de la epizootia') (n= 66) y (ii) individuos para los que se desconoce la causa de la muerte ('muestras de no epizootia') (n= 62).

Se aplicó un PERMANCOVA para investigar los efectos de la longitud del hospedador, sexo y periodo sobre la estructura de la comunidad helmíntica para ambos grupos de delfines. Además, se aplicó un test BIO-ENV para tratar de detectar si existía una relación significativa entre la estructura de la comunidad helmíntica y la dieta.

0.7.3. RESULTADOS

De los 128 individuos examinados, sólo cuatro no estaban infectados. Se recogió un total de 6.498 individuos parásitos pertenecientes a cuatro especies: tres especies de cestodos tetrabotrídeos: *Tetrabothrius forsteri*, *Trigonocotyle globicephalae* y *Strobilocephalus triangularis*, e individuos inmaduros de la especie de acantocéfalo *Bolbosoma vasculosum*. La especie más abundante fue *Tetrabothrius forsteri* (n= 6.046) y se encontró todos los años a lo largo de las últimas dos décadas. *Trigonocotyle globicephalae* se encontró sólo en cinco de los 20 años estudiados (n= 31), mientras que *Strobilocephalus triangularis* parecía ser más abundante durante los últimos diez años (2000-2010) (n= 348). Sólo se detectó la presencia del acantocéfalo *Bolbosoma vasculosum* durante 1990, amén de un único individuo encontrado en 1997 (n= 73).

Ninguno de los PERMANCOVA realizados (tanto para las 'muestras de la epizootia', como para las de 'no epizootia') detectó interacciones entre ninguno de los factores y la covariable. Hubo diferencias entre las 'muestras de la epizootia', debidas a *B. vasculosum* ya que su presencia se detectó en la primera epizootia de 1990, pero no en 2007. Sin embargo, no se detectó un efecto temporal sobre la estructura de la comunidad cuando se analizaron las muestras de 'no epizootia'. En esta muestra, sí se detectaron efectos del tamaño del hospedador. Hubo una relación negativa significativamente entre la longitud total del hospedador y la abundancia de *T. forsteri* (r_s= -0.327; n= 61; p= 0.010) y *T. globicephalae* (r_s= -0.306; n= 61; p= 0.016); la relación con la abundancia de *S. triangularis*, aunque negativa, no fue significativa (r_s= -0.207; n= 61;

p= 0.109), y la de *B. vasculosum* fue cercana a cero (r_s = 0.007; n= 61; p= 0.55). Según el análisis BIO-ENV, la correlación entre los datos de dieta y la comunidad helmíntica intestinal del delfín listado no era estadísticamente significativa (r_s = 0.11; p= 0.265).

0.7.4. DISCUSIÓN

Nuestros resultados indican que existen diferencias significativas en las faunas parásitas del delfín listado entre epizootias causadas por los niveles de infección de *B. vasculosum*, ya que dicha especie se encontró frecuentemente en la primera epizootia de 1990 pero no en la segunda de 2007. Dos hipótesis podrían explicar estas diferencias: (i) el cambio de dieta sugerido por Gómez-Campos *et al*. (2011) puede haber afectado al consumo del hospedador intermediario y/o paraténico de este acantocéfalo, modulando el contacto entre parásito y hospedador, y/o (ii) 1990 fue un año extraordinario durante el cual *B. vasculosum* fue capaz de encontrar e infectar al delfín listado. Un estudio reciente sugiere que la dieta del delfín listado del Mediterráneo occidental ha sufrido un cambio hacia presas más neríticas (Bosch de Castro, 2014). La mayoría de referencias de *B. vasculosum* en odontocetos, se restringe a odontocetos oceánicos (ver Pendergraph, 1971; Zam *et al*., 1971; Dailey y Perrin, 1973; Raga y Carbonell, 1985; Mignucci-Giannoni *et al*., 1998; Costa *et al*., 2000; Aguilar-Aguilar *et al*., 2001; Fernández *et al*., 2004), por lo que dicho cambio de dieta podría haber disminuido la probabilidad de encuentro entre parásito y hospedador. Sin embargo, no podemos descartar la idea de que 1990 fuera un año excepcional. De hecho, Raga y Carbonell (1985) tan sólo encontraron un espécimen de *B. vasculosum* en 10 delfines listados examinados en la misma área de estudio durante la década previa. Por lo tanto, la cuestión sigue siendo si fuimos incapaces de detectar cambios a largo plazo en la helminto fauna del delfín listado porque sólo se analizaron animales altamente parasitados (una muestra no aleatoria), o porque realmente no hay cambios que detectar. En cualquier caso, parece claro que los niveles de infección de *B. vasculosum* eran significativamente más altos en 1990. Sin embargo, como la identidad de los hospedadores paraténicos de *B. vasculosum* sigue siendo desconocida, no se puede comprobar si nuestros resultados concuerdan con cambios en la dieta del delfín listado.

Para las especies de cestodos encontradas, no se encontraron cambios significativos a largo plazo. El hecho de que el muestreo sea oportunista podría haber afectado a nuestros resultados ya que no representa una muestra aleatoria de la población. Por lo tanto, no se puede determinar si no se detectaron cambios porque no los hay o porque sólo disponemos de individuos altamente parasitados. La abundancia de los cestodos tetrabotrídeos disminuyó con la longitud total (edad) del hospedador, de forma similar a lo que se ha observado en otras especies del género *Stenella*.

La relevancia de este trabajo es que representa el primer estudio parasitológico de cetáceos que investiga tendencias a largo plazo en la abundancia y composición de la comunidad helmíntica intestinal. Además, también es la primera vez que se comparan datos de dieta con datos de infección para investigar los posibles efectos que el cambio de dieta puede haber tenido sobre la comunidad helmíntica.

0.8. EL PAPEL DE LOS MICTÓFIDOS (MYCTOPHIDAE) Y LOS CEFALÓPODOS EN LOS CICLOS VITALES DE PARÁSITOS DE CETÁCEOS EN EL MEDITERRÁNEO OCCIDENTAL.

0.8.1. INTRODUCCIÓN

Los peces de la familia Myctophidae y los cefalópodos son una parte fundamental de la comunidad mesopelágica debido a su gran abundancia y biomasa (Olivar *et al*., 2012). Además, varios estudios subrayan que la dieta de muchos cetáceos se compone por peces y cefalópodos mesopelágicos (Clarke, 1996; Barlow *et al*., 2008), por lo que no sería de extrañar que dichas presas formaran parte del ciclo vital de los parásitos de cetáceos.

El objetivo de este estudio es tratar de identificar hospedadores intermediarios y/o paraténicos de los parásitos de cetáceos. Para ello, se examina, por primera vez en el Mediterráneo, la fauna parásita de ocho especies de peces mesopelágicos de la familia Myctophidae y dos especies de cefálopodos bentopelágicos.

0.8.2. MATERIAL Y METÓDOS

Se recogió un total de 1.012 individuos pertenecientes a ocho especies de mictófidos: *Ceratoscopelus maderensis* (n= 390), *Lampanyctus crocodilus* (n= 295), *Notoscopelus elongatus* (n= 222), *Benthosema glaciale* (n= 70), *Myctophum punctatum* (n= 14), *Lobianchia dofleini* (n= 9), *Diaphus holti* (n= 8) y *Hygophum benoiti* (n= 4), y 792 individuos de dos especies de cefalópodos: *Alloteuthis media* (n= 670) y *Sepietta oweniana* (n= 122), de las aguas del Golfo de Valencia y el Mar de Alborán.

Inmediatamente después de su captura, las muestras se fijaron en etanol al 70%. Una vez en el laboratorio, todos los individuos fueron pesados y medidos. Se examinaron, por separado, las branquias, gónadas, estómago e intestino, hígado, corazón y cerebro de los peces, y las gónadas y el tracto digestivo de los cefalópodos en solución salina bajo la lupa (20-100x). Además, la musculatura de los peces y el manto de los cefalópodos se examinaron por transparencia.

Se recogieron todos los helmintos encontrados, excepto en el caso de unos pequeños nematodos enquistados en la pared estomacal identificados como pertenecientes a la familia Raphidascarididae, para los que sólo se contabilizó y recogió una muestra (véase Resultados). Los especímenes que se recogieron fueron fijados en etanol absoluto (análisis molecular) o etanol al 70% (estudio morfológico). Para su identificación, los nematodos fueron transparentados con lactofenol y, en el caso de *Anisakis*, asignados a tipo I o II. Los digeneos fueron teñidos con acetocarmín, se deshidrataron y se montaron en preparaciones permanentes con bálsamo de Canadá.

Se realizó el análisis molecular de seis especímenes de *Anisakis* obtenidos de siete individuos de *N. elongatus*. Se extrajo ADN mitocondrial usando el protocolo previamente descrito por Valentini *et al*. (2006) y Mattiucci *et al*. (2009, 2014) y se comparó el citocromo c oxidasa II (*cox2*) para su correcta identificación (véase Nadler y Hudspeth, 2000). Para los nematodos encontrados en la pared estomacal (véase Resultados), tan sólo se consiguió extraer ADN de un individuo. En este caso se secuenció ADN ribosomal (SSU rRNA) usando el kit de extracción Chelex®.

Se investigó si la abundancia de *Anisakis* spp. incrementaba con la longitud del hospedador mediante una correlación de Spearman y se compararon diferencias en las prevalencias entre las distintas especies de peces usando un test de Fisher.

0.8.3. RESULTADOS

No se encontraron parásitos en ninguna de las especies de cefalópodos, mientras que se identificaron cuatro taxones en las distintas especies de mictófidos. Se recogieron un total de 21 larvas de *Anisakis* en 18 de los 222 *N. elongatus* y en dos de los 390 *C. maderensis* examinados, con una intensidad de 1-2 larvas por individuo. El test de Fisher mostró que *N. elongatus* estaba significativamente más parasitada por *Anisakis* spp. que cualquiera de las otras especies de mictófidos con n>50 individuos (p≤ 0.009 en todas las comparaciones). No se halló una relación significativa entre el tamaño del pez y la cantidad de *Anisakis* spp. (p> 0,15 en todos los casos). También se encontraron 89 digeneos juveniles de la familia Didymozoidae en el intestino de 41 de los 222 *N. elongatus* y en 3 de los 70 *B. glaciale*, que fueron asignados a juveniles del tipo Torticaeum (*sensu* Pozdnyakov y Gibson, 2008). Además, se encontraron larvas muy pequeñas de nematodo (≤ 300 μm) enquistadas en la pared estomacal, pero su tamaño impidió que pudieran ser identificadas incluso a nivel de familia. También se encontró un individuo de *Hysterothylacium* sp. y dos plerocerdoides en la cavidad de *N. elongatus*.

El análisis molecular permitió identificar dos especies de *Anisakis* en la muestra, *A. pegreffii* y *A. physeteris*. Las secuencias están disponibles en GenBank con número de acceso: KF972438 (*A. pegreffii*) and KF972439 (*A. physeteris*). El análisis molecular de uno de los nematodos encontrados en la pared estomacal permitió concluir que pertenecen a la familia Raphidascarididae (GenBank, número de acceso KF972437).

0.8.4. DISCUSIÓN

De los cuatro taxones de parásitos encontrados en mictófidos, tan sólo dos especies son parásitos de transmisión trófica que infectan cetáceos.

Por primera vez, se encuentran dos especies de *Anisakis* (*A. pegreffii* y *A. physeteris*) infectando mictófidos del Mediterráneo occidental. Los mictófidos son una parte importante de la dieta del delfín listado en aguas del Mediterráneo occidental. Puesto que hemos encontrado adultos de *A. pegreffii* en dicho hospedador (Mattiucci *et al*., 2014; ver Capítulo 6), sugerimos que *N. elongatus* podría formar parte de su ciclo vital. Se considera que el hospedador definitivo de *A. physeteris* es el cachalote, *Physeter macrocephalus* (Mattiucci y Nascetti, 2008) y se conoce que su dieta esta principalmente basada en cefalópodos. Nuestros resultados sugieren, por primera vez, que los mictófidos, particularmente *N. elongatus*, podría desempeñar un papel como hospedador paraténico en el ciclo vital de *A. pegreffii* y *A. physeteris* en el Mediterráneo occidental. Ninguna de las otras larvas encontradas infectan cetáceos, pero algunas podrían ser transmitidas a grandes peces depredadores. La extrema escasez de parásitos de cetáceos en este y otros estudios parasitológicos previos de peces y cefalópodos mesopelágicos, contrasta que el papel de estas presas en la dieta de los cetáceos oceánicos.

0.9. VARIACIÓN GEOGRÁFICA DE METAZOOS PARÁSITOS DE *BATHYPTEROIS MEDITERRANEUS* BAUCHOT, 1962 (OSTEICHTHYES: IPNOPIDAE) EN AGUAS DEL MEDITERRÁNEO OCCIDENTAL

0.9.1. INTRODUCCIÓN

Las larvas de helmintos de cetáceos de transmisión trófica circulan por las redes tróficas, por lo que pueden ser detectadas en distintas especies de peces y cefalópodos, aunque no sean presas de cetáceos. En este capítulo, se realizó un estudio parasitológico de *Bathypterois mediterraneus*, una especie que no es una presa del delfín listado, sin embargo (i) es la especie más abundante por debajo de los 1500m en aguas del mar Mediterráneo (Carrasson y Matallanas, 2001); (ii) habitas aguas del talud continental donde es muy frecuente la presencia del delfín listado (Gómez de Segura *et al*., 2008) y (iii) su dieta se compone principalmente de copépodos (Carrasson y Matallanas, 2001) que podrían actuar como hospedadores intermediarios y/o paraténicos.

Durante las últimas décadas algunos estudios se han centrado en distintos aspectos de la ecología de *B. mediterraneus*, como su distribución (Geistdoerfer y Rannou, 1972; Stefanescu *et al*., 1992a; Cartes *et al*., 2004), crecimiento (Morales-Nin, 1990; Stefanescu *et al*., 1992b; Morales-Nin *et al*., 1996; Moranta *et al*., 2004), dieta (Carrassón y Matallanas, 1990, 1994, 2001; Polunin *et al*., 2001), y reproducción (Fishelson y Galil, 2001; D'Onghia *et al*., 2004; Porcu *et al*., 2010). Sin embargo, la información disponible sobre los parásitos de *B. mediterraneus* es muy limitada (ver Tortonese y Orsi, 1970).

El objetivo de esta investigación es proporcionar, por primera vez, un estudio parasitológico de *B. mediterraneus* en aguas del Mediterráneo occidental. Se analizó la comunidad componente en dos áreas geográficas y a tres profundidades, con especial énfasis en las fases larvarias de parásitos de cetáceos, para tratar de avanzar en los conocimientos relacionados con los ciclos vitales de dichos helmintos. Además, se evaluó el uso de los parásitos como marcadores biológicos para discriminación de stocks.

0.9.2. MATERIALES Y METÓDOS

En julio de 2010 se capturaron 170 individuos de *B. mediterraneus* del talud continental, entre las costas de Cataluña y las Islas Baleares. Inmediatamente después de su captura todos los individuos fueron congelados a -20°C. Una vez en el laboratorio se descongelaron y fueron pesados y medidos. Todos los órganos fueron examinados por separado en solución salina bajo la lupa (20-100x). Además, la musculatura se examinó por transparencia. Se recogieron todos los parásitos encontrados, siendo fijados en etanol al 70%. Para su identificación, los digeneos fueron teñidos con acetocarmín, se deshidrataron y se montaron en preparaciones permanentes con bálsamo de Canadá, mientras que los nematodos se transparentaron con glicerina.

Para los análisis estadísticos, los datos se agruparon en dos grupos según la longitud del hospedador: individuos inmaduros, con un tamaño estándar <113mm, e individuos maduros, con un tamaño estándar ≥113mm (Carrassón y Matallanas, 2001). Además, cada tamaño se agrupó por localidad (Barcelona o Islas Baleares) y por profundidad (1000-1400m, 1400-2000m, 2000-2200m). Se llevó a cabo un análisis de correspondencia canónica (ACC) para generar una perspectiva general de las relaciones entre la abundancia de parásitos y las variables vinculadas con el hábitat (localidad y profundidad). Para investigar las diferencias señaladas por el ACC, se realizó un modelo lineal generalizado (MLG) entre las poblaciones parásitas de las cinco categorías de localidad y profundidad.

0.9.3. RESULTADOS

Se encontraron cinco especies de parásitos en *B. mediterraneus*: el digeneo *Steringophorus* cf. *dorsolineatum* (n= 135); una larva de cestodo asignada a *Scolex pleuronectis* (n= 4); el nematodo *Hysterothylacium aduncum* (n= 175), el anisákido *Anisakis* sp. (larva 3 tipo II) (n= 2) y el copépodo *Sarcotretes* sp. (n= 2).

No se detectaron diferencias significativas entre los individuos hospedadores inmaduros y maduros ($p > 0,05$ en todos los casos). Los valores máximos de abundancia media se detectaron en ambas localidades a una profundidad de 2000-2200m. Sin embargo, nuestros resultados sugieren que la riqueza media de especies es más alta en las Islas Baleares, a una profundidad de 2000-2200m, y que dicha riqueza va disminuyendo en las costas de Barcelona y a medida que las capturas se realizan a menor profundidad. La abundancia de *S.* cf. *dorsolineatum* parece significativamente más alta en el talud continental de las Islas Baleares que en las costas de Barcelona. Además, *H. aduncum* se encontró con una prevalencia y una abundancia más alta a mayores profundidades.

0.9.4. DISCUSIÓN

Este es el primer estudio parasitológico de *B. mediterraneus* en aguas del Mediterráneo occidental. Por lo que sabemos, sólo hay un estudio previo que cita dos individuos de copépodos (Tortonese y Orsi, 1970), por lo que esta investigación representa la primera cita de las cuatro especies helmínticas en *B. mediterraneus*. Nuestros resultados sugieren que la fauna parásita de *B. mediterraneus* es bastante pobre, con tan sólo cuatro especies helmínticas y una especie de copépodo. Esta fauna parásita, que incluye varias especies generalistas, es más común de peces meso y batipelágicos que de especies demersales como *B. mediterraneus*.

El resto de las especies parásitas encontradas en *B. mediterraneus* no son parásitos de cetáceos. No se han identificado, a nivel de especie, los individuos de *Anisakis* encontrados, por lo que no se puede descartar que *B. mediterraneus* pueda formar parte de su ciclo vital en la zona de estudio.

Las diferencias significativas en la abundancia de *S.* cf. *dorsolineatum* (según localidad) y de *H. aduncum* (según profundidad), sugiere que dichas especies podrían usarse para la discriminación de stocks de *B. mediterraneus*.

0.10. CONCLUSIONES GENERALES

1. La comunidad helmíntica del aparato digestivo del delfín listado en aguas del Mediterráneo occidental esta compuesta por dos brachicládidos infectando los conductos hepatopancreáticos, *Brachycladium atlanticum* y *Oschmarinella rochebruni*; un digeneo heterófido, *Pholeter gastrophilus*, y al menos un nematodo anisákido, *Anisakis pegreffii* en el estómago y tres cestodos tetrabotrídeos, *Tetrabothrius forsteri*, *Trigonocotyle globicephalae* y *Strobilocephalus triangularis*, e individuos inmaduros del acantocéfalo polimórfido *Bolbosoma vasculosum* en el intestino. Todos los taxones encontrados son específicos de cetáceos, un resultado que fue predicho con antelación basado en los efectos de los filtros de contacto y compatibilidad derivados en la distancia filogenética entre cetáceos y otros vertebrados simpátricos.

2. Las infracomunidades intestinales resultaron ser depauperadas y altamente impredecibles. Ambas características eran las esperadas en cetáceos y otros vertebrados oceánicos porque los estados infectivos de los helmintos de transmisión trófica están altamente “diluidos” en el hábitat oceánico y, por lo tanto, la probabilidad de transmisión al hospedador definitivo es baja. La baja tasa de reclutamiento ha sido relacionada con la falta de estructuras en la comunidad helmíntica intestinal en otros cetáceos oceánicos.

3. La longitud, edad o sexo de los individuos de delfín listado no parecen ejercer efectos significativos sobre las abundancias de las especies parásitas, ni sobre los descriptores de infracomunidad (abundancia total, riqueza o índice de diversidad de Brillouin), cuando el análisis se basó en una muestra compuesta principalmente de individuos adultos (n= 53). Este resultado apoyaría la idea de que la probabilidad de transmisión al hospedador definitivo es baja y, por lo tanto, el efecto del hospedador es débil. Sin embargo, un segundo análisis basado en un tamaño muestral mayor (n= 128) que incluía más individuos juveniles, reveló efectos significativos de la longitud (o edad) en la estructura de la comunidad helmíntica. En particular, la abundancia de los cestodos tetrabotrídeos tendía a disminuir con la longitud. Hay cambios significativos en la dieta del delfín listado del Mediterráneo occidental relacionados con la edad, que podrían explicar dicho patrón. La influencia de efectos ontogenéticos en los niveles de infección de los tetrabotrídeos se ha sugerido en otras especies del género *Stenella*.

4. *Oschmarinella rochebruni* se encontró con una alta prevalencia (61,2%). Sin embargo, dicha especie no se encontró en las otras especies de cetáceos analizadas (calderón gris, delfín mular, delfín común y calderón común). Además, no se encontraron individuos inmaduros a lo largo del intestino. Dichos resultados sugieren la posibilidad de que el filtro de compatibilidad (hábitos alimenticios) definen la especificidad de *O. rochebruni* en el delfín listado.

5. *Brachycladium atlanticum* se encontró con una prevalencia moderada (39,8%), pero también se encontró en 1 de los 8 delfines comunes analizados (prevalencia: 12,5%). En el delfín listado el hospedador individual ejerce efectos significativos en la morfología y fecundidad de *B. atlanticum*, que, aparentemente, no estaban relacionados con efectos por hacinamiento. Este resultado sugiere que habría que reexaminar el uso de rasgos morfométricos en estudios taxonómicos de digeneos de cetáceos. Los individuos de *B. atlanticum* en el delfín común eran enanos comparados con los individuos encontrados en el delfín listado, y eran menos fecundos debido a un efecto alométrico. Esto sugiere que el filtro de compatibilidad también podría ejercer un efecto sobre la especificidad de *B. atlanticum* en el delfín listado. Para confirmar dicha hipótesis se requieren datos morfológicos adicionales de individuos recogidos de otras especies de cetáceos.

6. El digeneo estomacal *Pholeter gastrophilus* se encontró con una alta prevalencia (más de 85%), especialmente en el estómago pilórico, mientras que los nematodos identificados como *Anisakis pegreffii* se encontraron en menos del 15% de los delfines, principalmente en el estómago pilórico. Los niveles de infección de *A. pegreffii* en el delfín listado del Mediterráneo occidental son sorprendentemente bajos comparados con los niveles de infección de distintas especies de *Anisakis* en otras regiones. Aparentemente, esto no es un efecto de la especie hospedadora, ya que también se han encontrado niveles de infección bajos en otros cetáceos y peces del Mediterráneo occidental, sugiriendo que hay una baja densidad de dicho nematodo en esta región.

7. El análisis de tendencias a largo plazo (1990-2010) en la abundancia y composición de la comunidad helmíntica intestinal del delfín listado, indica que sólo la abundancia de *B. vasculosum* cambió significativamente a lo largo de los años. En particular, los niveles de infección fueron más altos en 1990, y el parásito rara vez se encontró en años posteriores. Esto sugiere que 1990 fue un año excepcional o que la disminución en los niveles de infección de los años posteriores refleja un cambio de dieta del hospedador hacia presas que no son infectados por el acantocéfalo. La segunda hipótesis concuerda con los datos que indican un cambio significativo en la dieta del delfín listado durante el periodo de estudio. Sin embargo, no encontramos una relación significativa entre la dieta del hospedador y la estructura de la comunidad helmíntica. Por lo tanto, la cuestión sigue siendo si no fue posible detectar cambios a largo plazo porque sólo fueron analizados animales altamente parasitados (una muestra no aleatoria) o porque no hay cambios que detectar. Este es el primer estudio a largo plazo realizado sobre parásitos de cetáceos.

8. Se analizaron 1012 individuos de ocho especies de mictófidos (*Ceratoscopelus maderensis*, *Lampanyctus crocodilus*, *Notoscopelus elongatus*, *Benthosema glaciale*, *Myctophum punctatum*, *Lobianchia dofleini*, *Diaphus holti* y *Hygophum benoiti*) a nivel parasitológico. Los nematodos *Anisakis pegreffii* y *Anisakis physeteris* fueron detectados sólo en *N. elongatus* y *C. maderensis* con bajas prevalencias, siendo la prevalencia en *N. elongatus* significativamente más alta que en las otras tres especies de mictófidos con una n> 50. Se encontró un individuo de *Hysterothylacium* sp. en *N. elongatus* y Raphidascarididae gen. spp. en *N. elongatus* y *L. crocodilus*. Se encontraron dos plerocercoides sin identificar en *N. elongatus*. Este estudio proporciona el primer estudio parasitológico de mictófidos del Mediterráneo. Sólo *Anisakis* spp. son parásitos de cetáceos.

9. El hallazgo de *A. pegreffii* en *N. elongatus* apoyaría la hipótesis de que *A. pegreffii* circula por las redes tróficas mesopelágicas usando mictófidos como hospedadores paraténicos. Los mictófidos, incluyendo *N. elongatus* y *C. maderensis*, son una parte importante en la dieta del delfín listado en el área de estudio y *A. pegreffii* ha sido aislado del delfín listado. Los mictófidos podrían jugar un papel significativo en el ciclo vital de *A. physeteris*, pero sigue siendo una incógnita en qué medida son directamente consumidos por el hospedador definitivo, el cachalote, *Physeter macrocephalus*, o si son consumidos por cefalópodos que, a su vez, son consumidos por el cachalote.

10. A pesar del gran tamaño muestra analizado, no se encontraron helmintos parásitos en los cefalópodos *Alloteuthis media* y *Sepietta oweniana*.

11. Se encontraron cinco especies parásitas infectando *Bathypterois mediterraneus* en el área de estudio: el digeneo *Steringophorus* cf. *dorsolineatum*; cestodos tetrafilídeos recogidos bajo el nombre colectivo *Scolex pleuronectis*; dos larvas de nematodos, el rafidascárido *Hysterothylacium aduncum* y el anisákido *Anisakis* sp. larva 3 tipo II; y el copépodo penélido *Sarcotretes* sp. El hallazgo de todas las especies helmínticas representan nuevos registros en *B. mediterraneus*. Sólo *Anisakis* sp. es un parásito de cetáceos.

12. *Hysterothylacium aduncum* mostró diferencias significativas en la abundancia entre profundidades de 2000-2200m con 1000-1400m y 1400-2000m sin importar la localidad, mientras que *Steringophorus cf. dorsolineatum* mostró diferencias significativas entre localidades en todas las profundidades menos para 2000-2200m. Sugerimos el posible uso de dichas especies parásitas para la discriminación de stocks de *B. mediterraneus* en el Mediterráneo occidental.

13. De los 21 taxones helmínticos registrados en cetáceos en el área de estudio, sólo se detectaron estados infectivos de especies de *Anisakis* en peces mesopelágicos y batidemersales. La no detección de otros taxones es difícilmente atribuible al sesgo de detección. Es posible que algunas larvas de parásitos de cetáceos puedan haber sido pasadas por alto si sus niveles de infección en peces mesopelágicos y batidemersales, y cefalópodos son muy bajos en el área de estudio. Por otra parte, también podría ser que las especies seleccionadas para su análisis no albergaran otros estados infectivos de especies de helmíntos de cetáceos. Por lo tanto, dilucidar el ciclo vital de helmintos de cetáceos continua siendo, en su mayor parte, difícil. Este estudio destaca la necesidad de un mayor número de estudios parasitológicos de presas de cetáceos.

HELMINTHS FROM THE D STRIPED DOLPHIN (*STEN* IN THE WESTERN MEDIT PATTERNS WITHIN A

CHAPTER I:
GENERAL INTRODUCTION

DIGESTIVE TRACT OF THE
NELLA *COERULEOALBA*)
TERRANEAN: INFECTION
LONG-TERM SCALE

1.1. THE HELMINTH FAUNA OF CETACEANS

1.1.1. DIVERSITY

Cetaceans harbour a relatively diverse and highly specific fauna of trophically-transmitted metazoan parasites, including digeneans, cestodes, nematodes and acanthocephalans (Aznar *et al*., 2001; Raga *et al*., 2009). Around 40 species from four families of digeneans have typically been reported in cetaceans; species of Heterophyidae and Brauninidae infect dolphins, those of Notocotylidae whales, and those of Brachycladiidae both dolphins and whales. These digeneans occur mainly in the digestive system, although some species can also be found infecting the air sinuses and lungs (Raga *et al*., 2009). With regard to the cestodes, there are three families infecting cetaceans, namely, Tetrabothriidae, Diphyllobothriidae and Phyllobothriidae, which comprise, in overall, around 30 species (Aznar *et al*., 2001; Raga *et al*., 2009). Adults of species of the families Tetrabothriidae and Diphyllobothriidae have all been found along the intestine of cetaceans, from the duodenum to the terminal colon and rectum. Larvae of species of the family Phyllobothriidae are typically found, not only in the digestive tract, but also in the blubber and mesentery of many cetacean species. Rather interestingly, cetaceans seem to act as intermediate hosts for phyllobothriids, whose adults reproduce in large predatory sharks (Aznar *et al*., 2007). Among the nematodes, cetaceans harbor species of three main families, i.e. Anisakidae, Crassicaudidae and Pseudaliidae. Doubtless, species of the family Anisakidae are the most studied ones. Their economic and public health importance, together with the fact that their larvae are very common in a number of fish species, have probably contributed to a large number of studies about the ecology and health impact of members of this family. According to Mattiucci and Nascetti (2006), there are currently 9 species belonging to the genus *Anisakis*, i.e., *A. pegreffi*, *A. simplex* s.s., *A. berlandi*, *A. typica*, *A. ziphidarum*, *Anisakis* sp., *A. physeteris*, *A. brevispiculata* and *A. paggiae*, which have adapted to a number of hosts and environments (Mattiucci *et al*., 1997; Mattiucci and Nascetti, 2007) and are found in marine mammals all around the world (Mattiuci *et al*., 2005). Species of the family Crassicaudidae have been found infecting dolphins and whales, occurring in the kidneys, urogenital organs, muscles, mammary glands and even the placenta; whereas there are about 17 species of pseudaliids which are exclusively found in toothed whales, infecting mainly the lungs, air sinuses and heart (Raga *et al*., 2009). Finally, the fauna of acanthocephalans infecting cetaceans is relatively poor; nine species of *Bolbosoma* which infect whales, and secondarily dolphins worldwide (Raga *et al*., 2009), and three species of *Corynosoma* are specific to several dolphins, porpoises and whales (Sardella *et al*., 2005). Acanthocephalans are restricted to the digestive tract, especially the intestine, although *C. cetaceum* shows a preference for the stomach (Aznar *et al*., 2001).

A major problem for the study of cetacean helminths is sample availability. Most studies have relied on opportunistic sampling, which usually results in relatively low sample sizes of both hosts and parasites (Mateu *et al*., 2014). Furthermore, the preservation of helminth specimens is often poor because sampling is carried out on host carcasses. This hampers a proper description and identification (or classification, if necessary) of helminth taxa (Gibson, 2002, 2005). Accordingly, there are major gaps in our knowledge about the diversity of cetacean helminths. First, many host species have never been thoroughly analyzed for parasites (Aznar *et al*., 2001), or reports are extremely scarce, e.g., on most members of the family Ziphiidae (Fernández *et al*., 2004). Parasitological information is lacking, or very limited, in species which have recently gone extinct or that are on the verge of extinction, such as the Chinese lake dolphin (*Lipotes vexillifer*), which is considered to be "possibly extinct", or the vaquita (*Phocoena sinus*), which is considered critically endangered (IUCN Red List of Threatened Species, 2012). These cases indicate, not only that it may be too late to fill the gap about the parasitological fauna of these species but, more importantly, that specific unknown parasite species could have also become extinct. In addition, there are vast geographical areas where the parasite fauna of cetaceans remains fairly unexplored. A clear example is that of the Indian Ocean, where the information available about cetacean helminths is very scarce (see Kumarran, 2012 and references therein).

1.1.2. ORIGIN OF ASSOCIATIONS

During the Eocene (50 mya.), the ancestors of present-day cetaceans successfully colonized the marine realm (Berta *et al*., 2006). The transition from a terrestrial to a marine ecosystem has been considered to be a fundamental factor in structuring helminth fauna of cetaceans (Balbuena and Raga, 1993). Several authors suggest that the ocean represented an ecological barrier for the life-cycles of terrestrial parasites (Hoberg, 1987a; Balbuena and Raga, 1993; Aznar *et al*., 1994, 2001) so that the host's transition from land to sea may have resulted in the extinction of parasite species that were unable to adapt to the new environment (Delyamure, 1955; Aznar *et al*., 2001). According to this hypothesis, the present-day helminth fauna of cetaceans did not result from an "association by descent", i.e., they are not associated today because their terrestrial ancestors were associated in the past, but from an "association by colonization", i.e., parasites which emerged in other host taxa successfully colonized a cetacean species at sea (see Brooks and McLennan, 1991 and references therein). There are two major steps towards a successful colonization of new hosts. First, the parasite has to encounter the host, and second, the parasite must be able to survive in the new host (i.e., they must be compatible). The probability of encounter depends on behavioral, ecological and/or biogeographical factors; e.g., if parasite and host do not exploit the same habitat, there will be a low probability of encounter. Once contact has occurred, colonization will depend on the compatibility between parasite and host. Raga *et al*. (2009) argued that, since most parasites of marine mammals use food webs for transmission, it is highly probable that they end up in "right" and "wrong" host species. If the encounter with "wrong" host species occurs predictably throughout time, speciation or accommodation can be promoted and parasites may be able to overcome the compatibility filter. Sometimes, the parasite may incorporate a new host to its life-cycle as a result (Fernández *et al*., 1998). Available evidence indicates that all trophically-transmitted metazoan lineages infecting cetaceans became associated with them through colonization events at sea, being fish and marine birds major 'donor' hosts of parasites (see Hoberg, 1987a).

Phylogenetic studies suggest that the four main groups of gastrointestinal helminths of cetaceans that will be addressed in this study, namely, brachycladiids (Digenea), tetrabothriids (Cestoda), anisakids (Nematoda) and *Bolbosoma* species (Acanthocepahala) are taxa of marine origin which were transferred from other host groups such as teleosts, seabirds and pinnipeds, to cetaceans (see Aznar *et al*., 2001 and references therein; Fraija-Fernández *et al*., 2014). Fernández *et al*. (1998) suggested that the life-cycle of brachycladiids originally ended in fish and were able to switch to a cetacean hosts. Hoberg (1987a) argued that tetrabothriid cestodes evolved from a tetraphyllidean ancestor from elasmobrachs which speciated in seabirds and marine mammals, but recent phylogenetic evidence suggests that tetrabothriids suffered a host switching from marine archosaurians to early seabirds (Hoberg *et al*., 1997). Likewise, García-Varela *et al*. (2013) proposed that aquatic birds were the ancestral definitive host for acanthocephalans of the family Polymorphidae, with a secondary colonization of marine mammals (i.e., species of *Bolbosoma* and *Corynosoma*). Phylogenetic studies also suggest a marine origin for species of *Anisakis*, with a subsequent co-evolution between species of *Anisakis* and their definitive cetacean hosts (Mattiucci *et al*., 2005; Valentini *et al*., 2006). This would mean that the ancestor of cetaceans was already parasitized by a nematode which has evolved to the different *Anisakis* species we know today.

1.1.3. LIFE-CYCLES

Due to the obvious difficulties to work on hosts living in the marine realm, especially in oceanic waters, very little is known about the life-cycles of helminths from cetaceans (Aznar *et al*., 2001; Raga *et al*., 2009). In what follows, we will briefly describe the general life-cycle of each of the major helminth groups; then, we will point out the specific gaps about the identity of intermediate and paratenic hosts (if any) of the taxa infecting cetaceans.

All digeneans are parasitic and their life-cycles are among the most complex in nature, being usually linked to the feeding strategies of their definitive hosts (Bush *et al*., 2001). Unfortunately, very little is known about the life-cycles of species infecting cetaceans. As far as we are aware, there is only information about the free-living larvae of some brachycladiids. Dailey (1985) described a putative ciliated miracidium of *Nasitrema* sp., whereas several cercariae have been recorded infecting different species of gastropods (see Barnett *et al*., 2008 and references therein). Other aspects about the life-cycle of digeneans of cetaceans can be drawn based on the general life-cycle of digeneans. Adult digeneans release eggs to the environment, from which a miracium emerges and actively seeks for the first intermediate host, which almost invariably is a mollusk, usually a gastropod or a bivalve. Mollusks are therefore expected to be first intermediate hosts for digeneans of cetaceans. Within the mollusk, digeneans suffer a phase of asexual reproduction and, eventually, a free-living cercaria is released to the environment where, depending on the species, it can infect a second intermediate host, or the definitive host (Poulin, 1998; Bush *et al*., 2001). Cetaceans are top-predators and, therefore, we suspect that the life-cycle of their digeneans may require a second intermediate host, and even additional paratenic (transport) hosts, to fill the trophic gap between the first intermediate hosts and the definitive hosts (see Combes, 1995). Fish, cephalopods, or both, could act as second intermediate or paratenic hosts of digeneans infecting cetaceans, but no information exists on the specific identify of any of them.

The life-cycles of trophically-transmitted cestodes are complicated, often involving two or three hosts. Concerning taxa infecting cetaceans, no complete life-cycle has been elucidated for any species of the Tetrabothriidae. The first intermediate host of this group is believed to be a crustacean, where procercoids stay latent until being consumed by the second intermediate host, probably a cephalopod or a teleost. Once the second intermediate host is infected, the larva becomes a plerocercoid, which does also stay dormant until the definitive host preys upon this intermediate host (Bush *et al*., 2001). Hoberg (1987b) described a recently recruited plerocercoid of a tetrabothriid species found in the small intestine of greater shearwaters, *Puffinus gravis*, but no description of plerocercoids exists for species infecting cetaceans. Similar life-cycles have been predicted for tetraphyllideans, involving crustaceans, cephalopods and teleosts as intermediate/paratenic hosts, and elasmobrachs as definitive hosts (Hoberg, 1987a). However, no complete life-cycle has been elucidated to date for any species (Caira and Reyda, 2005). Four types of tetraphyllidean larvae do infect cetaceans, two plerocerdoids and two merocercoids, suggesting that cetaceans could act as intermediate hosts to complete the trophic gap between cephalopods and teleosts, and elasmobranchs (Aznar *et al*., 2007). The life-cycles of diphyllobothriids involve copepods and fish as intermediate hosts (Raga *et al*., 2009). The definitive hosts for most species are known to be pinnipeds, making up the bulk of their cestode fauna (Lauckner, 1985). However, there are also some species that use cetaceans as definitive hosts. Some plerocercoids are very common in fish species such as salmon, and have even been used to discriminate stocks (Mosquera *et al*., 2003).

Nematodes are among the most diverse creatures on earth and can be found as free living species, parasitic in plants, and parasitic in invertebrates and vertebrates (Bush *et al*., 2001). There are species of three common anisakid genera infecting marine mammals, namely *Pseudoterranova*, *Anisakis* and *Contracaecum*. The life-cycles of the three genera are quite similar. Generally, a free-living second stage larva (L2) emerges from the egg which is ingested by euphausiids and small copepods, where the third stage larvae (L3) develops. If the definitive host is a plankton-eating cetacean the life-cycle may not require additional intermediate hosts. However, species of teleosts and/or cephalopods may fill the trophic gap between the second intermediate host and many definitive cetacean hosts (Bush *et al*., 2001). The life-cycle of *Anisakis* has been elucidated in many geographical areas. For instance, at the St. Lawrence estuary (Canada), the euphausiid *Thysanoessa raschii* appears to be the main intermediate host, the fish *Mallotus villosus* and *Clupea harengus* function as paratenic hosts, and cetaceans, especially belugas, *Delphinapterus leucas*, are definitive hosts for *A. simplex* s.l. (Hays *et al*., 1998a, b). To give another example, in the Norwegian Deep (northern North Sea), Klimpel *et al*. (2004) identified the copepod *Paraeuchaeta norvegica* and the mesopelagic fish *Maurolicus muelleri* as obligatory first and second intermediate host, respectively, and cetaceans, particularly the harbor porpoise, *Phocoena phocoena*, as definitive hosts for *A. simplex* s.l. The life-cycle of species of the family Crassicaudidae is largely unknown and, as far as we know, no larvae stages have ever been found. Dailey (2005) suggested that a direct life-cycle may occur, with calves becoming infected by transplacental transmission. There are seven genera of pseudaliids whose species usually infect the respiratory tracts of toothed whales (Lehnert *et al*., 2010, and references therein). Most species are probably trophically-transmitted, but for species of the genus *Halocercus*, prenatal transmission has also been suggested (Dailey, 2005). As far as we are aware, there is a single study reporting pseudaliid larvae in cetacean prey. Using molecular techniques, Lehnert *et al*. (2010) were able to identify larvae of *Pseudalius inflexus* and *Parafilaroides gymnurus* infecting several fish species, mainly dab (*Limanda limanda*) and plaice (*Pleuronectes platessa*) from German waters.

Adult acanthocephalans have been found worldwide infecting amphibians, reptiles, birds, fish and mammals. The infective stage remains inside the egg waiting to be ingested by the intermediate host, which in the case of *Bolbosoma* spp., are copepods and euphausiids (Bush *et al*., 2001). Within the intermediate host the parasite becomes a cystacanth, which remains encysted until it reaches a paratenic host or the definitive host. There are reports of acanthocephalans of the genus *Bolbosoma* infecting putative paratenic fish hosts (Costa *et al*., 2000; Klimpel *et al*., 2006), but the identity of intermediate hosts remains elusive for most species. An exception is the euphausiid *Nyctiphanes couchii*, which has been identified as the intermediate host for *Bolbosoma balaenae* (Gregori *et al*., 2012). It is also interesting to note that *Bolbosoma vasculosum* occurs in dolphins as immature forms, but the identity of its specific definitive host, likely a whale, is still to be known. Marine species of *Corynosoma* use amphipods as intermediate hosts, fish as paratenic hosts and mostly marine mammals as definitive hosts (Aznar *et al*., 2006).

1.1.4. COMMUNITY STRUCTURE

Due to the origin of host-parasite associations (see Section 1.1.2) and the phylogenetic distance between cetaceans and other marine vertebrates, most helminth species found in cetaceans are specific to them. Therefore, the composition and species richness of helminth faunas from any cetacean species in any locality (i.e., the helminth component community) is expected to be restricted by two factors. First, the local diversity of helminth species would largely result from an interplay between regional history and local ecology (Holmes, 1990). Second, cetaceans would largely behave as an isolated group for parasitic exchange because they rarely share helminth species with other marine vertebrates. Therefore, the local community of cetacean species would be the most relevant factor setting the upper limits of diversity and composition of their helminth faunas (Aznar *et al*., 1998). Parasitological surveys of many cetacean species clearly illustrate the operation of these factors (Dailey and Perrin, 1973; Forrester *et al*., 1980; Balbuena and Raga, 1993; Hoberg *et al*., 1993; Gibson *et al*., 1998; Aguilar-Aguilar *et al*., 2001; Andrade *et al*., 2001; Fernández *et al*., 2003, 2004).

At the level of individual hosts (infracommunity level), species richness and composition primarily depends on the probability of encounter between hosts and parasites. It has been suggested that the infective stages of helminths in the marine realm are highly “diluted” (Hoberg, 1996, 2005; Raga *et al*., 2009; Valente *et al*., 2009; Santoro *et al*., 2010) and, therefore, the likelihood of contact between cetaceans and infected prey would be low. This could bring about two consequences. First, helminth infracommunities should be depauperate compared with the richness observed at component community; second, infracommunities should constitute random subsets of the pool of species of the component community. Although there are very few studies addressing this issue, some surveys report low mean helminth species richness per host in cetaceans compared to the total number of helminth taxa reported in the host sample (Balbuena and Raga, 1993; Dans *et al*., 1999; Fernández *et al*., 2003). However, the issue of predictability of infracommunities has been addressed only in two studies. Intestinal helminth infracommunities of oceanic, vagile long-finned pilot whales, *Globicephala melas*, were largely unpredictable (Balbuena and Raga, 1993), whereas those of coastal, sedentary franciscanas, *Pontoporia blainvillei*, were highly predictable (Aznar *et al*., 1995). Apparently, the likelihood of infection largely depends on both the degree of dispersion ("dilution") of infective stages in the environment and the mobility of all hosts involved in the cycle. Therefore, unpredictable infracommunities are expected to occur in oceanic cetaceans (Fernández *et al*., 2003, 2004), but this hypothesis should be explored through analysis of other cetacean species.

1.1.5. THE IMPORTANCE OF LONG-TERM SURVEYS

In ecology, it is a truism to claim that unveiling the works of nature, or the effect of natural or anthropogenic disturbances, requires long-term series of data; a clear example is that of climate change (Collins *et al*., 2013). Surprisingly, there are relatively few examples of such datasets (Likens, 1989). In fact, the tendency to provide 'snapshots' of ecological processes based on single samples, or short-term surveys, has been increasing over the last decades for sociological reasons: one of the greatest challenges facing any young academic is securing of tenure and, to accomplish this goal, he/she must publish many papers (Esch, 2004).

In parasitological studies, there are very good examples of long-term track of host-parasite systems, particularly in freshwater (e.g., Esch *et al*., 1988; Kennedy, 2001; Kennedy *et al*., 2002) and terrestrial (e.g., Cattadori *et al*., 2005; Hudson *et al*., 2006) habitats. A salient point of these studies is the importance of choosing a right (long-term) scale to correctly interpret patterns and processes. For instance, parasitological studies lasting for just 3 or 4 years failed to detect true cycles in the dynamics of several host-parasite systems (Esch, 2004). Punctual studies give no hint about changes that occur at an annual, not to mention decadal, scales.

To our knowledge, long-term (i.e., decadal) parasitological studies have never been carried out in the case of cetaceans due to the difficulties of getting host samples. Certainly, there are surveys collating parasitological data for long periods (e.g., Carvalho *et al*., 2010), but data have never been explored using an explicit framework to analyze temporal changes.

1.2. PARASITES AS ECOLOGICAL MARKERS

1.2.1. BACKGROUND

Parasites are excellent biological indicators due to their ecological ubiquity and strict dependence on their hosts (MacKenzie and Abaunza, 2005; Whiteman and Parker, 2005). Trophically-transmitted parasites, in particular, can also be used to trace interactions in trophic webs because their life-cycles usually include free-living stages and intermediate, paratenic (transport) and definitive hosts that are linked through predator-prey relationships. Therefore, significant changes in abiotic conditions, population size, habitat use or trophic interactions of their hosts can be reflected on changes of infection levels (Marcogliese and Cone, 1997; Marcogliese, 2004). These peculiarities make parasites ideal candidates to monitor not only trophic interactions, but also long-term changes in the ecosystem. Indeed, there are many studies which use parasites as biological indicators of pollution (i.e., MacKenzie *et al*., 1995; MacKenzie, 1999; Sures *et al*., 1999; Lewis *et al*., 2003; Malek *et al*., 2007), population structure and stock discrimination (i.e., Bower and Margolis, 1991; Williams *et al*., 1992; Balbuena *et al*., 1995; MacKenzie and Abaunza, 1998; Timi, 2007). Likewise, there are some studies that use parasites to detect variations associated to global change (i.e., Mackenzie, 1987; Khan and Chandra, 2006). Due to the complexity of host-parasite systems, it is speculated that global change may not only affect parasite transmission but also their virulence (Marcogliese, 2001). However, the use of helminths as biological indicators for global changes is far from understood and requires much more investigation.

1.2.2. PARASITES AS DIET INDICATORS AND TRACERS OF CHANGES IN THE TROPHIC WEB

The usefulness of trophically-transmitted helminths as trophic tags lies on the fact that they usually remain within the individual host for months or even years after infection (Polis and Hurd, 1996; Lafferty *et al*., 2008). Therefore, they provide certain advantages compared with gut content analysis as the latter represent just a ‘snapshot’ of the diet, i.e., the last meal. In this context, parasites represent a good complementary source of evidence of standard methods, including stomach content analysis or stable isotope analysis (Marcogliese, 2002, 2004). Even if there is no change in the diet of hosts, other factors such as trophic interactions and population size of intermediate host species could also have an effect on the helminth’s life-cycle and, therefore, on the parasitic load or composition of helminth communities in intermediate or definitive hosts. Note, however, that a correct interpretation of possible long-term changes requires a good knowledge about the life-cycles of the different helminth taxa, and the population dynamics of their hosts.

1.2.3. PARASITES AS BIOLOGICAL INDICATORS OF CETACEANS

In cetaceans, most studies have concentrated on the description of the parasite fauna (i.e., Zam *et al*., 1971; Dailey and Stroud, 1978; Raga and Carbonell, 1985; Balbuena and Raga, 1993; Cerioni and Mariniello, 1996; Gibson *et al*., 1998; Aguilar-Aguilar *et al*., 2001; Rosas *et al*., 2002; Fernández *et al*., 2003, 2004). Additionally, some authors have also used parasite species to investigate aspects of behaviour (Balbuena and Raga, 1991), social structure (Balbuena and Raga, 1993; Balbuena *et al*., 1995), phylogeography (Kaliszewska *et al*., 2005), stock discrimination (Aznar *et al*., 1995) and health assessment (Aznar *et al*., 1994, 2005) of their cetaceans hosts. However, parasitological studies are yet to reach their full potential. Aznar *et al*. (2010) extensively discussed the importance of the parasite fauna of cetaceans, with emphasis on the fact that parasites matter and that they are an invaluable source of information about their hosts which is yet to be exploited. Cetaceans are generally top predators within food webs, which means that they usually act as definitive hosts for many helminth species. If long-term changes are detected in the helminth community structure or composition, it would be reasonable to assume that there have been dietary changes and/or changes within the trophic web. However, as far as we are aware, there are no parasitological studies based on sample large enough, and collected over a time scale sufficiently long, to investigate this possibility.

1.3. CETACEANS AND CETACEAN HELMINTHS IN THE WESTERN MEDITERRANEAN

There are around 83 extant species of cetaceans, which, as a group, have colonized virtually all marine environments, from polar waters, e.g., beluga whales, *Delphinapterus leucas*, which inhabits arctic and sub-arctic waters to tropical waters, e.g., the tucuxi, *Sotalia fluviatilis*, which occurs in salt and fresh waters of northern South America (Borobia *et al*., 1991; Silva and Best, 1996). Toothed whales (Odontoceti) represent the largest of the two suborders, with 10 families, 40 genera and at least 70 species, whereas baleen whales (Mysticeti) is composed of 4 families, 6 genera and 13 species.

Notarbartolo di Sciara (2002) estimated that 21 cetacean species occur or have occurred in the Mediterranean along its recent history. Except for some occasional wanderers of Lessepsian origin, all species also occur in the Atlantic Ocean, and some of them cannot be considered to occur regularly in the Mediterranean, as they are present only at certain seasonal periods or at particular stages of their life-cycle. For instance, the continental shelf and offshore waters of the western Ligurian and Corsican Seas are known to be one of the most important feeding grounds in the Mediterranean basin, especially for Atlantic fin whales, *Balaenoptera physalus*, although there is evidence that there could also be a resident Mediterranean subpopulation (Castellote *et al*., 2012). Currently, seven species are known to have resident populations in the western Mediterranean: short-beaked common dolphin, *Delphinus delphis*, striped dolphin, *Stenella coeruleoalba*, bottlenose dolphin, *Tursiops truncatus*, Risso´s dolphin, *Grampus griseus*, long-finned pilot whale, *Globicephala melas*, sperm whale, *Physeter macrocephalus*, and Cuvier's beaked whale, *Ziphius cavirostris* (Notarbartolo di Sciara, 2002).

In western Mediterranean waters, at least 21 cetacean helminth taxa have been reported, including five digeneans, eight cestodes, seven nematodes and one acanthocephalan (Raga, 1985; Raga and Carbonell, 1985; Raga *et al*., 1992; Raga and Balbuena, 1993; Fernández *et al*., 2003, 2004; Aznar *et al*., 2006, 2007; Quiñones *et al*., 2013). Surveys generally deal with the specific identity of the parasite taxa found and, due to the problems of opportunistic sampling note above, there might be some helminth species yet to be described. Also, an effort is being made to ascertain ecological aspects of the host-parasite relationships (e.g., Aznar *et al*., 2006, 2007).

1.3.1. THE STRIPED DOLPHIN, *STENELLA COERULEOALBA*

The striped dolphin, *Stenella coeruleoalba* (Figure 1.1) is a cosmopolitan species distributed worldwide throughout temperate and tropical waters (Archer, 2009), including the Mediterranean Sea (Figure 1.2). In central Spanish Mediterranean waters, Gómez de Segura *et al*. (2006) concluded that the striped dolphin was the most abundant cetacean, with an estimated mean abundance of over 15,000 individuals. However, the Mediterranean population is currently considered as "vulnerable" by the IUCN Red List of Threatened Species (2012). Not surprisingly, there are a great number of studies about different aspects of the ecology of striped dolphins, including reproductive biology (Aguilar, 1991; Calzada *et al*., 1996, 1997), distribution and habitat use (Cañadas *et al*., 2002; Azzellino *et al*., 2008), diet composition (Blanco *et al*., 1995; Hassani *et al*., 1997) and health (Van Bressem *et al*., 1991, 2003; González *et al*., 2002; Muñoz *et al*., 2006; Raga *et al*., 2008).

Figure 1.1. Striped dolphins (*Stenella coeruleoalba*) from western Mediterranean waters. Source: Universitat de València.

The striped dolphin is considered to be a generalist predator that feeds on teleosts, cephalopods and crustaceans. The feeding ecology of striped dolphins, based on stomach contents, has been thoroughly investigated off the coasts of Japan, Atlantic Ocean and Mediterranean Sea (Miyazaki *et al*., 1973; Desportes, 1985; Pulcini *et al*., 1992; Sekiguchi *et al*., 1992; Würtz and Marrale, 1993; Blanco *et al*., 1995; Hassani *et al*., 1997; Meotti and Podestà, 1997; Ringelstein *et al*., 2006; Spitz *et al*., 2006; Öztürk *et al*., 2007; Santos *et al*., 2008). Other investigations are starting to use stable isotopes of carbon and nitrogen for studies on the diet of striped dolphins (Das *et al*., 2000; Gómez-Campos *et al*., 2011; Meissner *et al*., 2012). In Mediterranean waters, mesopelagic teleosts and cephalopods make up the bulk of prey species in terms of frequency and abundance (Würtz and Marrale, 1993; Blanco *et al*., 1995; Meotti and Podestà, 1997).

In 1990, western Mediterranean striped dolphins suffered an epizootic event caused by a dolphin morbillivirus (DMV). This die-off started in the summer of 1990 and affected the whole Mediterranean population (Aguilar and Raga, 1993). Although the total number of dead dolphins could not be determined, Aguilar and Raga (1993) suggested that several thousand individuals could have died due to encephalitis and/or pneumonia (Domingo *et al*., 1992; Duignan *et al*., 1992). The population apparently recovered at a fast rate, as the density of striped dolphins estimated in the Gulf of Valencia (Spain) in 2001-2003 was close to the maximum previously reported for this species in the western Mediterranean (Gómez de Segura *et al*., 2006). However, this high population density likely favored the propagation of morbillivirus infections (Black, 1991) and, indeed, a second epizooty occurred in 2007 (Raga *et al*., 2008). This new outbreak was geographically more restricted and affected a lower number of individuals (Castrillon *et al*., 2010; see also Aguilar and Raga 1993, Raga *et al*., 2008).

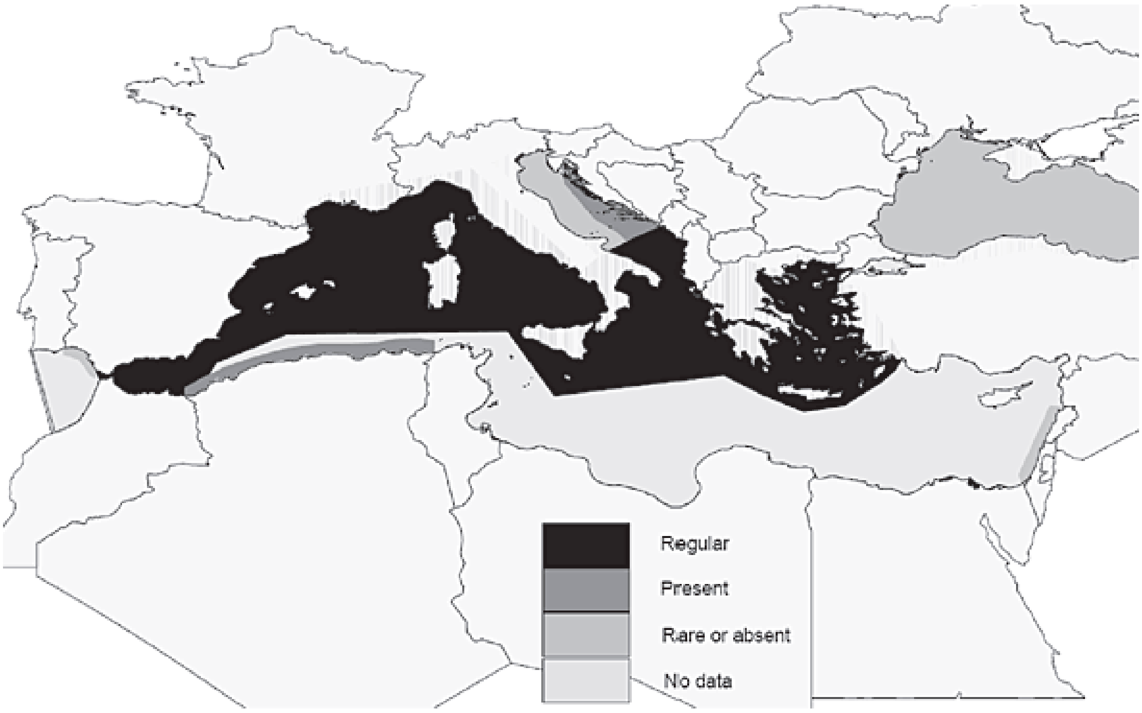

Figure 1.2. Distribution of the striped dolphin, *Stenella coeruleoalba*, in the Mediterranean Sea. Source: Reeves and Notarbartolo di Sciara (2006).

1.3.2. HELMINTH FAUNA OF THE STRIPED DOLPHIN

The helminth fauna of the striped dolphin has been surveyed in several geographical regions, including the north western Atlantic (Zam *et al*., 1971), the north eastern Atlantic (Abril *et al*., 1991; Abollo *et al*., 1998; Gibson *et al*., 1998), the north eastern Pacific (Dailey and Stroud, 1978; Dailey and Walker, 1978) and the Mediterranean Sea (Raga and Carbonell, 1985; Raga, 1986). These studies are based on low host sample sizes (n ≤ 15) and only provide information about the identity of the parasite taxa found. The nematode species *Skrjabinalius guevarai* and *Sterunus ovatus* have been found in the respiratory tract and lungs. The digeneans *Pholeter gastrophilus*, *Oschmarinella rochebruni* and *Brachycladium atlanticum*, together with the cestodes *Tetrabothrius forsteri*, *Strobilocephalus triangularis*, *Trigonocotyle* sp., the acanthocephalan *Bolbosoma vasculosum* and the nematodes *Anisakis* spp. have been reported within the digestive tract. The nematode *Crassicauda* sp. and the cestodes *Monorygma grimaldii* and *Phyllobothrium delphini* have been reported infecting the blubber and mesenteries (Raga and Carbonell, 1985: Fernández *et al*., 1991; Manfredi *et al*., 1992; Agustí *et al*., 1999). Although the specific identity of these species is generally well known there might be still taxa to be described. Furthermore, there are many other aspects about the ecology of these host-parasite systems for which information is lacking.

Up to date, there is a shortage of quantitative information about the helminth fauna of the striped dolphin based on a reasonably large sample size. Additionally, quantitative information from a helminth community perspective is scanty, and this would help to understand the role of phylogenetic and ecological factors on the dynamics of parasite faunas (Poulin, 1995). Studies on parasite communities from several cetacean species have suggested the fundamental role of phylogenetic factors in accounting for the diversity and composition of communities (Balbuena and Raga, 1993; Aznar *et al*., 1994). However, a relatively unexplored aspect in these studies is the potential role of host-related variables, i.e., species, total length, age and sex, in providing structure to helminth communities (Balbuena and Raga, 1993; Balbuena *et al*., 1994). Opportunistic sampling usually precludes such analyses because it is difficult to obtain large, homogeneous host samples to analyze. On the other hand, there is the need of long-term surveys that can provide insight, for the first time, about the dynamics of the system at a decadal scale. This goal is particularly interesting when there are clear disturbances in the system, both natural (i.e., viral epizootics) and anthropogenic (see the next section).

1.3.3. LONG-TERM IMPACTS ON MEDITERRANEAN STRIPED DOLPHIN POPULATIONS

Over the last decades the western Mediterranean population of striped dolphins has apparently suffered several anthropogenic and environmental impacts. Firstly, there is correlational evidence that overfishing of sardine, *Sardina pilchardus*, which is, supposedly, one of the main prey species of striped dolphins in the area, may have caused a trophic cascade leading to a significant dietary shift of striped dolphins towards demersal prey, particularly hake, *Merluccius merluccius*, as well as to fish and squid species of high turnover rates (Gómez-Campos *et al*., 2011). Since a hake-dominated diet provides lower energy content than a sardine-dominated one, it has been hypothesized that this dietary shift could entail long-term problems for the energetic balance of animals in the population, particularly those having higher nutritional demands such as young individuals or pregnant and lactating females (Gómez-Campos *et al*., 2011). The overfishing of key species in the trophic web (i.e, hake, sardine and anchovy, *Engraulis encrasicolus*) has already been linked to a decrease in the population of other cetacean species, such as the bottlenose dolphin and the common dolphin in the Mediterranean Sea (Bearzi *et al*., 2006, 2008a, b).

Secondly, during the first epizootic event caused by the dolphin morbillivirus, abnormally high organochlorine compounds (OC) levels were observed in dolphins affected by the DMV. Significant evidence suggests that the immunosuppressive effects associated to these compounds could have increased susceptibility to infection (Aguilar and Borrell, 1994). However, during the second outbreak, OC concentrations in deceased dolphins were not more elevated than in presumably healthy individuals and, therefore, this second outburst was not believed to have been enhanced by OC pollutants (Castrillon *et al*., 2010). Interestingly, Aguilar and Raga (1993) also suggested that abnormally warm water temperatures during the winter previous to the first epizootic could have led to a decrease in productivity with the consequent reduction of available food and the lack of capacity by dolphins to overcome the morbillivirus. Heatwaves and other type of extreme weather conditions have increasingly been reported during the last decades, and the point is whether they are related to climate change (Coumou and Rahmstorf, 2012). In the western Mediterranean, there is clear evidence of an increase of temperature and salinity of sea surface waters (Vargas-Yáñez *et al*., 2010), which may impact, not only populations of apex predators, like striped dolphins, but also the function of the whole ecosystem (Bianchi, 2007).

Due to all these anthropogenic and environmental impacts associated to global change, it would be interesting to explore whether long-term changes in the helminth fauna of cetaceans may reflect the impact of the factors discussed above.

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CHAPTER II:
AIM AND OBJECTIVES

2.1. AIM

The aim of the present study is three-fold. First, to increase knowledge regarding the specific diversity and community structure of the helminth fauna from the digestive tract of the striped dolphin, *Stenella coeruleoalba,* in western Mediterranean waters. Second, to investigate whether natural and anthropogenic factors may have had a long-term effect on the structure of these helminth communities. Third, to investigate the life-cycles of cetacean helminths, using this information to interpret the ecological patterns found in the definitive host.

2.2. OBJECTIVES

2.2.1. To describe the helminth fauna infecting the digestive tract of the striped dolphin, *Stenella coeruleoalba*, from the western Mediterranean.

2.2.2. To investigate the factors which determine the structure and composition of the helminth communities:

a) Specificity patterns

b) Community richness and predictability

2.2.3. To explore long-term changes in the structure of helminth communities.

2.2.4. To examine cetacean helminth larvae down the trophic web aiming to elucidate life-cycles and routes of infection:

a) Prey

b) Other organisms present in the ecosystem

Each of these objectives will be assessed in the different chapters which are briefly described below.

2.3. BRIEF CHAPTERS’ DESCRIPTION

2.3.1. Chapter 4 describes the helminth fauna found in the intestine of 52 striped dolphins. A total of 2,517 helminth specimens was collected; adult individuals of three cestode species (*Tetrabothrius forsteri*, *Trigonocotyle globicephalae* and *Strobilocephalus triangularis*) and immature individuals of an acanthocephalan species (*Bolbosoma vasculosum*). This study covers three main aspects: (i) it describes the intestinal helminth fauna of the striped dolphin in this region based on a large sample size; (ii) provides community parameters at both infracommunity and component community levels; and (iii) explores the effects of total length, age, and sex of dolphins on the structure of the helminth community.

2.3.2. Chapter 5 determines host specificity of the two hepato-pancreatic helminths, namely, the digeneans *Brachycladium atlanticum* and *Oschmarinella rochebruni*, in striped dolphins and 4 additional cetacean species from western Mediterranean waters. We examine the liver and pancreas of 103 striped dolphins, 18 Risso’s dolphins, 14 bottlenose dolphins, 8 common dolphins and 5 long-finned pilot whales. *Oschmarinella rochebruni* was exclusively found in striped dolphins, whereas *B. atlanticum* was found infecting striped dolphins and common dolphins. To date, *B. atlanticum* had only been reported in striped dolphins from the Canary Islands (Abril *et al*., 1991) and, therefore, its finding in a common dolphin constitutes a new host record. Additionally, a principal component analysis, using morphometric variables, was used to compare the specimens of *B. atlanticum* found in both host species in order to shed light on host species effects on the parasites’ fitness.

2.3.3. Chapter 6 describes the helminth fauna infecting the stomach of 47 striped dolphins and investigates the effects of host length and age on the abundance of helminth species. Additionally, this study identifies, for the first time, to a species level, larvae and adult specimens of the genus *Anisakis* which infect striped dolphins in the western Mediterranean.

2.3.4. Chapter 7 investigates long-term changes in the structure of the intestinal helminth community of striped dolphins. We examine 128 individuals, stranded during the last twenty years (1990-2010) to analyze, for the first time, possible long-term trends in the abundance and composition of the intestinal helminth community. Additionally, we combine these results with dietary data attempting to identify potential intermediate and/or paratenic hosts for helminths of the digestive tract of striped dolphins from western Mediterranean waters.

2.3.5. Chapter 8 sheds light on the hypothesis raised in the previous chapter, trying to find cetacean helminth larvae down the trophic web. For the first time, a large sample size of myctophids and cephalopods is examined for parasites. Molecular identification is attempted for all taxa whose adults could potentially infect cetaceans, comparing the identities with the individuals found in striped dolphins. This study examines 1,012 individuals of eight species of lantern fish (Myctophidae) and 793 individuals of two cephalopod species to evaluate the role of these species in the transmission of cetacean parasites.

2.3.6. In Chapter 9 we investigate, for the first time, the parasite fauna of the Mediterranean spiderfish, *Bathypterois mediterraneus*, a demersal deep-sea fish that is not consumed by striped dolphins but represents the most common fish below 1500m in the western Mediterranean and feeds on benthopelagic planktonic calanoid copepods. The rationale of this choice lies on the fact that infective stages of trophically-transmitted helminths of cetacean circulate through oceanic food webs and can be found also in non-prey species. A total of 170 individuals of *B. mediterraneus* were examined for parasites

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CHAPTER III:
GENERAL MATERIALS AND METHODS

A detailed account of materials and methods is given in each chapter. Here we present a brief summary of materials and methods used in this Ph.D. thesis.

3.1. HOST SAMPLING

3.1.1. CETACEAN SPECIES

Individuals of five cetacean species were analyzed for helminths infecting the digestive tract. In Chapter 4, the intestine of 28 male and 24 female striped dolphins, *Stenella coeruleoalba*, were examined for helminths. Mean total length (in cm) (±SD) of males and females was 198.5±9.9 (range: 179-213) and 191.8±11.4 (range: 163-211), respectively. In Chapter 5, the liver and pancreas of 103 striped dolphins (mean total length (±SD): 187.9±20.0; range: 110-213; number of males: 59; number of females: 44) were examined for helminths. These data were compared with these from 18 Risso's dolphins, *Grampus griseus,* (mean total length (±SD): 259.6±51.5; range: 170-308; number of males: 8; number of females: 10), 14 bottlenose dolphins, *Tursiops truncatus* (mean total length (±SD): 249.9±37.8; range: 196-324; number of males: 7; number of females: 7), 8 common dolphins, *Delphinus delphis* (mean total length (±SD): 164.2±39.1; range: 102-201; number of males: 2; number of females: 6), and 5 long-finned pilot whales, *Globicephala melas* (mean total length (±SD): 402.8±35.6; range: 375-455; number of males: 1; number of females: 3 [plus an unsexed individual]). In Chapter 6, the stomach of 47 striped dolphins (26 males and 20 females [plus an unsexed individual]) (mean total length (±SD): 194.2±12.4; range: 158-213) were examined for helminths.

Necropsies were performed to all but highly decomposed individuals following a standardized protocol (Geraci and Lounsbury, 2005) during 1990-2010, by the staff of the marine mammal stranding network. Before necropsy, all individuals were sexed and standardized measurements were taken to the nearest centimeter (Geraci and Lounsbury, 2005). During the necropsy, all organs were removed from the carcass and kept frozen at -20°C until their examination was possible.

In Chapter 4, animals were found stranded dead or alive along the coasts of Catalonia, Valencian Community and Murcia (between 42°25'57''N, 3°10'24''E and 37°23'70''N, 1°37'33''W) (Figure 3.1). Cetaceans were collected thanks to the marine mammal stranding network of the Marine Zoology Unit, University of Valencia. Detailed biological data of cetaceans examined for intestinal, hepato-pancreatic and stomach parasites are summarized in Appendix 1, Appendix 2 and Appendix 3, respectively.

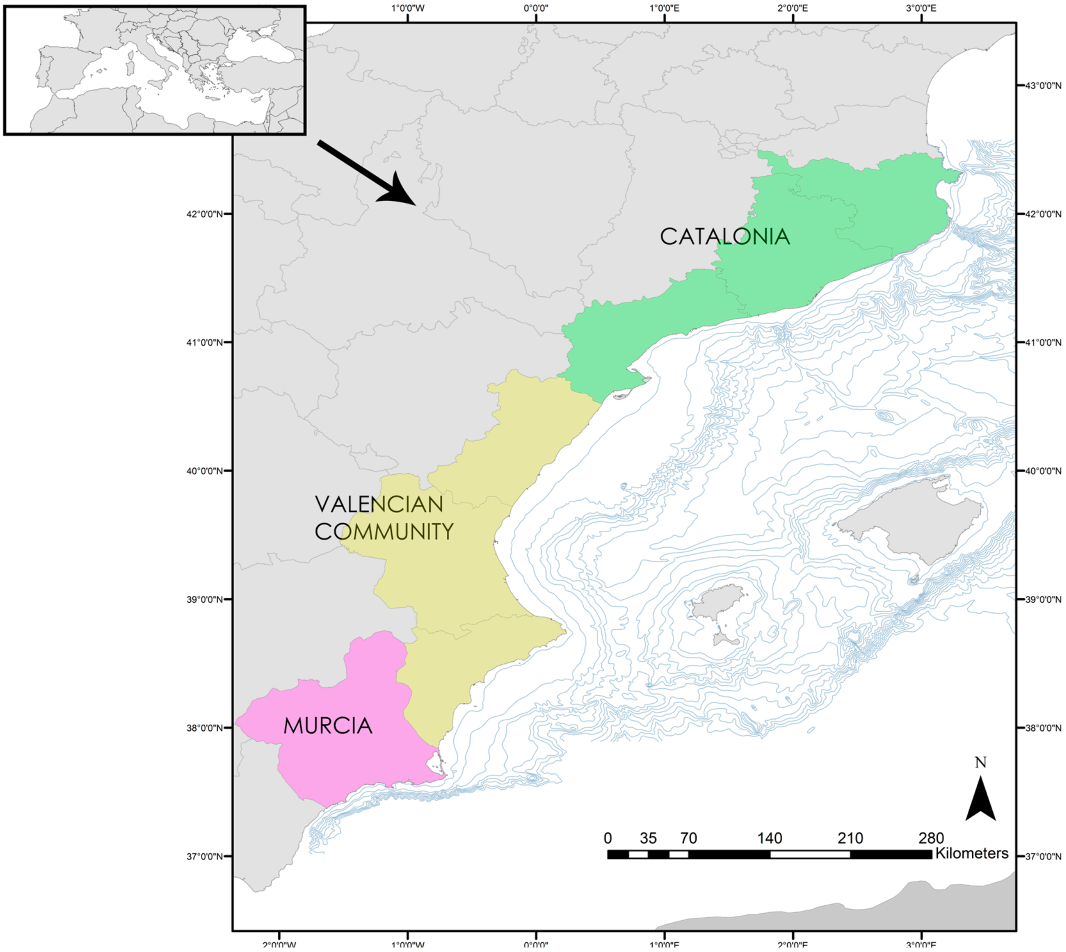

Figure 3.1. Sampling area of striped dolphins and other cetacean species analyzed in this study (Catalonia, Valencian Community and Murcia).

3.1.2. SPECIES OF TELEOSTS AND CEPHALOPODS

Eight species of the family Myctophidae and two cephalopod species analyzed in Chapter 8, were collected in the Gulf of Valencia and the Alboran Sea (Table 3.1 and Figure 3.2). Both regions include areas suggested to be Protected Areas of Mediterranean Importance due to the high cetacean diversity and abundance (Raga and Pantoja, 2004). Captures at the Gulf of Valencia were carried out during November 2010 by the fishing trawler "PAUSEP" at depths between 400m and 430m. Captures in the Alboran Sea were carried out during "MEDITS" survey (Bertrand *et al.*, 2002) in May 2011 and April/May 2012 on board of the research vessel "Cornide de Saavedra" with a GOC 73 bottom trawl sampler (Fiorentini *et al.*, 1999), operating at depths between 44m and 790m.

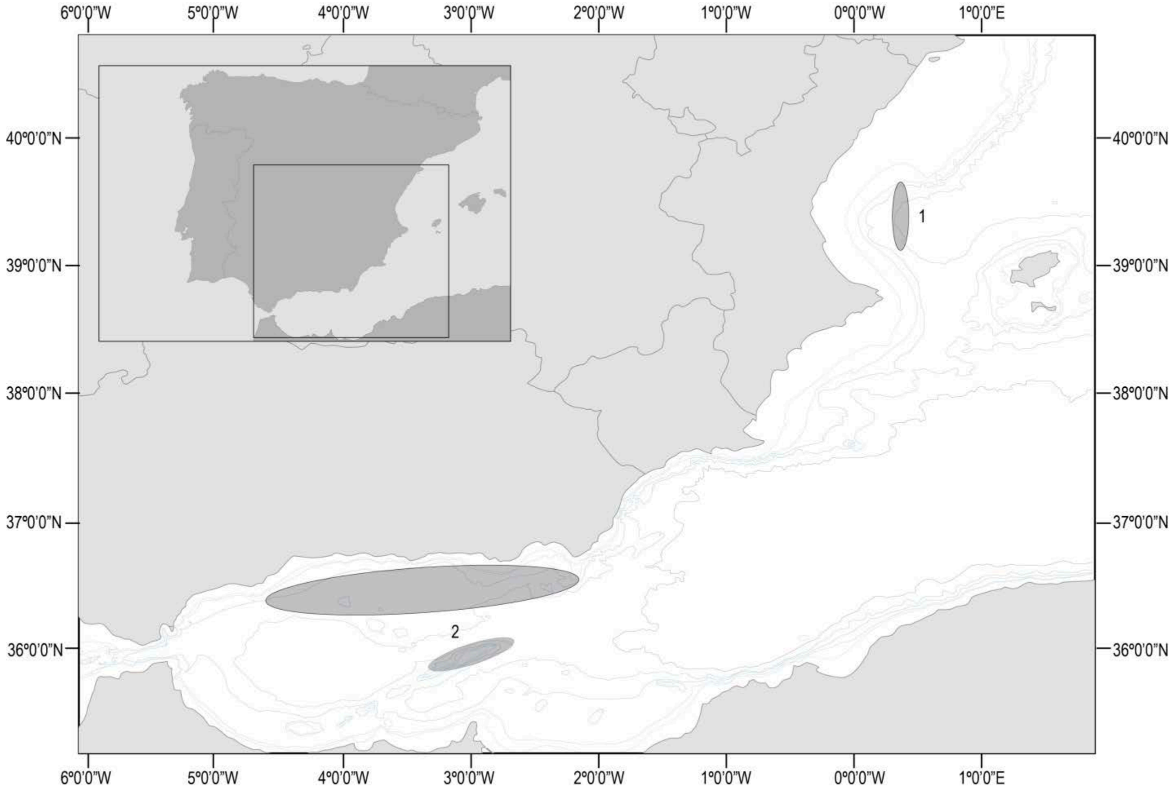

Figure 3.2. Sampling areas of myctophid and cephalopod species analyzed in this study: Gulf of Valencia (1) and Alboran Sea (2).

A total of 1,012 individuals of 8 species of the family Myctophidae was collected from both areas and examined for larval helminths, i.e. *Ceratoscopelus maderensis* (n= 390), *Lampanyctus crocodilus* (n= 295), *Notoscopelus elongatus* (n= 222), *Benthosema glaciale* (n= 70), *Myctophum punctatum* (n= 14), *Lobianchia dofleini* (n= 9), *Diaphus holti* (n= 8) and *Hygophum benoiti* (n= 4). A total of 792 individuals of two cephalopod species, namely, *Alloteuthis media* (n= 670) and *Sepietta oweniana* (n= 122), from the Alboran Sea was also examined for larval helminths (Table 3.1). Immediately after capture, all specimens were fixed and preserved in 70% ethanol on board.

Table 3.1. Sampling details for each of 8 species of myctophid fish and 2 cephalopod species collected from the western Mediterranean. GV: Gulf of Valencia; AS: Alboran Sea; *n*, number of specimens analyzed (see also Figure 3.2).

Area	Date	Species	*n*
Teleostei			
GV	November 2010	*Ceratoscopelus maderensis*	4
GV	November 2010	*Lampanyctus crocodilus*	1
GV	November 2010	*Notoscopelus elongatus*	221
GV	November 2010	*Benthosema glaciale*	26
AS	May 2011	*C. maderensis*	386
AS	May 2011 - April/May 2012	*L. crocodilus*	294
AS	May 2011	*N. elongatus*	1
AS	May 2011	*B. glaciale*	44
AS	May 2011	*M. punctatum*	14
AS	May 2011	*L. dofleini*	9
AS	May 2011	*D. holti*	8
AS	May 2011	*H. benoiti*	4
Cephalopoda			
AS	April/May 2012	*Alloteuthis media*	670
AS	April/May 2012	*Sepietta oweniana*	122

A total of 170 individuals of the Mediterranean spiderfish, *Bathypterois mediterraneus* (Osteichthyes: Ipnopidae), analyzed for Chapter 9, were collected from the continental slope in western Mediterranean waters (Figure 3.3). Captures were carried out during the oceanographic project "ANTROMARE" in July 2010, on board of the research vessel "García del Cid" with an OTSB14 bottom trawl (Merret and Marshall, 1981), operating at depths between 1000m and 2200m.

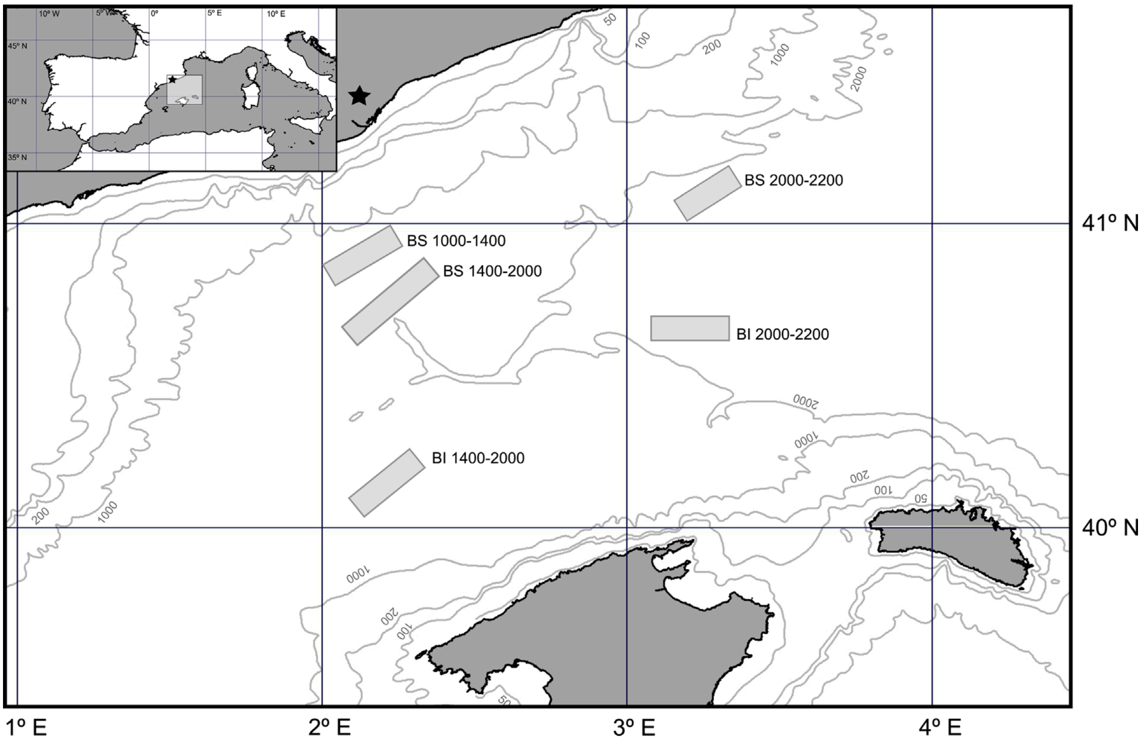

Figure 3.3. Localities where individuals of the Mediterranean spiderfish, *Bathypterois mediterraneus*, were sampled in the western Mediterranean waters.

3.2. COLLECTION AND PRESERVATION OF PARASITES

3.2.1. CETACEANS

3.2.1.1. Intestine

After thawing, the intestine of each cetacean individual was measured and divided into 20 sections of equal length. Each section was washed with tap water separately under a 0.02mm mesh and the solid content was collected in a Petri dish. The intestine wall was also thoroughly examined for attached helminths. Parasites were then collected under a stereomicroscope, washed in saline, identified, counted and fixed and preserved in 70% ethanol for morphological description or 100% ethanol for molecular work.

3.2.1.2. Liver and pancreas

After thawing, the liver and pancreas were weighted, cut into pieces and the hepato-pancreatic ducts opened. A first filtering was carried out with tap water under a 0.02mm mesh. After 5-10 minutes in cold water, a second filtering was carried out and the solid content was collected in a Petri dish. Parasites were then collected under a stereomicroscope, washed in saline, identified, counted and fixed and preserved in 70% ethanol for morphological purposes or 100% ethanol for molecular analysis.

3.2.1.3. Stomach

After thawing, the stomachs were opened and rinsed with water under a sieve of 0.02mm mesh. Worms were collected under a stereomicroscope, counted, cleaned in saline and fixed and preserved in absolute (for molecular analysis) or 70% ethanol (for morphometric analysis). The stomach walls were also examined for the presence of cysts.

3.2.2. FISH AND CEPHALOPODS

Myctophids and cephalopods were fixed in 70% ethanol on board. Once in the laboratory, all individuals were weighed and measured to the nearest millimeter (standard length for fish and mantle length for cephalopods). Gills, gonads, stomach and intestine, liver, kidney, heart and brain of fish, and gills, gonads and digestive tract of cephalopods, were individually put in saline, torn apart and examined under a stereomicroscope (20-100x). Epiaxial and hypoaxial muscle of fish, and mantle and (opened) siphon of cephalopods, were pressed between two glasses and examined by candling under a stereomicroscope. All parasites were collected, fixed and preserved in either 70% ethanol for morphological purposes or absolute ethanol for molecular analysis.

Individuals of *Bathypterois mediterraneus* were frozen at -20°C on board. After thawing, each animal was weighed and measured (standard length, to the nearest millimeter). Using a standardized protocol all organs were then taken apart, weighed and examined separately in a Petri dish with saline under a stereomicroscope. Muscle tissues were also examined by candling with transmitted light. All parasites were collected, fixed and preserved in 70% ethanol.

3.3. IDENTIFICATION OF PARASITES

Digeneans infecting the hepato-pancreatic ducts of dolphins were dehydrated through a graded ethanol series, stained with aluminium carmine (Georgiev *et al.*, 1986) and mounted on Canada balsam. Specimens were identified according to Raga (1986), Abril *et al.* (1991) and Gibson (2005). Additionally, to confirm that specimens from different host species were conspecific, the internal transcribed spacer 2 (ITS2) region of the rDNA gene was sequenced (see Nolan and Cribb, 2005). Total DNA was extracted following a standard phenol-chloroform methodology (Holzer *et al.*, 2004). Sequences are available in GenBank under accession numbers: GQ226035 (*Brachycladium atlanticum* from striped dolphin) and FJ211250 (*B. atlanticum* from common dolphin).

Specimens of cestodes from dolphins were mounted following the same protocol as digeneans (see above), and drawn using a light microscope connected to a drawing tube. The identification was based on Hoberg (1987a, 1989, 1990) and Nikolov *et al.* (2010).

Acanthocephalans from dolphins were cleared in lactophenol, mounted on temporary slides and drawn using a light microscope connected to a drawing tube. Identification was carried out according to Petrochenko (1958) and Costa *et al.* (2000).

Voucher specimens are deposited in the Natural History Museum, London, UK with accession numbers: *Tetrabothrius forsteri*, NHMUK 2013.5.31.6-7 and *Strobilocephalus triangularis*, NHMUK 2013.5.31.8-9 (for specimens from 1990) and *Tetrabothrius forsteri*, (2011) NHMUK 2013.5.31.1-2, *Trigonocotyle globicephalae*, (2011) NHMUK 2013.5.31.3 and (2007) *Strobilocephalus triangularis*, NHMUK 2013.5.31.4-5 (for specimens collected in later sampling). Additional material can be found in the collection of the Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain.

In the case of anisakid nematodes, the mitochondrial cytochrome oxidase subunit II (mtDNA *cox2*) gene was amplified. Previous studies have already confirmed the suitability of the *cox2* for the identification of anisakid nematode species (Valentini *et al.*, 2006; Mattiucci and Nascetti, 2008; Mattiucci *et al.*, 2010). Total DNA was extracted by using cetyltrithylammonium bromide method (CTAB) previously described in detail by Valentini *et al.* (2006) and Mattiucci *et al.* (2009, 2014). Sequences are available in GenBank under accession numbers: KF972438 (*A. pegreffii* infecting myctophids), KF972439 (*A. physeteris* infecting myctophids) and KR149283 (*A. pegreffii* infecting the striped dolphin).

For the molecular identification of other nematode larvae found in the stomach wall of some fish (see Chapter 8) that could not be identified based on morphological features, we sequenced the small-subunit ribosomal DNA (SSU rRNA) according to Carreno and Nadler (2003), Holterman *et al.* (2006) and Nadler *et al.* (2007). DNA from a single individual could be extracted using Chelex®. The sequence is available in GenBank under accession number: KF972437 (Raphidascarididae sp.).

For the identification of helminths collected from *Bathypterois mediterraneus*, adult platyhelminths were stained with aluminium carmine, dehydrated in ethanol, cleared in dimethylphthalate, and mounted in Canada balsam. In the case of nematodes, individuals were cleared with glycerol, whereas parasites from other phyla were examined on wet ethanol mounts.

3.4. TERMINOLOGY AND STATISTICAL ANALYSES

Ecological terms follow Bush *et al*. (1997). ‘Prevalence’ is the percentage of hosts in the sample infected by a helminth species, ‘intensity’ is the number of individuals of a helminth species in an infected host, and ‘mean abundance’ is the average number of individuals of a helminth species in a sample of hosts, regardless of whether they are infected or not. The 95% confidence intervals (CI) for prevalences were calculated with Sterne’s exact method (Reiczigel, 2003), and for mean abundances and mean intensities of each helminth taxa with the bias-corrected and accelerated bootstrap method using 20,000 replications (Rózsa *et al*., 2000), using the statistical software Quantitative Parasitology v. 3.0 (Rózsa *et al*., 2000). Most statistical analyses were carried out using the statistical package SPSS v.19. Specific analyses for each study or other statistical programs used will be indicated in each chapter. Statistical significance was set at $p< 0.05$.

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CHAPTER IV:

INTESTINAL HELMINTH FAUNA OF STRIPED DOLPHINS (*STENELLA COERULEOALBA*): NO EFFECTS OF HOST BODY LENGTH, AGE AND SEX

Marine Mammal Science

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Intestinal helminth fauna of striped dolphins (*Stenella coeruleoalba*) in the western Mediterranean: No effects of host body length, age and sex

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ABSTRACT

The intestine of 52 (28 males and 24 females) striped dolphins (*Stenella coeruleoalba*) from western Mediterranean waters was examined for helminths. Animals were found stranded along the Spanish coasts during the morbillivirus epizootic in 1990. In accordance with observations from other pelagic tetrapods, including cetaceans from other geographical regions, the intestinal helminth community was depauperate. Four helminth species were found: three tetrabothriid cestodes, *Tetrabothrius forsteri* (prevalence: 96.2%; mean intensity [95% CI]: 47.4 [34.4–68.2]), *Trigonocotyle globicephalae* (9.6%; 1.8 [1.0–2.2]), and *Strobilocephalus triangularis* (23.1%; 5.3 [2.9–10.2]), and immature individuals of the acanthocephalan *Bolbosoma vasculosum* (51.9%; 2.7 [2.0–3.4]). Schluter's variance test indicated a weak, but statistically significant association of occurrence of helminth species in dolphins, suggesting that some tetrabothriid species might use the same intermediate or paratenic hosts. Neither the abundance of helminth species nor infracommunity descriptors were significantly affected by sex, host body length or age. The absence of predictable effects of body size and age on the recruitment rate of helminths could be related to the fact that the host sample was largely composed of adults. This study provides quantitative evidence about helminth community structure of striped dolphins, based on a large sample size.

Key words: *Stenella coeruleoalba*, Cetacea, morbillivirus, *Tetrabothrius forsteri*, *Trigonocotyle globicephalae*, *Strobilocephalus triangularis*, Cestoda, *Bolbosoma vasculosum*, Acanthocephala, host effect.

The striped dolphin, *Stenella coeruleoalba*, is a cosmopolitan species distributed worldwide throughout temperate and tropical waters (Archer 2009). In central Spanish Mediterranean waters Gómez de Segura *et al.* (2006) concluded that this species was the most abundant cetacean, with an estimated abundance of 15,778 individuals. However, the Mediterranean population is currently considered as "vulnerable" by the IUCN Red List of Threatened Species (2012).

The helminth fauna of the striped dolphin has been surveyed in several geographical regions, including northwestern Atlantic (Zam *et al.* 1971), northeastern Atlantic (Abollo *et al.* 1998, Gibson *et al.* 1998), northeastern Pacific (Dailey and Stroud

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1978, Dailey and Walker 1978) and the Mediterranean Sea (Raga and Carbonell 1985, Raga 1986, Abril *et al.* 1991). These studies are based on low host sample sizes ($n \leq 15$) and provide information about the identity of the parasite taxa found. There is, however, a shortage of quantitative information from a helminth community perspective, which would help to understand the role of phylogenetic and ecological factors on the dynamics of parasite faunas (Poulin 1995). Studies on parasite communities from several cetacean species have suggested the fundamental role of phylogenetic factors in accounting for the diversity and composition of communities (Balbuena and Raga 1993, Aznar *et al.* 1994). However, a relatively unexplored aspect in these studies is the potential role of host-related variables, *i.e.*, total length, age, and sex, in providing structure to helminth communities (Balbuena and Raga 1993, Balbuena *et al.* 1994). Opportunistic sampling usually precludes such analyses because of the difficulty to obtain large, homogeneous host samples to analyze.

In 1990, western Mediterranean striped dolphins suffered an epizootic event caused by a morbillivirus (DMV) which killed thousands of individuals (Aguilar and Raga 1993) due to encephalitis and/or pneumonia (Domingo *et al.* 1992, Duignan *et al.* 1992). Because a large number of individuals across several age and sex classes could be collected during a short period of time and all stranded individuals had a known cause of death, this outbreak provided a unique opportunity to (1) describe the intestinal helminth fauna of the striped dolphin in this region based on a large sample size; (2) provide community parameters at both infracommunity and component community levels; and (3) explore the effects of total length, age, and sex of dolphins on the structure of the helminth community.

Materials and Methods

Sample Collection and Examination

A total of 52 striped dolphins were analyzed for intestinal helminths. Dolphins were found stranded along the Mediterranean coast of Spain (Valencian Community, between 40º31′00″N, 0º31′00″E and 37º50′00″N, 0º45′42″W) between July and December of 1990. Stranded dolphins were found either recently dead ($n = 40$) or moribund ($n = 12$). Live animals had neurologic symptoms and died shortly after being rescued.

Before necropsy, all individuals were sexed and measured (to the nearest centimeter) according to Geraci and Lounsbury (2005). Teeth were sent to the Department of Animal Biology, University of Barcelona (Spain), for age determination by counting growth layer groups in dentine (see Calzada *et al.* 1994 for procedure details). Age was available from 35 of the 52 dolphins.

During necropsy, the intestine was removed (from the distal end of duodenal ampulla to the anus) and stored at −20°C. After thawing, the intestines were measured and divided into 20 sections of equal length. Each section was washed with tap water separately under a 0.02 mm mesh and the solid content was collected in a Petri dish. The intestine wall was also thoroughly examined for attached helminths. Parasites were then collected under a stereomicroscope, washed in 0.9% saline, identified, counted, and fixed and preserved in 70% ethanol. Tetraphyllidean plerocercoids were reported on elsewhere (Aznar *et al.* 2007) and were not considered as members of the intestinal community because they use dolphins as intermediate hosts and occur in the intestine only temporarily (Aznar *et al.* 2007).

Cestodes were stained with iron acetocarmine (Georgiev *et al.* 1986) and drawn using a light microscope connected to a drawing tube to identify them based on Hoberg (1987*a*, 1989, 1990) and Nikolov *et al.* (2010). In the case of *Tetrabothrius forsteri* and *Trigonocotyle globicephalae*, Hoberg (1987*a*) reported high intraspecific variability in several characters among individuals. Therefore, we chose three discriminatory features to distinguish between both species, namely, number and aspect of auricles, size and position of bothridia, and number of testes. Acanthocephalans were cleared in lactophenol, drawn using the same procedure as with cestodes and identified according to Petrochenko (1958) and Costa *et al.* (2000).Voucher specimens have been deposited at the Natural History Museum of London with accession numbers: *Tetrabothrius forsteri*, NHMUK 2013.5.31.6-7 and *Strobilocephalus triangularis*, NHMUK 2013.5.31.8-9. Additional material can be found at the Collection of the Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain.

Definitions and Statistical Analysis

Ecological terms follow Bush *et al.* (1997) where "prevalence" is the percentage of hosts in the sample infected by a helminth species, "intensity" is the number of individuals of a helminth species in an infected host and "mean abundance" is the average number of individuals of a helminth species in a sample of hosts, regardless of whether they are infected. Total abundance, species richness, and Brillouin's index of diversity were used as overall descriptors of infracommunities. Total abundance is the number of individuals of all helminth species, and species richness the number of helminth species harbored by each individual dolphin. The 95% confidence interval (CI) for prevalence was calculated with Sterne's exact method (Reiczigel 2003). The 95% CI for mean abundance and intensity of each helminth taxa, mean total abundance, mean species richness and mean Brillouin's index was obtained with the bias-corrected and accelerated bootstrap method using 20,000 replications (Rózsa *et al.* 2000).

Two-tailed Spearman correlation tests were performed to investigate whether abundance of each helminth species and infracommunity variables varied with host length or age. Mann-Whitney tests were carried out to test for differences between males and females.

Overall association between helminth species was investigated with a variance ratio test (Schluter 1984). This test compares the observed variance in helminth species richness per dolphin, with the variance expected under the null hypothesis that occurrence of each species is independent of the others; the theoretical expected value is 1 (see Schluter 1984 for details). To create a null distribution, we fixed the observed value of species' occurrences and randomized the occurrence of each species among the dolphins, assuming they were equiprobable; we repeated this process 20,000 times. We were particularly interested in variance ratios that were high relative to the null model because this would suggest that several helminth species use the same intermediate host and/or co-occur in the same type of habitats where the dolphin forages (Lotz *et al.* 1995). Pairwise Fisher tests were performed to pinpoint significant associations between heminth species. Probability values were corrected by the sequential Bonferroni procedure (Rice 1989).

Permutational multivariate analysis of covariance (PERMANCOVA) based on a similarity matrix (Anderson *et al.* 2008) was used to investigate the effects of body length, age, and sex on the structure of parasite infracommunities. Sex was treated as a fixed factor, and body length or age was used as covariate. Because sample size were different for body length and age, two models were built, *i.e.*, one including sex and body length, and another including sex and age. We firstly included interaction terms of the covariates with the factor but when interactions were not significant, they were removed from models to increase the sensitivity of the analysis and to correctly interpret main effects (Engqvist 2005). To build the models, raw intensity data for each helminth species was fourth-root transformed, and then a Bray-Curtis similarity matrix between infracommunities was obtained. Because parasite species had zero values in most dolphins, a zero-adjusted Bray-Curtis matrix was obtained by adding a value of 1 to all cells (Clarke and Warwick 2001).

Pseudo-*F* statistics under a true null hypothesis were obtained by using a permutation procedure, *i.e.* group labels were randomly shuffled onto different simple units, and this procedure was repeated 10,000 times, according to the method described by Anderson *et al.* (2008) in models including covariates. Our design was unbalanced and, therefore, we used a type III sum of squares to ensure a complete independence of all effects being tested (Anderson *et al.* 2008).

The package Permanova for Primer v. 6 was used to calculate infracommunity parameters and to carry out the PERMANCOVAs (Clarke and Gorley 2006); the free software Quantitative Parasitology v. 3 (Reiczigel and Rózsa 2005) to set 95% CIs of parameters, the free software EcoSimR to perform the Schluter's test (Gotelli and Ellison 2013), and the statistical package SPSS v. 19 for the remaining analyses (SPSS Inc., Chicago, IL). Statistical significance was set at $P < 0.05$.

Results

Condition of Animals

Mean total length (± SD) of males and females were 198.5 ± 9.9 (range: 179–213; $n = 28$) and 191.8 ± 11.4 (range: 163–211; $n = 24$), respectively (see Fig. 1). Based on previously published work on these animals, all dolphins were in poor body condition, with a thin blubber layer that, in some animals, showed the outline of the ribs (Duignan *et al.* 1992). It was estimated that the lipid reserves of these animals had been reduced to about 60% of the normal values for the species in the area (Aguilar and Raga 1993). Out of 34 animals that were examined for pathology, 20 (58.8%) exhibited histopathological lesions consistent with morbillivirus infection, involving primarily the respiratory, lymphoreticular, and nervous system (Duignan *et al.* 1992). Antigens of morbillivirus were detected in pulmonary tissue of four out of five affected dolphins (Van Bressem *et al.* 1991). The morbillivirus was antigenically and genetically identified as the dolphin morbillivirus (DMV) (see Raga *et al.* 2008). Based on the above evidence, we assume that all the dolphins analyzed in this study were affected by an epizootics caused by DMV.

Infection Patterns

A total of 2,517 helminth specimens was collected, and all examined dolphins were infected by at least one helminth species (Table 1). Adult individuals were ascribed to three cestode species of the family Tetrabothriidae. *Tetrabothrius forsteri* was the most frequent and abundant species ($n = 2,372$) and was found throughout the intestine. However, the bulk of the infrapopulation was concentrated in the

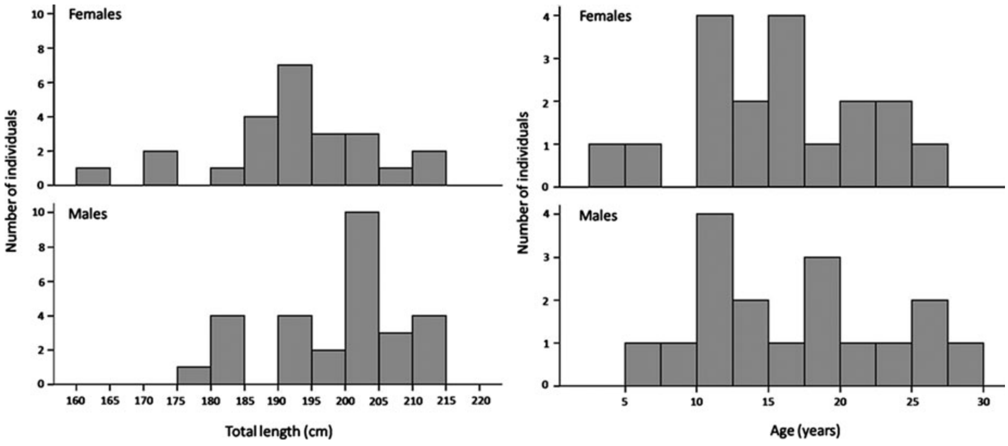

Figure 1. Frequency of females and males of striped dolphin (*Stenella coeruleoalba*) collected in this study grouped by total length (cm) and age (yr).

Table 1. Infection parameters of intestinal helminths found in 52 striped dolphins (*Stenella coeruleoalba*) from the western Mediterranean. CI: Confidence interval.

Species	Prevalence (%) (95% CI)	Mean intensity (95% CI) [range]	Mean abundance (95% CI)
Cestoda			
Tetrabothrius forsteri	96.2 (86.8–99.3)	47.4 (34.4–68.2) [1–294]	45.6 (33.1–66.4)
Trigonocotyle globicephalae	9.6 (3.9–20.9)	1.8 (1–2.2) [1–3]	0.2 (0.0–0.4)
Strobilocephalus triangularis	23.1 (13.2–36.4)	5.3 (2.9–10.2) [1–22]	1.3 (0.5–2.8)
Acanthocephala			
Bolbosoma vasculosum	51.9 (38.4–65.5)	2.7 (2.0–3.4) [1–7]	1.4 (0.9–2.0)

duodenum and numbers decreased monotonically from the first to the last intestinal section (Fig. 2a). *Trigonocotyle globicephalae* was found in low numbers (1–3 individuals per dolphin, total n = 9) in the first half of the intestine, more frequently in the most anterior part of the duodenum (Fig. 2b). No apparent gross pathological lesions were associated with either of these species. Specimens of *Strobilocephalus triangularis* (n = 64) were found in the posterior end of the colon (section 20) except in one case (Fig. 2c); the scolex was embedded in the colon wall and was typically surrounded by a fibrotic capsule. Mild inflammatory reaction was commonly observed in the region where scolices were attached. Apart from cestodes, only immature individuals of the polymorphid acanthocephalan *Bolbosoma vasculosum* (n = 72) were found throughout the intestine (Fig. 2d), either attached to the wall, or free in the lumen. Local inflammation of the mucosa and wall were found in the attachment point of proboscis.

Values of mean total abundance, species richness, and Brillouin's diversity index of helminth infracommunities for male and female striped dolphins are shown in Table 2. No significant differences were detected between sexes in the abundance of

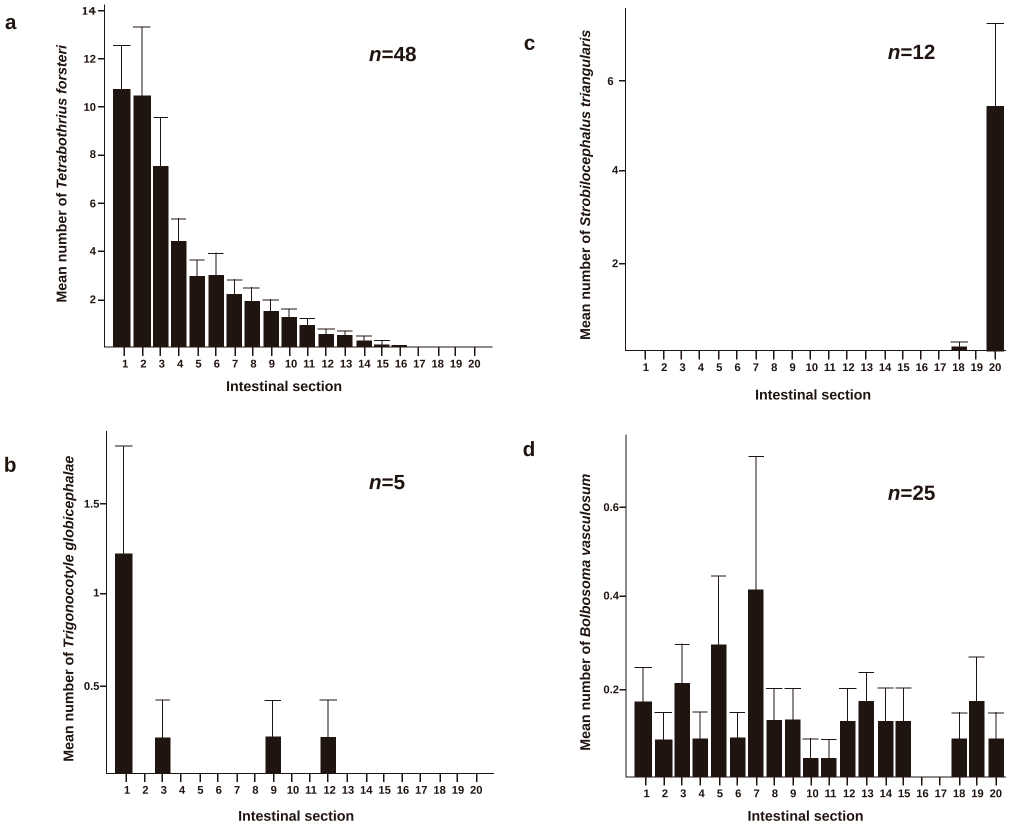

Figure 2. Mean number of individuals per intestinal section of four helminth species collected from striped dolphins (*Stenella coeruleoalba*) in the western Mediterranean. (a) *Tetrabothrius forsteri*, (b) *Trigonocotyle globicephalae*, (c) *Strobilocephalus triangularis* and (d) *Bolbosoma vasculosum*. Section 1 begins at the distal end of the duodenal ampulla and section 20 ends at the anus. *n* is the number of infected dolphins for each helminth species. Bars represent SD.

any helminth species or infracommunity descriptors (Mann-Whitney tests, $P > 0.25$ in all cases). Overall, infracommunities had low values of richness, with less than two species per dolphin on average and a maximum of four species (Table 2). A total of 22 dolphins (42.3%) had one helminth species, 20 (38.5%) had two, 8 (15.4%) had three, and only 2 dolphins (3.8%) had four species. Mean total abundance was also low (<60 helminth individuals per dolphin) with a maximum of 294 individuals (Table 2). Brillouin's diversity index was also very low (Table 2), reflecting both the low species richness per dolphin and the fact that infracommunities were numerically dominated by *T. forsteri*. In fact, only two dolphins in the sample did not harbor *T. forsteri*. Also, this species co-occurred with other helminth taxa in 30 dolphins and, in all but one dolphin, *T. forsteri* had a higher intensity than any other species and made up, on average, 86.6% (SD: 15.7) of total abundance.

Schluter's variance test indicated a weak, but statistically significant positive association of occurrence of helminth species in dolphins (observed V [variance] = 1.259, mean V in simulations: 0.950 [0.022], $P = 0.035$). However, none of the pair-wise associations between species was statistically significant (all nominal P-values > 0.185).

Table 2. Mean values of infracommunity descriptors of intestinal helminth communities of female and male striped dolphins (*Stenella coeruleoalba*) from the western Mediterranean.

Sex	Total abundance (CI 95%) [range]	Species richness (CI 95%) [range]	Brillouin's index (CI 95%) [range]
Female ($n = 24$)	58.7 (37.9–97.9) [2–227]	1.67 (1.38–1.88) [1–3]	0.177 (0.105–0.282) [0–0.630]
Male ($n = 28$)	39.6 (27.5–62.8) [1–294]	1.93 (1.57–2.29) [1–4]	0.168 (0.104–0.246) [0–0.817]
Total ($n = 52$)	48.4 (36.1–68.9) [1–294]	1.81 (1.58–2.04) [1–4]	0.172 (0.123–0.233) [0–0.817]

Spearman correlation tests showed no statistically significant relationship between the abundance of any helminth species and host body length or age; correlation coefficients ranged from -0.224 to 0.064, with nominal $P > 0.05$ in all cases (Table 3). Infracommunity descriptors also had very low correlations with host age (range from -0.181 to -0.110) and length (range from -0.147 to -0.048), and none was statistically significant (Table 3, nominal $P > 0.05$ in all cases). PERMANCOVA models also indicated that host body length, age, and sex did not significantly influence the structure of infracommunities (Table 4).

DISCUSSION

The striped dolphins analyzed in this study were obtained from a population affected by DMV which killed thousands of individuals (Aguilar and Raga 1993). An important question that arises is the extent to which the disease may have affected the abundance of helminths present in the intestine of sampled animals. Morbilliviruses typically impair the immune system of their hosts (Kennedy 1998 and references therein) and, therefore, the establishment of metazoan parasites could be easier than expected in healthy individuals. This possibility has indeed been suggested for ectoparasites and phoronts colonizing striped dolphins affected by DMV (Aznar *et al.*

Table 3. Spearman correlation coefficients (r_s) between infection parameters or infracommunity descriptors of intestinal helminths, and age or length of striped dolphins, *Stenella coeruleoalba*, from the western Mediterranean.

	Age ($n = 35$)		Body legnth ($n = 52$)	
	r_s	P	r_s	P
Cestoda				
Tetrabothrius forsteri	–0.068	0.699	–0.001	0.996
Trigonocotyle globicephalae	0.064	0.714	–0.130	0.357
Strobilocephalus triangularis	–0.224	0.195	–0.118	0.406
Acanthocephala				
Bolbosoma vasculosum	–0.105	0.549	0.026	0.857
Infracommunity descriptors				
Total abundance	–0.181	0.299	–0.048	0.738
Species richness	–0.110	0.528	–0.107	0.452
Brillouin's index	–0.176	0.311	–0.147	0.297

Table 4. Results of PERMANCOVA models for the effects of host variables on the structure of intestinal helminth communities striped dolphins, *Stenella coeruleoalba*, from the western Mediterranean. Two separate models were built for length and age because the number of aged animals was smaller (see text for details).

	Variable	Pseudo-*F*	*P*	df1[a]	df2[a]
Full model	Length	0.431	0.669	1	48
	Sex	1.535	0.239	1	48
	Length × sex	0.334	0.716	1	48
Main effects model	Length	0.437	0.670	1	49
	Sex	1.556	0.238	1	49
Full model	Age	0.493	0.734	1	31
	Sex	1.303	0.280	1	31
	Age × sex	2.248	0.069	1	31
Main effects model	Age	0.475	0.750	1	32
	Sex	1.254	0.295	1	32

[a] Degrees of freedom associated with the test for each parameter.

2005). However, dolphins developing severe symptoms of the illness were also presumably unable to feed, in which case the influence of an impaired immune system would be inconsequential because fasting would stop recruitment of trophically transmitted helminths. Stomachs of most striped dolphins affected by DMV only contained hard remains of prey, including otoliths of small fish species (Blanco *et al.* 1995, Blanco[2]), suggesting that dolphins may have stopped feeding a few hours to 1–2 wk prior to death (see Sekigushi and Best 1997). We can speculate that this is indeed the period between the manifestation of clinical symptoms of DMV and hosts' death. Although exact data from DMV are not available (Kennedy 1998), evidence from allied morbilliviruses infecting pinnipeds and mustelids suggest that most animals die 1–2 wk after the first clinical symptoms appear (Harder *et al.* 1990, Evermann *et al.* 2001). If so, we would neither expect an obvious decrease on helminth infection levels in ill animals because the period between the manifestation of clinical symptoms of DMV and hosts' death would be shorter than helminths' life span, *i.e.*, months (see Wittner and Tanowitz 2006). In summary, we suggest that inferences made in this study are not severely distorted by the potential impact of DMV on the recruitment and abundance of helminths because the disease runs its course quickly.

Perhaps it is not coincidental that infection levels of the same helminth species studied here are surprisingly similar to those observed in other *Stenella* dolphins (*S. graffmani* and *S. longirostris*) caught in fisheries (thus, presumably healthy) in Pacific waters (Dailey and Perrin 1973).

Intestinal Helminth Fauna

Tetrabothrius forsteri (Krefft 1871) has been reported in several species of delphinids from the Atlantic and the Pacific, including *Lagenodelphis hosei*, *Lagenorhynchus acutus*, *Steno bredanensis*, *Stenella attenuata*, *S. longirostris*, and *Tursiops truncatus* (Forrester and

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[2] Personal communication from Carmen Blanco, Unitat de Zoologia Marina, Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, PO Box 22085, Valencia 46071, Spain, May 2013.

Robertson 1975, Dailey and Otto 1982, Van Waerebeek *et al.* 1990, Mignucci-Giannoni *et al.* 1998, Olson and Caira 1999). In Mediterranean waters, *T. forsteri* has been reported infecting *S. coeruleoalba* (Raga and Carbonell 1985), *Delphinus delphis* (Raga and Balbuena 1987, Quiñones *et al.* 2013), *T. truncatus* (Quiñones *et al.* 2013), and a nondelphinid cetacean, *Mesoplodon bidens* (Raga and Balbuena 1987). The pattern of distribution along the intestine suggests that *T. forsteri* would favor the anterior duodenum. However, individuals of this species are apparently able to mature and reproduce in the anterior half of the intestine.

Likewise, *Strobilocephalus triangularis* (Diesing 1850) has also been found in Atlantic and Pacific waters, infecting mainly delphinids, *i.e.*, *Cephalorhynchus commersonii*, *D. delphis*, *Hyperoodon ampullatus*, *L. hosei*, *L. acutus*, *M. bidens*, *S. attenuata*, *S. longirostris*, and *S. bredanensis* (Baer 1932, Delyamure 1955, Dailey and Otto 1982, Raga 1985, Berón-Vera *et al.* 2001). In western Mediterranean waters it has also been reported infecting *S. coeruleoalba* (Raga and Carbonell 1985) and *T. truncatus* (Quiñones *et al.* 2013). In all host species reported to date, *S. triangularis* typically occurs in the wall of the colon and rectum, usually provoking an intense inflammatory reaction and fibrosis in the peripheral tissues (Howard 1983, Raga and Carbonell 1985).

Trigonocotyle globicephalae (Baer 1954) seems more specific than *T. forsteri* and appears to be typically associated only with teuthophagous delphinids. Host records include *G. griseus* off the coasts of Japan, *Globicephala melas* in the North Atlantic, and *G. macrorhynchus* from Japan (Yamaguti 1959, Kikuchi *et al.* 1988, Balbuena and Raga 1993). In the Mediterranean Sea, it has been reported to be a common intestinal parasite of *G. melas* and *G. griseus* (Raga and Balbuena 1993, Fernández *et al.* 2003). Accordingly, this study reports for the first time, *T. globicephalae* infecting a species from the genus *Stenella*. The intestinal distribution of *T. globicephalae* in striped dolphin is similar to that described in *G. melas*, with most individuals occurring in the first half of the intestine (Balbuena 1991).

Finally, individuals of *Bolbosoma vasculosum* (Rudolphi 1819) were found throughout the intestine, from the anterior-most duodenum to the rectum, without a preferential region of attachment. Many individuals were still attached to the wall at the time of finding, but all individuals were sexually immature. These findings suggest that *B. vasculosum* is able to establish, but unable to reproduce in striped dolphins. This observation is common to nearly all records of *B. vasculosum* found worldwide to date. Apart from records of cystancaths in paratenic fish hosts (*e.g.*, Bakay and Zubchenko 1984, Panebianco and Sebastio 1988, Costa *et al.* 2000, Klimpel *et al.* 2006, Garcia *et al.* 2008), juvenile individuals of *B. vasculosum* have been reported from the intestine of many odontocetes, mainly oceanic, in Pacific (Pendergraph 1971, Zam *et al.* 1971), Atlantic (Dailey and Perrin 1973, Mignucci-Giannoni *et al.* 1998, Costa *et al.* 2000, Aguilar-Aguilar *et al.* 2001), and Mediterranean waters (Raga and Carbonell 1985, Fernández *et al.* 2004). However, as far as we are aware, there are only three studies reporting gravid individuals of *B. vasculosum*. Williams and Bunkley-Williams (1996) found a single adult individual in a king mackerel, *Scomberomorus cavalla*, from Puerto Rico. Additionally, Harada (1935) found three individuals of *B. thunni* (synonym of *B. vasculosum* according to Petrochenko 1958) with developing acanthors in tuna, *Thunnus thynnus* from Japan. However, it is unlikely that either king mackerel or tuna are normal definitive hosts for *B. vasculosum*. First, no additional records of gravid individuals of this acanthocephalan have been reported in king mackerel or tuna (Williams and Bunkley-Williams 1996, Munday *et al.* 2003). Second, all species of *Bolbosoma* whose description is based on adult specimens are specific, either to baleen whales (Delyamure 1955, 1961; Zdzitowiecki 1991) or toothed whales (Amin and Margolis 1998). Therefore, the definitive host for *B. vasculosum* could be predicted to be a cetacean. In fact, Yamaguti (1963) also reported some gravid individuals of *B. vasculosum* in common dolphins, *D. delphis*, and Sowerby's beaked whales, *M. bidens*. However, the absence of additional records of adult worms of *B. vasculosum* in any cetacean species makes the identity of its definitive host still elusive.

Community Patterns

All helminth species reported in this study are almost exclusively found in cetaceans, mainly members of the family Delphinidae inhabiting oceanic waters. This pattern of specificity is common to other oceanic cetaceans, the intestinal helminth fauna of which is dominated by tetrabothriids and/or *Bolbosoma* spp. worldwide. Thus, at the community component level, the upper limit of diversity is predicted to be largely set by local diversity of these species (see *e.g.*, Dailey and Perrin 1973; Forrester *et al.* 1980; Balbuena and Raga 1993; Hoberg *et al.* 1993; Gibson *et al.* 1998; Aguilar-Aguilar *et al.* 2001; Andrade *et al.* 2001; Fernández *et al.* 2003, 2004). In other words, oceanic cetaceans behave as an isolated group for exchange of parasites, if at all (Aznar *et al.* 1998). At infracommunity level, however, species richness depends on the probability of encounter between these specific parasite taxa and their potential hosts. It has been suggested that probability of transmission is especially low for trophically transmitted helmiths in the oceanic realm because infective stages are highly "diluted" (Valente *et al.* 2009 and Santoro *et al.* 2010 for marine turtles; Hoberg 1996, 2005 for marine birds; and Raga *et al.* 2009 for marine mammals). In fact, 80% of striped dolphins in our sample harbored only 1–2 species, and helminth abundance was very low (<60 individuals) compared with aquatic mammals (references in Bush *et al.* 1990) including coastal cetaceans (Aznar *et al.* 1994, Herreras *et al.* 1997, Dans *et al.* 1999). However, it seems clear that one species, namely, *T. forsteri*, has a great potential for colonization because nearly all dolphins were infected with this species. A proper test for the "dilution" hypothesis would require, firstly, identifying the intermediate hosts for tetrabothriids and *Bolbosoma* spp. and, secondly, quantifying infection levels. In this respect, information is extremely scanty. Recently, Gregori *et al.* (2012) found low prevalence (0.1%) of a *Bolbosoma* species in its euphausiid intermediate host and suggested that such low infection levels could be related to the effect of dilution in the pelagic realm and be typical to helminths using zooplanktonic hosts. Crustaceans, cephalopods, and teleosts have been suggested as intermediate and/or paratenic hosts for tetrabothriid cestodes, but no data are available about the specific identity of intermediate hosts for any tetrabothriid species (Hoberg 1987*b*).

Low recruitment rates in the oceanic realm have also been related to the absence of community structure in intestinal helminth communities in cetaceans (Balbuena and Raga 1993) and marine turtles (Santoro *et al.* 2010). In other words, when the probability of infection is low, the resulting pattern would be one of independent colonization of each helminth species, regardless of whether some helminth species may use the same intermediate hosts (Santoro *et al.* 2010). In individual striped dolphins, however, communities are predicted to be composed by *T. forsteri* plus other tetrabothriids or *Bolbosoma*. As a result, co-occurrence of helminth species in individual dolphins tends to be nonrandom, with a slight excess of positive associations between species. Note, however, that statistical support is weak (see Results). Overall, these data could be interpreted as evidence that some helminth species (most likely tetrabothriid species) use the same intermediate/paratenic hosts, or simply as evidence that some species tend to co-occur simply because one of them (*T. forsteri*) is highly prevalent. In this respect, specific data about intermediate hosts is crucial (see above).

Host Effects

In cetaceans, we are aware of a single study that has examined changes in helminth infracommunity parameters according to host traits. In long-fined pilot whales, *Globicephala melas*, Balbuena and Raga (1993) found a positive relationship between host's age and the species' diversity and total abundance of infracommunities of intestinal helminths. These authors suggested that the observed trend might result from the relationship between age and body size, *i.e.*, larger whales would require more prey to satisfy their metabolic demands, thereby increasing the chances of infection with tropically-transmitted helminths. Potential ontogenetic changes in diet were considered to be of less importance (Balbuena and Raga 1993).

However, in striped dolphins, we failed to detect significant effects of host body length, age, or sex on the abundance of any of the helminth species, nor on infracommunity descriptors. The sample of striped dolphins contains a reduced number of juvenile individuals (based on age/body length data, see Calzada *et al.* 1997) and, therefore, we could infer that, among adult hosts, infection parameters seem to vary idiosyncratically regardless of host's body length or age. Interestingly, a major difference between our sample and that from Balbuena and Raga (1993) is that the latter included a much wider range of ages (from 1 to 37 yr), with a sizeable portion of the sample (n = 95, or 64.6% of the total sample) being juvenile individuals (age ≤ 10 yr) in the growing phase (Balbuena and Raga 1993, Balbuena,[3] Bloch *et al.* 1993). When young individuals (≤10 yr old) were removed from the pilot whale sample, the relationship between host's age and helminth infracommunity parameters also vanished.[3]

The latter finding can make one wonder whether host age or body length could also act as a significant structuring force of helminth infracommunities in striped dolphins at a greater ontogenetic scale. In fact, Dailey and Perrin (1973) reported, based on circumstantial evidence, higher prevalence and abundance of *T. forsteri* and *S. triangularis* in calves and subadults of two *Stenella* spp. In our study, the relationship between infection parameters of both cestode species and host's age or total length, though not significant, might suggest a negative correlation. Given the significant dietary differences reported between juvenile and adult individuals of striped dolphins in the study area (Gómez-Campos *et al.* 2011), the hypothesis of a host's age effect cannot be ruled out and should be addressed in future studies.

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CHAPTER V:

HOST SPECIFICITY OF *OSCHMARINELLA ROCHEBRUNI* AND *BRACHYCLADIUM ATLANTICUM* (DIGENEA: BRACHYCLADIIDAE) IN FIVE CETACEAN SPECIES FROM WESTERN MEDITERRANEAN WATERS

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Host specificity of *Oschmarinella rochebruni* and *Brachycladium atlanticum* (Digenea: Brachycladiidae) in five cetacean species from western Mediterranean waters

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Abstract

We investigated patterns of specificity of liver flukes (fam. Brachycladiidae) in a community of cetaceans from the western Mediterranean. The liver and pancreas of 103 striped dolphins, *Stenella coeruleoalba*, 18 Risso's dolphins, *Grampus griseus*, 14 bottlenose dolphins, *Tursiops truncatus*, 8 common dolphins, *Delphinus delphis*, and 5 long-finned pilot whales, *Globicephala melas*, were analysed for brachycladiid species. Two species were found: *Oschmarinella rochebruni* in striped dolphins (prevalence (P): 61.2%; mean intensity (MI) (95% CI): 34.2 (25.7–45.6)), and *Brachycladium atlanticum* in striped dolphins (P: 39.8%; MI: 7.1 (4.8–13.1)) and a single individual of common dolphin (P: 12.5%; intensity: 19), which represents a new host record. A molecular analysis using the internal transcribed spacer 2 (ITS2) region of the rDNA gene confirmed that specimens of *B. atlanticum* were conspecific regardless of host species. Available dietary data suggest that Risso's dolphins, bottlenose dolphins and long-finned pilot whales would contact rarely, if at all, the infective stages of *O. rochebruni* and *B. atlanticum*. Neither the prevalence nor the mean abundance of *B. atlanticum* differed significantly between striped and common dolphins, but a principal component analysis using seven morphometric variables indicated that specimens collected from the common dolphin were stunted. These worms also had fewer eggs compared with specimens typically found in striped dolphins, although the size of the eggs was similar in both host species. Dwarfism and low fecundity have typically been found in helminths infecting unusual host species, and might reflect the lower compatibility of *B. atlanticum* for common dolphins. In summary, both *O. rochebruni* and *B. atlanticum* appear to exhibit a narrow specificity for striped dolphins in the western Mediterranean.

Introduction

The adult stage of digeneans from the family Brachycladiidae occurs mainly in hepatic and pancreatic ducts, but also in the intestine, lungs and head sinuses of marine mammals worldwide (Fernández *et al.*, 1998a, b; Gibson, 2005). The bulk of their diversity is found in cetaceans (35 of *c.* 42 described species), with additional species infecting pinnipeds and the sea otter (Aznar *et al.*, 2001; Dailey, 2007). Very little is known about the biology of brachycladiids. No intermediate or paratenic host has been identified for any species (Fernández, 1996; Barnett *et al.*, 2008), and data on patterns of specificity are

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fragmentary. Available evidence indicates that there are contrasting patterns in the occurrence of brachycladiid species within host communities, with some species being widespread among many sympatric marine mammals and others being restricted to single host species. For instance, *Orthosplanchnus arcticus* has been reported in six pinniped species and two cetacean species from arctic and subarctic waters (Adams, 1988; Raga & Balbuena, 1993), whereas *Lecithodesmus goliath* occurs in at least five species of baleen whales in the northern North Pacific (Deliamure, 1961; Yurakhno, 1967). In contrast, a parasitological survey of over 300 cetacean individuals from 13 species stranded on the coast of England and Wales (Gibson *et al.*, 1998) revealed that *Campula oblonga* occurred almost exclusively in harbour porpoises, *Phocoena phocoena*. Likewise, *Synthesium pontoporiae* is restricted to the franciscana dolphin, *Pontoporia blainvillei*, in the South American Atlantic (Andrade *et al.*, 2001; Melo *et al.*, 2006; Berón-Vera *et al.*, 2008; Marigo *et al.*, 2008, and references therein).

The reasons for these sharp differences in host specificity are unknown. However, they could be investigated using a simple yet powerful conceptual framework, i.e. the encounter/compatibility paradigm (Euzet & Combes, 1980; Combes, 2001; Kuris *et al.*, 2007). This paradigm states that specificity is determined by two sequential filters. The encounter filter would prevent infections of potential hosts that cannot contact the parasite, whereas the compatibility filter would: (1) exclude those contacted hosts in which the parasite is unable to find the appropriate resources and/or escape or deter the host's defences; or (2) impact other fitness components of the parasite if the contacted host is not unsuitable but suboptimal. In the case of brachycladiids, marine mammals become infected by consuming infected prey (probably fish and/or squid, see Fernández *et al.*, 1998b) and, therefore, dietary patterns are expected to determine the likelihood and frequency of host–parasite contacts. In any event, brachycladiids are expected to end up in a variety of potential hosts that overlap their diets (e.g. cetaceans, pinnipeds, marine birds) but differences in their suitability as habitats will prevent establishment in some hosts or affect parasite growth and/or reproduction in others (Raga *et al.*, 2009). In summary, differences of specificity between brachycladiid species could be understood by investigating the possibility of contacts within a marine mammal community, and the degree of compatibility with the contacted hosts.

In this paper, we adopt the encounter/compatibility filter paradigm to investigate, for the first time, the patterns of specificity of species of the Brachycladiidae. We focus on the species infecting the hepato-pancreactic ducts of a community of cetaceans in the western Mediterranean. First, we determine infection levels of each brachycladiid species in each cetacean species and discuss differences of infection in the light of existing data on diet overlap among hosts. Second, we compare fitness components (size, fecundity) of the individual parasites found in different host species. The overall results will provide a starting point to analyse patterns of specificity in other brachycladiid species and, by extension, in helminths from wild hosts for which parasitological research is based on opportunistic sampling.

Materials and methods

Sample collection

A total of 103 striped dolphins, *Stenella coeruleoalba*, 18 Risso's dolphins, *Grampus griseus*, 14 bottlenose dolphins, *Tursiops truncatus*, 8 common dolphins, *Delphinus delphis*, and 5 long-finned pilot whales, *Globicephala melas*, was analysed for hepato-pancreatic digeneans. Dolphins were found stranded along the Mediterranean coast of Spain (Comunidad Valenciana, between 40°25′N, 0°26′E and 37°58′N, 0°41′W) during 1982–2008. During necropsy, the liver and pancreas were removed and stored at −20°C. After thawing, the hepato-pancreatic ducts were opened and rinsed with water under a sieve of 0.02 mm mesh. Worms were collected, counted, cleaned in 0.9% saline and fixed and preserved in absolute (for molecular analysis) or 70% (for morphometric analysis) ethanol. Published parasitological surveys on part of the samples of long-finned pilot whales and Risso's dolphins used in this study can be found in Raga & Balbuena (1993) and Fernández *et al.* (2003), respectively.

Molecular analysis

To confirm that specimens of *Brachycladium atlanticum* from striped dolphins and the common dolphin were conspecific, the internal transcribed spacer 2 (ITS2) region of the rDNA gene was compared (see Nolan & Cribb, 2005). Two specimens of *B. atlanticum* from a common dolphin and four from a striped dolphin were cut in half and one piece was used to extract rDNA following a standard phenol–chloroform methodology (Holzer *et al.*, 2004). The ITS2 region was amplified using primers ITS2.2 (5′-CCTGGTTAGTTTCTTTTCCTCCGC-3′; Anderson & Barker, 1993) and GA1 (5′-AGAACATCGACATCTTGAAC-3′; Anderson & Barker, 1998) in 10 µl polymerase chain reactions (PCR). The thermocycling profile used was 35 cycles of 94°C for 50 s, 56°C for 50 s and 72°C for 1 min and 20 s, a single extension of 72°C for 4 min and a final hold at 10°C. Sequences were aligned using the BioEdit sequence alignment editor (Hall, 1999). Sequences are available in GenBank under accession numbers: GQ226035 (*B. atlanticum* from striped dolphin) and FJ211250 (*B. atlanticum* from common dolphin).

Comparison of infection levels

Ecological terms follow Bush *et al.* (1997). The 95% confidence intervals (CI) for prevalence were set with Sterne's exact method (Reiczigel, 2003) and the 95% CIs for mean intensity and mean abundance with 20,000 bootstrap replications (Rózsa *et al.*, 2000). Prevalence of each parasite was compared between pairs of host species based on difference of prevalence (DP) statistic expressed as a percentage. Exact 95% confidence limits for DP were set by inverting a two-sided unconditional test for difference of proportions (see Agresti & Min, 2001 for details). Pairwise differences of mean abundance between host species were tested with a bootstrap 2-sample Welch's *t*-test (Rózsa *et al.*, 2000).

Compatibility analysis of B. atlanticum

We selected 106 gravid specimens of *B. atlanticum* from 29 striped dolphins, and 17 from the only infected common dolphin. All specimens had been collected from fresh carcasses and were processed in a standardized manner. Worms were cleaned in 0.9% saline and fixed in 70% ethanol, dehydrated through a graded ethanol series, stained with aluminium carmine (Georgiev *et al.*, 1986) and mounted on Canada balsam. For each specimen, we measured the area of body, vitellarium, uterus, testes (combined), oral sucker, ventral sucker and egg. An average egg area was obtained by randomly selecting 10 eggs *in utero*. The areas of these seven elements were the only ones that could readily be measured in most specimens (e.g. the ovary was often covered by a dense mass of eggs). Areas provide more objective and precise measurements than distances when organs are irregular in shape. Drawings of specimens were taken using a light microscope connected to a drawing tube. The areas were calculated from scanned drawings using Image Tool 3.00 (University of Texas Health Science Center at San Antonio, available at http://ddsdx.uthscsa.edu/dig/download.html). Counts of eggs *in utero* were considered as a surrogate of fecundity. However, direct counting of eggs was impossible and, therefore, a coarse estimation was made by calculating the ratio between the area of the uterus filled with eggs and mean egg area.

We estimated, in striped dolphins, the amount of intraspecific variability of size and fecundity of *B. atlanticum* that was attributable to host individual effects. We selected striped dolphin individuals harbouring at least two worms ($n = 21$), and performed one-way ANOVAs using 'host individual' as a random factor, and body area (as a surrogate of size), egg area, or fecundity of *B. atlanticum* as dependent variables. The variability accounted for by host individual was assessed with the coefficient of determination (r^2). In addition, we used Spearman correlation tests (one-tailed) to investigate whether average body area, egg area and/or fecundity of *B. atlanticum* per host was reduced at increasing population sizes, either of *B. atlanticum* alone or of *B. atlanticum* + *Oschmarinella rochebruni* (the other co-occurring species).

For the comparison between the two host species, we examined whether average log-transformed values of body area, egg area and fecundity of *B. atlanticum* specimens from the common dolphin were within the distribution range of the mean values obtained in the 21 striped dolphins. Also, a principal component analysis (PCA) based on the variance–covariance matrix of the seven morphometric variables was conducted to visualize differences in static multivariate allometry of *B. atlanticum* between the two host species (see Bray & Des Clers, 1992; Pérez Ponce de León, 1995; Perdiguero-Alonso *et al.*, 2006). Both analyses indicated that specimens of *B. atlanticum* from the common dolphin exhibited both a smaller size and lower fecundity (see Results). Thus, we further examined whether the putative host species effect upon body size and fecundity were independent. A regression equation was obtained using mean values of body area and fecundity per host in striped dolphins. We used this equation to predict mean fecundity in worms from the common dolphin based on their mean body area. An independent host species effect on fecundity could be assumed if the observed fecundity was outside the 95% confidence interval of the predicted value.

Results

Molecular analysis

Two morphospecies of the Brachycladiidae, namely, *B. atlanticum* and *O. rochebruni*, were found in the whole sample of hosts, but only *B. atlanticum* was found in more than one host species (table 1). A sequence of 362 bp of the ITS2 was obtained from single specimens of *B. atlanticum* from striped dolphin and common dolphin, and their sequences were identical.

Infection patterns

Infection parameters for *B. atlanticum* and *O. rochebruni* are shown in table 1. Striped dolphins were infected with both species, and a single common dolphin was infected with 19 individuals of *B. atlanticum*. The other cetacean species were all uninfected. In an inferential context, statistical evidence suggests that striped dolphins were significantly more infected with *B. atlanticum* than the other species except the common dolphin, although the difference with long-finned pilot whales was just marginally significant due to the small sample in the latter (table 2). Also, the mean abundance of *B. atlanticum* did not differ significantly between striped dolphins and

Table 1. Infection parameters for *Brachycladium atlanticum* and *Oschmarinella rochebruni* in five cetacean species from the western Mediterranean.

	B. atlanticum			*O. rochebruni*		
Host species	P (95% CI)	MI (95% CI) [range]	MA (95% CI)	P (95% CI)	MI (95% CI) [range]	MA (95% CI)
Stenella coeruleoalba (*n* = 103)	39.8 (30.5–49.5)	7.1 (4.8–13.1) [1–69]	2.8 (1.8–5.2)	61.2 (51.5–70.5)	34.2 (25.7–45.6) [1–187]	20.9 (15.2–29.2)
Delphinus delphis (*n* = 8)	12.5 (0.6–50.0)	19	2.4 (0.0–7.1)	0 (0.0–36.5)	–	–
Tursiops truncatus (*n* = 14)	0 (0.0–23.8)	–	–	0 (0.0–23.8)	–	–
Grampus griseus (*n* = 18)	0 (0.0–18.5)	–	–	0 (0.0–18.5)	–	–
Globicephala melas (*n* = 5)	0 (0.0–50.0)	–	–	0 (0.0–50.0)	–	–

n, number of hosts analysed; P, prevalence (%); MI, mean intensity; MA, mean abundance. Range of intensity is in square brackets and 95% confidence interval for parameters in parentheses.

Table 2. Percentage differences in prevalence (DP) with its associated 95% confidence interval (CI), and probability value (*P*), in pairwise comparisons between values of prevalence of the digeneans *Brachycladium atlanticum* and *Oschmarinella rochebruni* in striped dolphins, *Stenella coeruleoalba*, and each of four delphinid species from the western Mediterranean.

	B. atlanticum			*O. rochebruni*		
Pair	DP	95% CI	*P*	DP	95% CI	*P*
Sc–Dd	27.3	(− 12.3–43.3)	0.141	61.7	(− 0.01–70.4)	0.029
Sc–Tt	39.8	(17.2–50.4)	0.015	61.7	(37.1–70.2)	< 0.001
Sc–Gg	39.8	(21.3–49.5)	0.008	61.7	(40.8–70.0)	< 0.001
Sc–Gm	39.8	(− 10.3–50.8)	0.092	61.7	(− 0.02–70.1)	0.041

Sc, *Stenella coeruleoalba*; Dd, *Delphinus delphis*; Tt, *Tursiops truncatus*; Gg, *Grampus griseus*; Gm, *Globicephala melas*.

common dolphins (*t*-test, $t = -0.176$, 109 df, $P = 0.822$). In the case of *O. rochebruni*, there was evidence that striped dolphins were significantly more infected than any other species, although the differences with common dolphins and long-finned pilot whales were just marginally significant (table 2).

Compatibility analysis

In striped dolphins, there were highly significant differences among individual hosts in all seven morphometric variables, and number of eggs, of *B. atlanticum* (one-way ANOVAs, $P < 0.008$ in all cases). In morphometric variables, the variance accounted for by 'host individual' ranged from $r^2 = 0.426$ (testes area) to $r^2 = 0.705$ (ventral sucker area), with values for body area and number of eggs being $r^2 = 0.596$ and $r^2 = 0.461$, respectively. We did not observe significant decreases of average values of body area (Spearman correlation, $r_s = -0.16$, one-tailed $P = 0.199$), egg area ($r_s = -0.24$, $n = 106$, one-tailed $P = 0.105$) or number of eggs per host ($r_s = 0.04$, one-tailed $P = 0.420$), at increasing intensities of *B. atlanticum* (fig. 1); the same occurred using the combined intensity of *B. atlanticum* + *O. rochebruni* (body area: $r_s = -0.24$, one-tailed $P = 0.103$; egg area: $r_s = -0.17$, one-tailed $P = 0.194$; egg number: $r_s = -0.08$, one-tailed $P = 0.348$). Therefore, we assumed that there were no density-dependent effects on these variables.

Morphometric values of specimens of *B. atlanticum* collected from the common dolphin were, on average, roughly half of those observed in specimens from striped dolphins, except in the case of egg area (table 3). Results from the PCA confirmed the existence of size differences associated with host species. The two first components accounted for 75.8% of the total morphometric variation, and all variables except egg area loaded positively on the first component (fig. 2). There was a clear segregation of individuals of *B. atlanticum* from the common dolphin only along the first (size) axis (fig. 2). Note, however, that specimens of *B. atlanticum* from common dolphins were collected from a single host. Thus, we also compared the average value of body size, egg size and egg number in the common dolphin with those obtained from individual striped dolphins. Average body area and egg number, but not egg area, were in the lower tail of the distribution of mean values obtained from the sample of striped dolphins (fig. 1).

In striped dolphins, mean body area of *B. atlanticum* per individual host was a significant predictor of mean number of eggs (regression equation: no. eggs $= 17.24 + 26.55 \times$ body area; $r^2 = 0.562$, $P < 0.0001$). The mean body area observed in individuals of *B. atlanticum* from the common dolphin was 643 μm^2, and the predicted number of eggs according to the above equation was 188 (95% CI: 79–279). The actual mean number of eggs (178) was close to the predicted value, and lay within the confidence interval.

Discussion

Infection data suggest that two species of the Brachycladiidae infect delphinid cetaceans in the western Mediterranean. The striped dolphin appears to be the main definitive host for *O. rochebruni*. Although sample sizes for other cetacean species were small, prevalence was significantly higher in striped dolphins, with potential population differences with other species being as high as 70% (see upper 95% CIs in table 2). *Oschmarinella rochebruni* has also been reported in striped dolphins from the central Mediterranean (Vegni-Talluri, 1982; Manfredi *et al.*, 1992; Di Cave *et al.*, 1993). Surveys in other localities, however, indicate that *O. rochebruni* does not show a strict specificity to striped dolphins worldwide, as it infects other members of the subfamily Delphininae (Gibson *et al.*, 1998; see also LeDuc, 2009). In the North Pacific, *O. rochebruni* was found in common dolphins, spinner dolphins, *Stenella longirostris*, and spotted dolphins, *Stenella attenuata*, but not in as many as 31 striped dolphins (Dailey & Perrin, 1973; Dailey & Otto, 1982). Likewise, in the eastern North Atlantic, Gibson *et al.* (1998) reported *O. rochebruni* in common dolphins, but not in striped dolphins. There is also an additional record of *O. rochebruni* in spotted dolphins from South Africa (Gibson & Harris, 1979).

Striped dolphins also had the highest prevalence of *B. atlanticum* in the study area, but there was lower statistical power to detect population differences with other host species. Prevalence was significantly higher than in Risso's dolphins ($n = 18$) and bottlenose dolphins ($n = 14$), but evidence was not conclusive in the comparison with long-finned pilot whales ($n = 5$). Although sample size was also low in the case of common dolphins ($n = 8$), we detected a positive infection of *B. atlanticum*, and neither the prevalence nor the mean

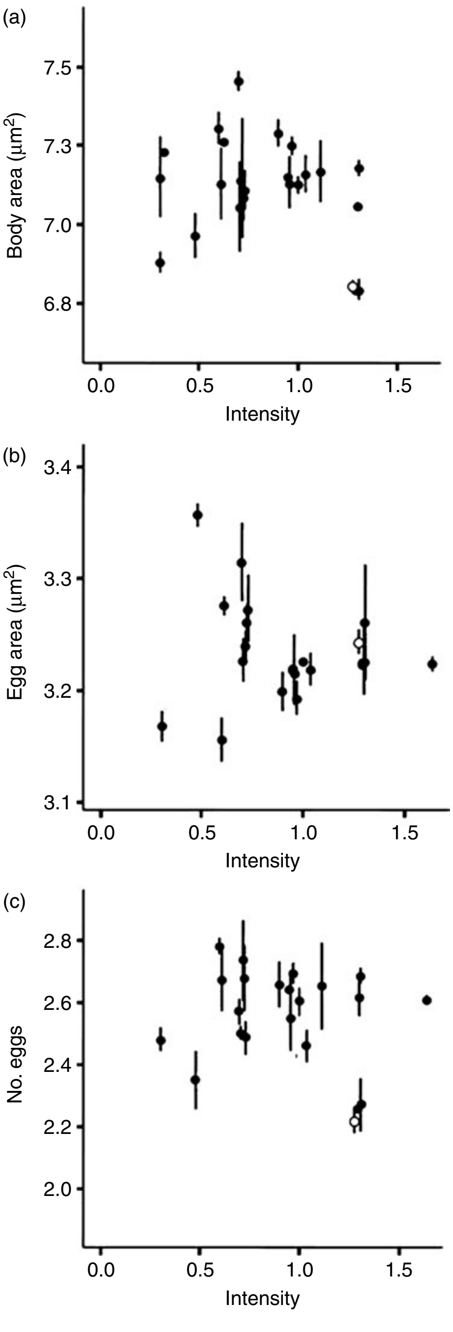

Fig. 1. Scatterplot showing the relationship between intensity and (a) body area, (b) egg area and (c) number of eggs of the digenean *Brachycladium atlanticum* from striped dolphin, *Stenella coeruleoalba* (black dots) and common dolphin, *Delphinus delphis* (white dot) from the western Mediterranean. Each point is an average value per host, and bars represent standard deviation. *x* and *y* axes are presented in a log-scale.

abundance differed from those from striped dolphins. A conservative interpretation would therefore be that *B. atlanticum* infects both striped dolphins and common dolphins, and more rarely, if at all, other delphinids. To date, *B. atlanticum* had only been reported in striped dolphins from the Canary Islands (Abril *et al.*, 1991) and, therefore, its finding in a common dolphin constitutes a new host record.

The restriction of *O. rochebruni* and *B. atlanticum* to 1–2 host species must result from a different probability of contact (encounter filter) and/or establishment (compatibility filter) among cetacean species. Obviously, the contribution of each filter cannot be quantified in our system, but some insight can be gained from two lines of evidence. First, it is likely that both *O. rochebruni* and *B. atlanticum* infect Mediterranean striped dolphins via mesopelagic cephalopods and/or mesopelagic fish, which are their two major prey groups in the study area (Blanco *et al.*, 1995, 2009). In contrast, the individuals of bottlenose dolphin that we analysed for liver flukes had consumed mainly demersal neritic fish (Blanco *et al.*, 2001), whereas Risso's dolphins and long-finned pilot whales had fed on mesopelagic cephalopods almost exclusively (Blanco *et al.*, 2006; Blanco, pers. comm.). These dietary differences might suggest that *O. rochebruni* and *B. atlanticum* use mesopelagic fish to complete their life cycle (see Fernández *et al.*, 1998b), thus rarely contacting, if at all, these three cetacean species. The hypothesis that contacts did not occur is also supported by the observation that immature, transient individuals of *O. rochebruni* or *B. atlanticum* were not detected throughout the gut of any individual of bottlenose dolphins, Risso's dolphins or long-finned pilot whales (Raga & Balbuena, 1993; Fernández *et al.*, 2003; Fernández, pers. comm.). However, we cannot rule out the possibility that the passage of food could be so fast (see, e.g., Kastelein *et al.*, 1997) that there was a low likelihood of detecting unsuccessful infections.

We currently lack data on the diet of common dolphins because the stomach of most individuals analysed in the present study was empty (Blanco, pers. comm.). However, in other geographical areas, common dolphins are known to exploit both neritic and oceanic resources, including a substantial amount of mesopelagic fish in their diet (Pusineri *et al.*, 2007 and references therein). The association between diet and the probability of infection by *O. rochebruni* and *B. atlanticum* will be more firmly established when more cetacean samples, particularly of common dolphins, are available.

In striped dolphins, we observed a highly significant effect of the individual host on the morphology and fecundity of *B. atlanticum*: about half of the variability of the body area and fecundity was accounted for by this factor. Strong host-individual effects on morphometry have also been reported for the brachycladiid *Synthesium tursionis* from Mediterranean bottlenose dolphins (Fernández *et al.*, 1995). Without experimental data it is difficult to determine the factors that induce this type of variability. However, for a range of intensity of 1–69 worms we found no evidence for potential effects of inter- or intraspecific competition on morphometric variables and/or the fecundity of *B. atlanticum*. Crowding effects on body size and/or fecundity have been reported for

Table 3. Areas (in mm^2 unless otherwise stated) of seven elements of the digenean *Brachycladium atlanticum* measured in 106 specimens collected from 29 striped dolphins, *Stenella coeruleoalba*, and 17 specimens collected from one common dolphin, *Delphinus delphis*.

	Striped dolphin ($n = 106$)		Common dolphin ($n = 17$)	
Area	Mean (CV)	[Range]	Mean (CV)	[Range]
Body	14.15 (31.7)	[5.36–30.77]	6.43 (17.7)	[3.89–8.13]
Vitellarium	8.00 (44.5)	[2.83–26.30]	3.85 (30.1)	[1.92–6.60]
Uterus	0.69 (31.8)	[0.16–1.36]	0.31 (29.7)	[0.13–0.45]
Testes	1.12 (41.0)	[0.38–3.56]	0.58 (33.5)	[0.35–0.94]
Oral sucker	0.36 (30.0)	[0.10–0.62]	0.19 (27.0)	[0.13–0.35]
Ventral sucker	0.54 (25.3)	[0.22–0.91]	0.26 (29.2)	[0.15–0.39]
Egg (μm^2)	1713 (12.0)	[1296–2370]	1762 (10.7)	[1497–2295]

CV, Coefficient of variation.

several digenean species under experimental conditions (e.g. Nollen, 1983; Yao *et al.*, 1991; Stillson & Platt, 2007, and references therein) but usually at intensities not commonly observed under natural conditions (Bush & Lotz, 2000).

Individuals of *B. atlanticum* collected from a single common dolphin represented a dwarf version of those commonly observed in striped dolphins, and apparently were less fecund. The lowered fecundity could result from the physical constraint relating to the accommodation of eggs in a smaller uterus. First, egg size did not differ between specimens of *B. atlanticum* from both species. Indeed, previous studies on brachycladiids have also shown that egg size is a highly conserved character regardless of the worm's body size and host individual (Fernández *et al.*, 1995). Second, the observed fecundity is what should be expected according to body size. On the other hand, the low intensity of *B. atlanticum* observed in the common dolphin (just 19 worms) would suggest that dwarfism has not resulted from crowding effects.

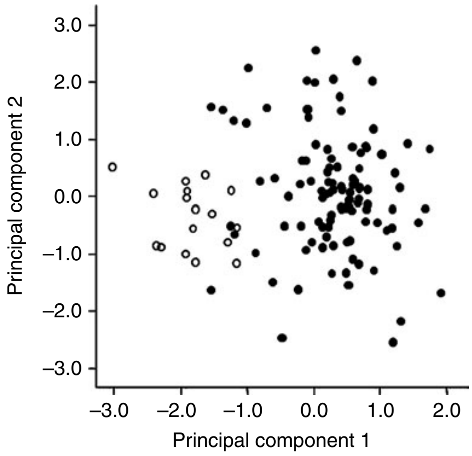

Fig. 2. Scatterplot of the first two principal components of the variance–covariance matrix of seven morphometric variables obtained from the digenean *Brachycladium atlanticum* from striped dolphin, *Stenella coeruleoalba* (black dots) and common dolphin, *Delphinus delphis* (white dots) from the western Mediterranean. Principal components 1 and 2 accounted for 62.3% and 13.5% of variance, respectively. The first eigenvector has the following loadings: body, 0.157; vitellarium, 0.165; uterus, 0.165; testes, 0.121; oral sucker, 0.121; ventral sucker, 0.125; egg, − 0.009.

The question remains, however, whether these traits are related to a 'host individual' or a 'host species' effect. In our study, both effects are inevitably confounded, but there is clear evidence that the mean values of body size and fecundity observed in the common dolphin are extreme compared with the distribution of the mean values obtained in a sample of striped dolphins. In other words, common dolphins appears to be less suitable as hosts for *B. atlanticum* than striped dolphins. Other studies report surprisingly similar effects of apparently unsuitable host species on worm morphology and fecundity. Adams *et al.* (1998) found a single mature specimen of the brachycladiid *Campula oblonga* in the liver of a thresher shark, *Alopias vulpinus*. This parasite is otherwise specific to porpoises (Phocoenidae) and, therefore, its occurrence in sharks was exceptional (Adams *et al.*, 1998). Interestingly, the specimen collected from the shark was dwarf and exhibited a reduced fecundity but an egg size comparable to that in its typical hosts. Likewise, single specimens of *Diclidophora merlagi*, a typical monogenean from whiting, *Merlangius merlangus*, were found in two cod, *Gadus morhua*, from two localities in the North Atlantic (Perdiguero-Alonso *et al.*, 2006). Both monogenean specimens exhibited a stunted growth, but one of them had developed an egg of normal size (Perdiguero-Alonso *et al.*, 2006).

In conclusion, this is the first study in which an assessment of host–parasite specificity has been attempted in a community of cetaceans. Available data suggest that, in the western Mediterranean, both *O. rochebruni* and *B. atlanticum* exhibit narrow specificity for striped dolphins. We conclude that these parasites contact other cetaceans less frequently, if at all, and/or exhibit a reduced fitness in them. Admittedly, our study suffers from several drawbacks associated to opportunistic sampling. However, we believe that it represents a starting point to properly address the study of specificity, not only in the Brachycladiidae, but also in any helminth group for which host sampling must, by necessity, be opportunistic.

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CHAPTER VI:

GASTRIC HELMINTH FAUNA OF STRIPED DOLPHINS (*STENELLA COERULEOALBA*): NO EFFECTS OF HOST BODY LENGTH AND AGE

6.1. INTRODUCTION

Out of the seven species of odontocete cetaceans which regularly occur in Spanish Mediterranean waters, the striped dolphin, *Stenella coeruleoalba*, is considered the most abundant cetacean species off Valencia (Gómez de Segura *et al*., 2006). However, the Mediterranean population is currently considered as "vulnerable" by the IUCN Red List of Threatened Species (2012). Many studies have reported the presence of helminths in striped dolphins from this area. In fact, at least 12 trophically-transmitted metazoan parasite taxa are known to infect this cetacean, including 3 digeneans, 5 cestodes, 3 nematodes and 1 acanthocephalan (Raga, 1985; Raga and Carbonell, 1985; Raga *et al*., 1992; Raga and Balbuena, 1993; Aznar *et al*., 2006, 2007; Mateu *et al*., 2011, 2014). Only two of these helminth species have been found in the stomach of striped dolphins, i.e., the digenean *Pholeter gastrophilus* and nematodes of the genus *Anisakis*. *Pholeter gastrophilus* has been reported in at least 17 cetacean species worldwide (Aznar *et al*., 1992, 2006; Raga, 1994), including coastal (Dollfus, 1974; Van Waerebeek *et al*., 1993; Aznar *et al*., 1994; Gibson *et al*., 1998; Berón-Vera *et al*., 2001) and oceanic species (Aznar *et al*., 1992; Raga and Balbuena, 1993; Fernández *et al*., 2003). As far as we know, all studies have reported the presence of *P. gastrophilus* within cysts in the digestive tract of cetaceans (i.e., Raga and Carbonell, 1985; Fernández *et al*., 1991; Manfredi *et al*., 1992; Gibson *et al*., 1998; Aznar *et al*., 2006) as the host forms fibrotic nodules that surround the parasite in response to infection (Woodard *et al*., 1969; Migaki *et al*., 1971; Howard *et al*., 1983).

According to Mattiucci and Nascetti (2006) there are currently 9 species belonging to the genus *Anisakis* (i.e., *A. pegreffii, A. simplex* s.s., *A. berlandi, A. typica, A. ziphidarum, Anisakis* sp., *A. physeteris*, *A. brevispiculata* and *A. paggiae*). Unfortunately, anisakid larvae and sibling species are morphologically indistinguishable and, therefore, the application of molecular techniques during the last decades has contributed to the taxonomic reorganization of these nematodes and the description of new species (Paggi *et al*., 1998; Mattiucci *et al*., 1997, 2005, 2009). In western Mediterranean waters, several studies have reported *Anisakis* spp. in striped dolphins (Raga and Carbonell, 1985; Fernández *et al*., 1991; Manfredi *et al*., 1992). However, the specific identity of these individuals remains unknown.

During the summer of 1990, western Mediterranean striped dolphins suffered an epizootic event caused by the Dolphin Morbillivirus (DMV) that killed thousands of individuals (Aguilar and Raga, 1993). This outbreak provided a unique opportunity to study a large number of individuals during a short period of time and with a known cause of death. The aim of this study is to describe the helminth fauna from the stomach of striped dolphins in this region based on a large sample size and to explore the effects of host's total length and age on infection parameters. Further details about infections of *P. gastrophilus* in striped dolphins can be found in Aznar *et al*. (2006).

6.2. MATERIALS AND METHODS

6.2.1. SAMPLE COLLECTION AND EXAMINATION

A total of 47 striped dolphins, *Stenella coeruleoalba,* was examined for gastric helminths. Dolphins were found stranded along the Mediterranean coast of Spain (off Catalonia and the Valencian Community) between August of 1990 and March of 1991. Before necropsy, all individuals were sexed and measured (to the nearest cm) according to Geraci and Lounsbury (2005). Teeth were sent to the Department of Animal Biology, University of Barcelona (Spain), for age determination by counting growth layer groups in dentine (see Calzada *et al*., 1994 for procedure details). Age was available from 36 of the 47 dolphins (see Appendix 3 for details).

During necropsy, the stomach was removed and stored at -20ºC. After thawing, the stomachs (i.e., forestomach, fundic stomach and pyloric stomach) were opened and rinsed with water under a sieve of 0.02mm mesh. The connecting channel was considered as part of the pyloric stomach (Harrison *et al*., 1970). In the case of anisakid nematodes, worms were collected, counted, cleaned in saline and fixed and preserved in absolute (for molecular analysis) or 70% ethanol (for morphometric analysis). The stomach wall was also thoroughly examined for the presence of the characteristic nodules of *P. gastrophilus* that are formed in response to the infection. For this study, only a census of nodules was attempted. There is evidence that the number of cysts is a gross indicator of infrapopulation size of this parasite (F.J. Aznar, unpub. data).

Ecological terms follow Bush *et al*. (1997). "Prevalence" is the percentage of hosts in the sample infected by a helminth species, "intensity" is the number of individuals of a helminth species in an infected host and "mean abundance" is the average number of individuals of a helminth species in a sample of hosts, regardless of whether they are infected or not. The 95% confidence interval (CI) for prevalence was calculated with Sterne's exact method (Reiczigel, 2003). The 95% CI for mean abundance and intensity for *Anisakis* spp. were obtained with the bias-corrected and accelerated bootstrap method using 20,000 replications (Rózsa *et al*., 2000). Two-tail Spearman correlation tests were performed to investigate whether the abundance of *Anisakis* spp. changed with host length or age.

6.2.2. MOLECULAR ANALYSIS

Molecular identification was carried out in order to identify larval and adult stages of *Anisakis* spp., because morphological characters do not allow their recognition to the species level. The mitochondrial cytochrome oxidase subunit II (mtDNA *cox2*) gene was sequenced from 6 specimens (5 larvae and 1 adult) obtained from 5 individuals of *S. coeruleoalba*. The total DNA was extracted from 2mg of tissue from each single nematode by using cetyltrithylammonium bromide method (CTAB), as previously described in detail by Valentini *et al*. (2006) and Mattiucci *et al*. (2009, 2014). The mtDNA *cox2* gene from *Anisakis* spp. was amplified using primers no. 210R (5' CACCAACTCTTAAAATTATC 3') and no. 211F (5' TTTTCTAGTTATATAGATTGRTTYAT 3') (Nadler and Hudspeth, 2000), spanning the mtDNA nucleotide position 10,639-11,248, as defined in *Ascaris suum* [GenBank X54253]. The PCR was carried out using the following conditions: 34 cycles of 94°C for 30 s, 46°C for 1min, and 72°C for 1min and 30 s, followed by a post amplification extension of 10 min at 72°C and a final hold at 4°C. Sequence identity was checked using the Basic Local Alignment Search Tool (BLAST) and aligned with previous characterized sequences of *Anisakis* and available from GenBank, by using Clustal X.

6.3. RESULTS

6.3.1. INFECTION PATTERNS

Mean total length (± SD) of dolphins was 194.2±12.4 (range: 158-213; *n*= 43) and mean age was 16.1±6.7 years (range: 2-28; *n*= 36). Out of the 47 stomachs analyzed, 40 were infected with *P. gastrophilus* (prevalence (95% CI): 85.1% (71.5-92.9)). All stomach chambers were infected, but the bulk of fibrotic nodules was found in the fundic stomach (average number of nodules in infected dolphins ± SD, forestomach: 1.5±1.1; fundic stomach: 4.1±5.0; pyloric stomach: 1.7±1.0). A total of 46 individuals of *Anisakis* spp. were found infecting only 7 out of the 47 stomachs analyzed (prevalence (95% CI): 14.9% (7.2-28.5); mean abundance (95% CI): 0.98 (0.2-4.0); mean intensity [range]: 6.6 [1-35]). Most worms (*n*= 40) were collected in the pyloric stomach, whereas the remaining worms were found in the forestomach; no worms were detected within the fundic stomach. All two-tail Spearman correlations tests showed no statistically significant correlations between the abundance of anisakid nematodes, or between the number of nodules of *P. gastrophilus*, and host total length or age ($p > 0.05$ in all cases).

6.3.2. MOLECULAR IDENTIFICATION

Sequences of the mtDNA *cox2* were obtained for 6 *Anisakis* specimens collected from 5 individuals of *S. coeruleoalba*. Sequences (629 bp) were compared with those from all *Anisakis* spp. that are deposited in GenBank. All sequences obtained were identical, and matched 100% those sequences of *A. pegreffii* collected from short-beaked common dolphin, *Delphinus delphis*, in NE Atlantic, and bottlenose dolphins, *Tursiops truncatus*, in SE Atlantic (Valentini *et al*., 2006) (DQ116428.1). The sequence obtained is available in GenBank under accession number: KR149283.

6.4. DISCUSSION

Pholeter gastrophilus has been reported in several species of odontocete species from the Atlantic Ocean, Pacific Ocean and Mediterranean Sea, including *Phocoena phocoena*, *Pontoporia blainvillei*, *Lagenorhynchus albirostris*, *L. obscurus*, *L. acutus*, *Globicephala melas*, *Grampus griseus*, *Cephalorhynchus commersonii*, *Steno bredanensis*, *Tursiops truncatus*, *Delphinus delphis*, *Stenella frontalis* and *S. coeruleoalba* (Zam *et al*., 1971; Dollfus, 1974; Raga and Carbonell, 1985; Aznar *et al*., 1992, 1994; Van Waerebeek *et al*., 1993; Raga and Balbuena, 1993; Raga, 1994; Gibson *et al*., 1998; Berón-Vera *et al*., 2001; Fernández *et al*., 2003). The high infection level reported in this study contrasts, for example, with the study of Van Waerebeek *et al*. (1993) who reported a low prevalence (8%) in 212 stomach of the dusky dolphin (*L. obscurus*) from Peru; or Zam *et al*. (1971) who found a prevalence of 19.6% in *T. truncatus* from Northeastern Florida. However, Aznar *et al*. (2006) reported prevalences > 50% in four odontocete species (*P. phocoena*, *T. truncatus*, *G. melas* and *S. coeruleoalba*) from Atlantic and Mediterranean waters. Additionally, these authors suggested that *P. gastrophilus* is largely associated to the glandular region of the stomach, a conclusion which agrees with our results.

Adult individuals of *Anisakis pegreffii* occur in several odontocete species, more rarely mysticetes, in Mediterranean and South Atlantic waters (Mattiucci *et al*., 2005, 2009, 2014; Mattiucci and Nascetti, 2006, 2007, 2008). In the Mediterranean, *A. pegreffii* is considered to be the most widespread species of *Anisakis* (Mattiucci and Nascetti, 2008), and its main definitive hosts are dolphins, including striped, common, and bottlenose dolphins (Mattiucci *et al*., 1997, 2004, 2014; Mattiucci and Nascetti, 2008). Larvae have been reported in a number of demersal and pelagic fish species from the western and eastern basins (Farjallah *et al*., 2008; Mattiucci and Nascetti, 2008; Pekmezci *et al*., 2014), suggesting that *A. pegreffii* is widespread in the Mediterranean Sea. This is the first report of *A. pegreffii* in cetaceans from the western Mediterranean.

Rather surprisingly, *A. pegreffii* showed low infection levels in Mediterranean striped dolphins compared with infection of *Anisakis* spp. in other cetaceans. For instance, Torres *et al*. (1992) reported high prevalences of *A. simplex* in *Phocoena spinipinnis* (61.1%) from the coast of southern Chile and *Anisakis* sp. in *Cephalorhynchus eutropia* (43.5%). Carvalho *et al*. (2010) found a prevalence of 59.8% within a cetacean community from the northeastern coasts of Brazil, whereas in striped dolphins, Abollo *et al*. (1998) reported a prevalence of 37.5% in individuals from northwestern Spanish Atlantic coasts. Additionally, off England and Wales, Gibson *et al*. (1998) also reported a higher prevalence (57%) of *A. simplex* (s.s.) infecting the striped dolphin. The question remains why striped dolphins from western Mediterranean waters show such low infection levels. This pattern does not seem associated to a specific cetacean species; analysis of bottlenose dolphins, common dolphins, Risso's dolphins, long-finned pilot whales and Cuvier's beaked whales from the same area also points to a general scarcity of *Anisakis* infections in this area (Fernández *et al*., 2003, 2004; Quiñones *et al*., 2013). Interestingly, a similar pattern is observed in fish. In the European hake (*Merluccius merluccius*), Valero *et al*. (2006) compared infection parameters of *Anisakis* spp. between fish caught in Atlantic and Mediterranean waters, and found that individuals from Mediterranean waters showed lower prevalences (85.7% for *A. simplex sensu lato* in Atlantic waters, compared to 41.3% in the Mediterranean; and 3.8% for *Anisakis* larvae type II in Atlantic waters and 1.6% in the Mediterranean).

The life-cycle of *A. pegreffii* is suggested to involve mesopelagic food webs (Mattiucci and Nascetti, 2008). In particular, Mateu *et al*. (2015) (Chapter 8) suggest that the myctophid species *Notoscopelus elongatus* could act as a paratenic and/or intermediate host for *A. pegreffii*. Arguably, the probability of transmission is especially low for trophically-transmitted helmiths in the oceanic realm because infective stages are highly "diluted" (Valente *et al*., 2009 and Santoro *et al*., 2010 for marine turtles; Hoberg, 1996, 2005 for marine birds; and Raga *et al*., 2009 for marine mammals). As Mateu *et al*. (2015) (Chapter 8) report a prevalence of 1.4% of *A. pegreffii* in *N. elongatus*, the present study could support the idea that striped dolphins in western Mediterranean waters become infected with *A. pegreffii* by the ingestion, *inter alia*, of parasitized myctophid species.

Gómez-Campos *et al*. (2011) suggested that western Mediterranean dolphins suffer a dietary shift with age and size, and also indicated an increased consumption of hake and sardine as dolphins mature. In contrast with these dietary changes, we were unable to detect significant differences in the abundance of *A. pegreffii* and the number of nodules of *P. gastrophilus* with host total length or age. Regarding *P. gastrophilus*, we acknowledge that the present study only uses a gross indicator of parasite abundance and, therefore, we cannot rule out the possibility that a significant correlation could exist if a complete census of worms had been made. In any event, we are left with two non-exclusive hypotheses. First, ontogenetic dietary changes would not affect the consumption of intermediate hosts for both *A. pegreffii* (see Chapter 7) and *P. gastrophilus* (whose intermediate and paratenic hosts are unknown). Second, the absence of correlation between the amount of *A. pegreffii* and host's total length or age could be related just to the scarcity of infection events occurring in protracted periods. This indeed appears to be the case for intestinal helminths (Mateu *et al*., 2015) (Chapters 4 and 8).

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HELMINTHS FROM THE D
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CHAPTER VII:

LONG-TERM CHANGES IN THE INTESTINAL HELMINTH COMMUNITY OF STRIPED DOLPHINS, *STENELLA COERULEOALBA*, FROM THE WESTERN MEDITERRANEAN

7.1. INTRODUCTION

Over the last decades, concern has been raised about the effects of global change on the long-term stability of marine mammal populations (Tynan and DeMaster, 1997; Learmonth *et al*., 2006). Firstly, overexploitation of fish stocks is causing changes on the distribution of marine mammals (Bearzi *et al*., 2003, 2004, 2006, 2008; Cañadas and Hammond, 2008) and may cause a depletion of populations due to inadequate energy intake (Gulland *et al*., 2005; Rosen and Trites, 2005; Rosen, 2009). Secondly, environmental contaminants are known to accumulate in marine mammals (Tanabe *et al*., 1994; Watanabe *et al*., 2002; Wafo *et al*., 2005; McHugh *et al*., 2007; Lailson-Brito *et al*., 2010) and may enhance disease and high mortality events (Van Bressem *et al*., 2001; Gulland and Hall, 2007). Thirdly, there is increasing evidence that climate change is also affecting the population distribution (MacLeod *et al*., 2005; Azzellino *et al*., 2008; Salvadeo *et al*., 2010) and predator-prey relationships (McLean *et al*., 2001; Walther *et al*., 2002) of marine mammals.

The striped dolphin, *Stenella coeruleoalba*, is an extensively studied dolphin distributed throughout temperate and tropical waters (Archer, 2009). Over the last decades, the western Mediterranean population of this species has suffered environmental impacts associated to global change. Firstly, there is correlational evidence that overfishing of sardine, *Sardina pilchardus*, one of the putative main prey species of striped dolphins in the area, may have caused a significant dietary shift of dolphins towards demersal prey, particularly hake, *Merluccius merluccius* (Gómez-Campos *et al*., 2011; see also below). Since a hake-dominated diet provides lower energy content than a sardine-dominated one, it is hypothesized that such dietary shift could entail long-term problems for the energetic balance of animals in the population, particularly those having higher nutritional demands, such as young individuals or pregnant and lactating females (Gómez-Campos *et al*., 2011).

Secondly, the western Mediterranean population of striped dolphins has suffered two epizootic events caused by the Dolphin Morbillivirus (DMV) (Aguilar and Raga, 1993; Raga *et al*., 2008). The first die-off started in the summer of 1990 and affected the whole Mediterranean population (Aguilar and Raga, 1993). Although the total number of individuals killed could not be calculated, Aguilar and Raga (1993) suggested that several thousands of individuals could have died. Interestingly, abnormally high organochlorine compound (OC) levels were observed in dolphins killed, and there is significant evidence that the immunosuppressive effects associated to these compounds could have increased susceptibility to infection (Aguilar and Borrell, 1994). The population apparently recovered at a fast rate: the density of striped dolphins estimated in the Gulf of Valencia (Spain) in 2001-2003 was again close to the maximum reported for this species in the western Mediterranean (Gómez de Segura *et al*., 2006).

However, this high population density was likely to favor the propagation of morbillivirus infections (Black, 1991) and, indeed, a second epizooty occurred in 2007 (Raga *et al*., 2008). This new outbreak was geographically more restricted and affected a lower number individuals (Castrillon *et al*., 2010; see also Aguilar and Raga 1993, Raga *et al*., 2008). Also, OC concentrations in deceased dolphins were not more elevated than in presumably healthy individuals and, therefore, this second outburst was not believed to have been enhanced by OC pollutants (Castrillon *et al*., 2010).

Interestingly, Aguilar and Raga (1993) also suggested that abnormally warm water temperatures during the winter previous to the first epizootic could have led to a decrease in productivity, with the consequent reduction of available food and the lack of capacity by dolphins to overcome the pathogenic effects of the morbillivirus. Heatwaves and other type of extreme weather conditions have increasingly been reported during the last decades, and the point is whether they may be related to climate change (Coumou and Rahmstorf, 2012). In the western Mediterranean, there is clear evidence of an increase of temperature and salinity of the sea surface (Vargas-Yáñez *et al*., 2010), which may impact not only populations of apex predators, like striped dolphins, but also the function of the whole ecosystem (Bianchi, 2007).

Parasites are excellent biological markers of their hosts due to their ecological ubiquity and strict trophic dependence on their hosts (MacKenzie and Abaunza, 2005; Whiteman and Parker, 2005). Trophically-transmitted parasites, in particular, can be used to trace interactions in trophic webs because their life cycles include free-living stages, and intermediate, paratenic (transport) and definitive hosts that are linked through predator-prey relationships. Therefore, significant changes in abiotic conditions, population size, habitat use or trophic interactions of their hosts can be reflected in changes of infection levels (Marcogliese and Cone, 1997; Marcogliese, 2004). This peculiarity makes parasites ideal candidates to monitor long-term changes in the ecosystem. Indeed, studies have started to use parasites to detect changes associated to global change (i.e., Marcogliese, 2001; Khan and Chandra, 2006).

Information on the intestinal helminth community of the striped dolphin in western Mediterranean waters has already been reported (Raga and Carbonell, 1985; Manfredi *et al*., 1992; Mateu *et al*., 2014). However, due to obvious difficulties when studying cetaceans, these studies are based on relatively low sample sizes. Moreover, none of them has used long-term data to investigate changes in the structure of the parasite community. However, over the last decades, both natural and anthropogenic factors have impacted, not only the population of striped dolphins, but also the whole ecosystem and, therefore, the question arises as to whether these impacts could be traced by changes in the structure of parasite communities of dolphins.

In this paper we use a large sample size, 128 individuals, stranded during the last twenty years (1990-2010) to analyze, for the first time, possible long-term trends in the abundance and composition of the intestinal helminth community of the striped dolphin in western Mediterranean waters. We also examine whether there is a significant relationship between changes in the diet and parasite loads based on a relatively large dataset of dolphins from which data on both stomach contents and intestinal helminths are gathered.

7.2. MATERIALS AND METHODS

7.2.1. SAMPLE COLLECTION AND EXAMINATION

A total of 128 striped dolphins (Appendix 1) was analyzed for intestinal helminths. Dolphins were found stranded along the Mediterranean coast of Spain (Valencian Community, between 40°31'00''N, 0°31'00''E and 37°50'00N, 0°45'42''W) during a twenty-year period (1990-2010). All but highly decomposed individuals (see Geraci and Lounsbury, 2005) were examined. Fifty-nine out of the 128 individuals examined were females (total length (TL) (in cm), mean ± standard deviation (SD) [range], 184.5 ± 20.8 [106-211]), 68 were males (TL: 186.7 ± 22.2 [123-220]), and one unsexed dolphin (TL=203cm). During necropsy the intestine was removed and stored at -20°C. After thawing, each intestine was measured and divided into 20 sections of equal length. Each section was washed with tap water under a 0.02mm mesh and the solid content was collected in a Petri dish. The intestine wall was also thoroughly examined for attached helminths. Parasites were collected under a stereomicroscope, washed in saline, identified, counted, and fixed and preserved in 70% ethanol. Tetraphyllidean plerocercoids were not considered as members of the intestinal helminth community because they use dolphins as intermediate host and occur in the intestine only temporarily (see Aznar *et al.*, 2007 for details).

For helminth identification cestodes were stained with acetocarmine (Georgiev *et al.*, 1986) and drawn using a light microscope connected to a drawing tube. Additionally, acanthocephalans were cleared in lactophenol and drawn using the same procedure as with cestodes (see Mateu *et al.*, 2014 and references therein for detailed information on species identification). Voucher specimens are deposited in the Natural History Museum, London, UK (accession numbers: *Tetrabothrius forsteri* (NHMUK 2013.5.31.1-2), *Trigonocotyle globicephalae* (NHMUK 2013.5.31.3) and *Strobilocephalus triangularis* (NHMUK 2013.5.31.4-5). Additional material can be found in the Collection of the Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain.

7.2.2. DIETARY DATA

Dietary data were available from 71 out of the 128 individuals of striped dolphin examined for intestinal helminths (Blanco *et al*., 1995; Míguez, 2009; Bosch de Castro, 2014; C. Blanco unpub. data). For each dolphin, fish otoliths and cephalopod beaks collected from the stomach were used to identify prey to the lowest possible taxonomic level. For fish, the number of individuals of each prey taxon per dolphin was estimated as the number of complete and incomplete otolith pairs plus half the number of those that could not be allocated to either side. For cephalopods, the total number was estimated as the highest number of either upper or lowest beaks (Blanco *et al*., 2001). Given the large amount of prey taxa, prey were grouped into six ecological categories: (1) neritic demersal fish; (2) neritic pelagic fish; (3) demersal fish from upper slope; (4) myctophids and other mesopelagic fish; (5) neritic cephalopods and (6) oceanic cephalopods (see Table 7.1 and Table 7.2 for details).

Table 7.1. Fish species included in the diet of 71 striped dolphins from western Mediterranean, grouped into four fish categories. Data were obtained from Míguez, 2009; Bosch de Castro, 2014; C. Blanco (unpub. data).

Neritic demersal fish	**Neritic pelagic fish**	**Demersal slope fish**	**Myctophids and other mesopelagic fish**
Lesueurigobius spp.	*Engraulis encrasicolus*	*Lepidorhombus* spp.	*Maurolicus muelleri*
Arnoglossus spp.	*Sardina pilchardus*	*Micromesistius potassou*	*Ceratoscopelus maderensis*
Cepola macrophthalma	*Sprattus sprattus*	*Phycis blennoides*	*Gadiculus argenteus*
Citharus linguatula	*Boops boops*	*Epigonus* spp.	*Lestidiops* spp.
Gobius spp.	*Sardinella* spp.		*Notoscopelus* spp.
Ophidion spp.	*Trachurus* spp.		*Arctozenus rissoi*
Trisopterus minutus			*Benthosema glaciale*
Merluccius merluccius			*Diaphus* spp.
Macrorhamphosus scolopax			*Lobianchia* spp.
			Electrona risso
			Hygophum hygomii
			Lampanyctus spp.
			Myctophum punctatum
			Notolepis rissoi
			Symbolophorus veranyi

Table 7.2. Cephalopod species included in the diet of 71 striped dolphins from western Mediterranean, grouped into two cephalopod categories. Data were obtained from Blanco *et al*., 1995; Míguez, 2009; Bosch de Castro, 2014.

Neritic cephalopods	**Oceanic cephalopods**
Alloteuthis spp.	*Abralia veranyi*
Eledone moschata	*Todarodes sagittatus*
Eledone cirrhosa	*Todaropsis eblanae*
Illex coindetii	*Abraliopsis pfefferi*
Loligo spp.	*Ancistroteuthis lichntensteinii*
Sepiola spp.	*Ancistrocheirus lesueurii*
	Brachioteuthis riisei
	Chiroteuthis veranyi
	Galiteuthis armata
	Onychoteuthis banksii
	Heteroteuthis dispar
	Histioteuthis reversa
	Histioteuthis bonnelli
	Ocythoe tuberculata
	Ommastrephes bartrami

7.2.3. STATISTICAL ANALYSIS

7.2.3.1. Infection parameters

Ecological terms follow Bush *et al*. (1997). 'Prevalence' is the number of host species infected with a given parasite taxon, 'mean intensity' is the mean number of individuals of a given helminth species in an infected host and 'mean abundance' is the average number of individuals of a helminth species in a sample of hosts, regardless of whether they are infected or not. The 95% confidence interval (CI) for prevalence was calculated with Sterne's exact method (Reiczigel, 2003), and for mean intensity and mean abundance of each helminth taxa with the bias-corrected and accelerated bootstrap method using 20,000 replications (Rózsa *et al*., 2000).

7.2.3.2. Influence of potential confounding variables

To minimize the influence of 'cause of death' as a confounding variable, two sets of data were defined: (i) dolphins which died from DMV outbreaks during 1990 and 2007 ('epizootic sample') (n= 66), and (ii) dolphins which stranded due to unknown causes during other years ('non-epizootic sample') (n= 62).

Out of the 66 dolphins analyzed during the epizooties (TL: 191.2 ± 17.03 [129-213]), 29 were females and 37 males. A Mann-Whitney was carried out to test for differences on dolphin's total length between both epizootic samples (years 1990 and 2007), and a Chi-square test to test for differences in sex ratios in both samples. Both epizooties took place during the same year period (summer to winter) and, therefore, season was not considered a potential confounding factor in this case.

The non-epizootic sample (TL: 180 ± 24.24 [106-220]) was composed of 30 females, 31 males and one unsexed individual. Since the number of dolphins examined was very low in some years, we grouped data in pairs of years. To test for significant differences on total length of dolphins, data were log-transformed and a one-way ANOVA was carried out, a Monte Carlo Chi-square was used to test differences in the amount of samples examined during each season and to test differences in the amount of males and females among pairs of years.

7.2.3.3. Temporal changes in helminth community structure

Permutational multivariate analysis of covariance (PERMANCOVA) based on a similarity matrix (Anderson *et al*., 2008) was used to investigate the effects of sex, host length (as a surrogate of age) and period (i.e., 1990 vs. 2007) on the structure of helminth communities. Sex and period were treated as fixed factors, and host length was used as a fixed covariate. We firstly included interaction terms of the covariate with the factors, but none was significant and were removed from final models to increase the sensitivity of the analysis and to correctly interpret main effects (Engqvist, 2005). To build the models, raw numerical data for parasite species were firstly fourth-root transformed, then a Bray-Curtis similarity matrix between infracommunities was obtained. As many prey and parasite species had zero values in dolphins, a zero-adjusted Bray-Curtis matrix was obtained by adding a value of 1 to all cells (see Clarke and Warwick, 2001). Pseudo-F statistics under a true null hypothesis were obtained by using a permutation procedure, i.e. group labels were randomly shuffled onto different sample units, and this procedure was repeated 10,000 times, according to the method described by Anderson *et al*. (2008) in models including covariates. Our design was unbalanced and, therefore, we used a type III sum of squares to ensure a complete independence of all effects being tested (Anderson *et al*., 2008). Differences in the abundance of individual helminth species between epizooties were tested with Mann-Whitney tests.

A PERMANCOVA was also used to investigate changes in the structure of the helminth community in the sample of 'non-epizootic' dolphins. In this case, dolphins were grouped into the following periods: 1991-1998 (n= 24), 1999-2006 (n= 18), and 2008-2010 (n= 20). This subdivision was also used to compare dietary data (Bosch de Castro, 2014). Sex, host length and period were used as predictors of community structure.

7.2.3.4. Relationship between helminth community structure and dietary data

Within the BEST procedure in PRIMER6 (Clarke and Gorley, 2006) the BIO-ENV method allows to test whether there is a link between species abundance and environmental factors. In particular, we investigated the relationship between the structure of the intestinal helminth community and the dietary data of the striped dolphin. Bray-Curtis similarity matrices of community structure and diet from 71 striped dolphins were used. Our null hypothesis was that there was no significant relationship between the two matrices measured with a Spearman correlation test, r_s. To construct the Bray-Curtis similarity matrices data were firstly fourth-root transformed. A null distribution of r_s was generated with 20,000 random permutations of labels before estimating r_s.

The free software Quantitative Parasitology v. 3 (Reiczigel and Rózsa, 2005) was used to set 95% CIs, the distance-based linear model and the BIO-ENV analysis were carried out with PERMANOVA+ for PRIMER (Anderson *et al*., 2008) and the statistical package SPSS v. 19 was used for the remaining analyses (SPSS Inc., Chicago, Illinois, USA). Statistical significance was set at $p < 0.05$.

7.3. RESULTS

For the 'epizootic' samples, there was a marginally significant difference on dolphin's total length between animals of 1990 and 2007 (Mann-Whitney test, $p < 0.05$). Therefore, a subsample of dolphins with a total length ≥ 160 cm was also generated (n= 61); in this case, dolphin length was comparable between samples (Mann-Whitney test, $p > 0.05$). A Chi-square test indicated that the relative number of males and females did not differ in both years, either using all data or the subsample of animals ≥ 160cm ($p > 0.05$).

For the 'non-epizootic' sample, a one-way ANOVA indicated that dolphin length did not differ between years ($p > 0.05$), nor were significant differences in the relative amount of males and females (Monte Carlo Chi-square $p > 0.05$). However, we found significant differences in the amount of strandings among seasons (Monte Carlo Chi-square $p > 0.05$). Therefore, the influence of season should be taken into account in the interpretation of potential changes of helminth community structure.

Out of the 128 dolphins examined, only 4 were uninfected, namely 3 females and the unsexed individual, with lengths ranging between 129cm and 203cm. A total of 6,498 helminth individuals were collected in the intestine. Four helminth species were found: three species of tetrabothriid cestodes, *Tetrabothrius forsteri*, *Trigonocotyle globicephalae* and *Strobilocephalus triangularis*, and immature individuals of the acanthocephalan *Bolbosoma vasculosum* (Table 7.3).

Table 7.3. Infection parameters of intestinal helminths found in 128 striped dolphins (*Stenella coeruleoalba*) from the western Mediterranean.

Species	**Prevalence (%) (95% CI)**	**Mean intensity (95% CI) [range]**	**Mean abundance (95% CI)**
Cestoda			
Tetrabothrius forsteri	95.3 (90.1-98.0)	49.2 (38.3-66.2) [1-518]	46.9 (36.4-63.0)
Trigonocotyle globicephalae	7.8 (4.2-13.8)	3.1 (2.0-4.2) [1-6]	2.4 (1.0-4.6)
Strobilocephalus triangularis	19.4 (13.2-27.1)	13.9 (8.6-24.4) [1-81]	2.7 (1.4-5.2)
Acanthocephala			
Bolbosoma vasculosum	20.2 (13.9-27.9)	2.4 (1.8-3.2) [1-7]	0.5 (0.3-0.7)

Tetrabothrius forsteri was the most abundant and prevalent species with a total of 123 infected dolphins (total n= 6,046) and was found every year throughout the study period (Fig. 7.1a). *Trigonocotyle globicephalae* was found in low numbers (1-6 individuals per dolphin, total n= 31) and only during 5 out of the twenty years sampled (Fig. 7.1b). Individuals of *S. triangularis* (total n=348) seemed more common during the second decade (2000-2010) (Fig. 7.1c). Finally, the acanthocephalan *B. vasculosum* was also found in low numbers (1-7 individuals per dolphin, total n= 73). Interestingly, this acanthocephalan was restricted to 1990, as all individuals (except a single specimen found in 1997) were collected during this year (Fig. 7.1d).

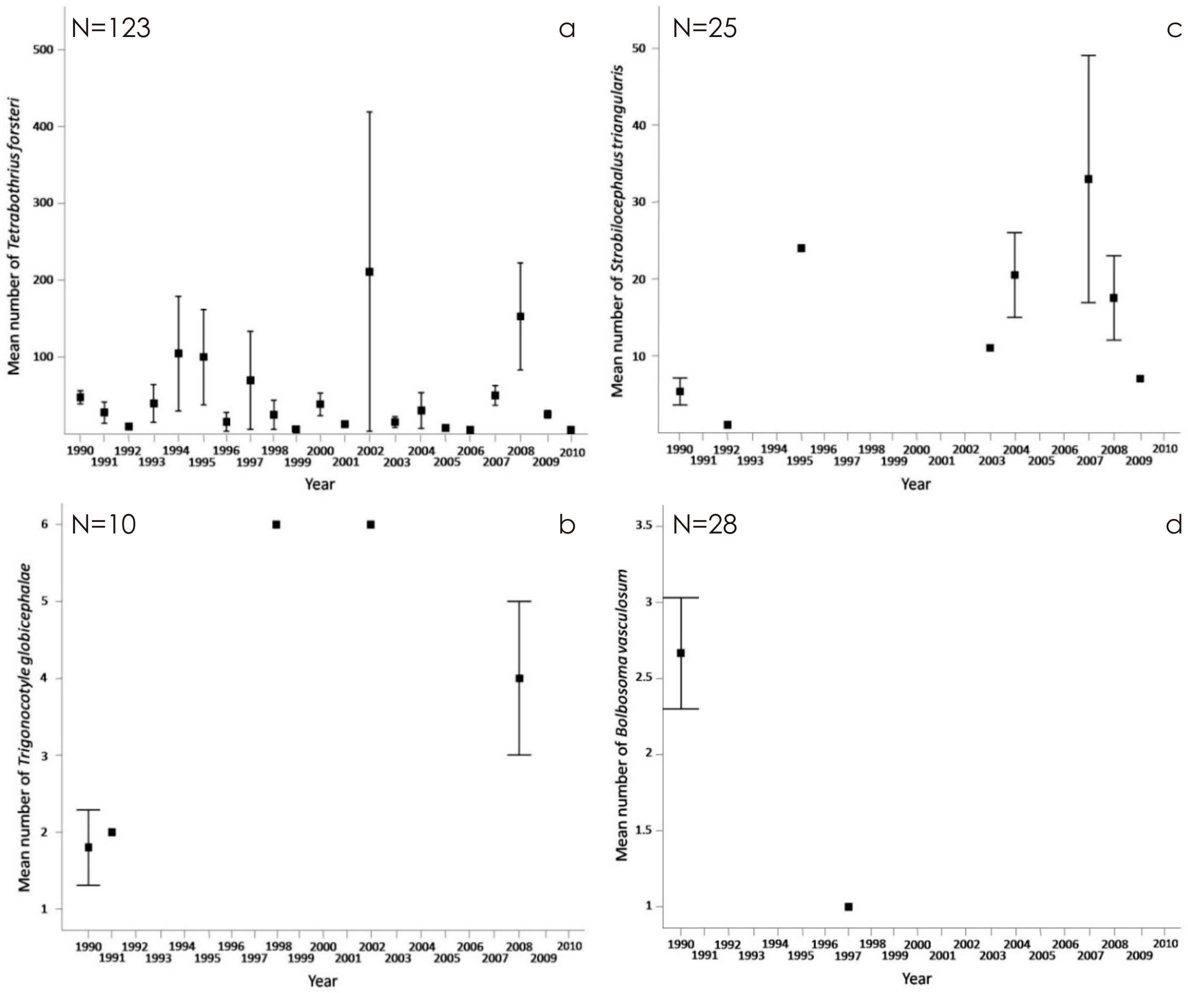

Figure 7.1. Mean number (±SD) of individuals per year of 4 helminth species collected from striped dolphins (*Stenella coeruleoalba*) in the western Mediterranean. (a) *Tetrabothrius forsteri*, (b) *Trigonocotyle globicephalae*, (c) *Strobilocephalus triangularis* and (d) *Bolbosoma vasculosum*. N is the number of infected dolphins for each helminth species.

The PERMANCOVA for the 'epizootic' samples indicated that none of the interactions between the factors and the covariable was significant. The final model is shown in Table 7.4. The only predictor was found to be statistically significant was 'Year'. Mann-Whitney tests indicated that the abundance of none of the cestode species differed between 1990 and 2007 ($p > 0.20$ in all cases), but that of the acanthocephalan did ($U= 195$; $p= 0.001$). These results were similar where the subsample of dolphins of dolphins with similar sizes was used (data not shown).

Table 7.4. Results of Permutational Multivariate Analysis of Covariance that accounts for the structure of the intestinal helminth community of two samples of striped dolphins affected by an epizootic in 1990 and 2007. df: degrees of freedom; SS: Sum of squares; MS: Mean sum of squares.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P (perm)**
Log body length	1	963.09	963.09	2.24	0.098
Year	1	1712.10	1712.10	3.98	0.014
Sex	1	4.86	4.86	$1.13e^{-2}$	0.917
Residual	62	26647	429.79		
Total	65	29327			

The PERMANCOVA for the 'non-epizootic' samples also indicated that none of the interactions between the factors and the covariable was significant. The final model is shown in Table 7.5. The only predictor that was found to be statistically significant was 'log body length'. Spearman correlation tests indicated significant negative relationships between this variable and the abundance of *T. forsteri* (r_s= -0.327; n= 61; p= 0.010) and *T. globicephalae* (r_s= -0.306; n= 61; p= 0.016); the relationship with *S. triangularis* was negative but not significant (r_s= -0.207; n= 61; p= 0.109), and that with *B. vasculosum* it was close to zero (r_s= 0.007; n= 61; p= 0.5).

Table 7.5. Results of a Permutational Multivariate Analysis of Covariance that accounts for the structure of the intestinal helminth community of three samples of striped dolphins stranded along the central coast of Spain in the period 1990-2010 (see text for details). df: degrees of freedom; SS: Sum of squares; MS: Mean sum of squares.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P (perm)**
Log body length	1	2346.40	2346.40	9.18	<0.001
Year	2	736.34	368.17	1.44	0.226
Sex	1	267.17	267.17	1.05	0.374
Residual	54	13805	255.65		
Total	58	17155			

According to BIO-ENV analysis, the correlation between the dietary data and the intestinal helminth community of striped dolphins was low and not statistically significant (r_s= 0.11; p= 0.265).

7.4. DISCUSSION

From a qualitative viewpoint, the composition of the helminth component community of *S. coeruleoalba* observed in this study is what should be expected for a cetacean. All helminth species reported here are specialists of cetaceans (Mateu *et al*., 2014) and, therefore, helminth diversity would depend on patterns of helminth exchange within the local community of cetaceans (see, e.g., Dailey and Perrin, 1973; Forrester *et al*., 1980; Balbuena and Raga, 1993; Hoberg *et al*., 1993; Gibson *et al*., 1998; Aguilar-Aguilar *et al*., 2001; Andrade *et al*., 2001; Fernández *et al*., 2003, 2004). In this context, oceanic cetaceans have been suggested to act as a small isolated group for exchange of helminths (see Aznar *et al*., 1998), and this would also result in depauperate communities. In fact, the composition of the intestinal helminth community in *S. coeruleoalba* is surprisingly similar to that found in other *Stenella* spp. in the Pacific regarding richness and composition (Dailey and Perrin, 1973). It is also remarkable that increasing host sampling effort for this study (n= 128 vs. 52 in the first parasitological analysis of *S. coeruleoalba*, see Mateu *et al*., 2014; Chapter 4) did not result in an increase of helminth richness. This strongly suggests that specificity likely prevents the contact and/or establishment of other helminths (Mateu *et al*., 2011; Chapter 5), i.e., there is a 'pool exhaustion' of all potential local immigrants to the community.

Changes in the community of trophically-transmitted helminths over the last two decades could have been expected because there is consistent evidence that the diet of *S. coeruleoalba* changed in the area during that period. A recent study based on stable isotope analysis investigated dietary changes of striped dolphins from the Western Mediterranean killed by the Dolphin Morbillivirus (DMV) in 1990 and 2007-08. Results suggests a change from a sardine-dominated diet to a juvenile hake-dominated one, with an increase of prey species of low trophic levels, such as lantern fish, or with high turnover rates, such as cephalopods (Gómez-Campos *et al*., 2011). Apparently, stock depletion of sardine, anglerfish and adult hake by fishing would have resulted in a trophic cascade provoking changes in prey availability (Gómez-Campos *et al*., 2011). This hypothesis was re-examined using stomach content analysis from two sets of samples, i.e., dolphins killed by the DMV in 1990 and 2007-08 (n= 44), and dolphins stranded in the period 1991-2012 (n= 75) on the central Mediterranean coast of Spain (Bosch de Castro, 2014; Aznar *et al*., 2015). Results indicated a significant increase in the consumption of both juvenile hake and the neritic squid *Illex coindetii*, which dominated the diet of striped dolphins in the second decade. Increased consumption of fish and cephalopods typical from the lower shelf was also noted, with a parallel, sharp decrease of consumption of oceanic cephalopods. These patterns suggest that striped dolphins might have relied more on oceanic prey in the 1990's, shifting to more neritic prey afterwards. Regardless of some contrasting dietary inferences obtained from stable isotope analysis vs. stomach content analysis (i.e., the importance of sardine in the diet of *S. coeruleoalba*), the hypothesis of a significant prey shift over the years is apparently solid.

However, results from this study offer contrasting results on whether parallel long-term changes in the helminth communities of *S. coeruleoalba* have occurred over the last decades. Regardless of host sample (i.e., epizootic and non-epizootic), there is no evidence of changes in the abundance of tetrabothriid cestodes, and significant variation in the abundance of the acanthocephalan *B. vasculosum* was only significantly detected in the comparison of 'epizootic' samples. A clear drawback for the interpretation of results is that no data are available about the specific identity of intermediate or potential paratenic hosts for any tetrabothriid species (Hoberg, 1987). In the case of *B. vasculosum*, several authors have reported the presence of cystancaths in paratenic fish hosts in other oceanic areas (see, e.g., Bakay and Zubchenko, 1984; Panebianco and Sebastio, 1988; Costa *et al*., 2000; Klimpel *et al*., 2006; Garcia *et al*., 2008). However, the identity of the putative fish hosts in our study area is still to be determined. Unfortunately, the analysis of the correlation between diet and parasites that we carried out in this study did not help either because we failed to find a significant association between both datasets. One possibility is that none of the prey species involved in dietary changes is a key intermediate/paratenic host for tetrabothriid cestodes and *B. vasculosum*. A more likely explanation is that stomach contents only reflect the last meal, which obviously does not carry the parasites already established in the dolphin. We should remember, however, that stable isotope analysis also revealed changes in the diet of *S. coeruleoalba*, and this method operates at a larger temporal scale, namely, several weeks (Gómez-Campos *et al*., 2011).

Opportunistic sampling based on stranded individuals is always associated to a number of caveats as it does not represent a random sample of the population. Thus, the question remains whether we are unable to detect clear long-term changes in the helminth fauna of *S. coeruleoalba* because we analysed only heavily parasitized animals or because there were really no changes to be detected. In fact, the infection patterns of intestinal helminths detected in this study could be interpreted according to two non-exclusive hypotheses. First, our samples of stranded dolphins could be relatively enriched with heavily parasitized animals. If so, there would be low probability of detecting changes in infection levels (i.e., there would be too much 'noise') even though a dietary change involving intermediate/paratenic hosts had occurred. This hypothesis alone, however, could not explain why infection levels of *B. vasculosum* were significantly higher in 1990; here, we should assume that the change was so strong that could be detected in spite of sampling 'noise'. A second possibility would be that 'epizootic' samples are less affected by sampling bias because the DMV likely killed dolphins regardless of parasite loads (see Aguilar and Raga, 1993; Raga *et al*., 2008, and references therein). If so, the comparison of helminth communities between 'epizootic' samples would be more meaningful, suggesting that only *B. vasculosum* was affected in the long-term tracking of infection levels.

Depending on which hypothesis is assumed, the interpretation of the significant differences on the abundance of *B. vasculosum* between 'epizootic' samples may differ. First, 1990 could have been an exceptional year for infections of *B. vasculosum*. In a parasitological study prior to 1990, Raga and Carbonell (1985) reported a single specimen of *B. vasculosum* in 10 striped dolphins stranded on the same study area. Likewise, we found a single specimen in years other than 1990. The absence of regular findings of *B. vasculosum* before and after 1990 would support that this year was extraordinary. Perhaps the exceptionality of 1990 could be related to a short-term change in the population of the intermediate, paratenic and/or definitive hosts of *B. vasculosum*. Interestingly, individuals infecting *S. coeruleoalba* were not gravid. Gravid individuals of putative *B. vasculosum* have been reported on single occasions in two fish species and common dolphins (Harada, 1935; Yamaguti, 1963; Williams and Bunkley-Williams, 1996). It seems clear that the typical definitive host must be a cetacean, possibly a baleen whale (see Mateu *et al*., 2014). Perhaps it is not coincidental that the only two additional studies which have reported the presence of *B. vasculosum* in the Mediterranean Sea (Raga and Carbonell, 1985; Fernández *et al*., 2004) are both from the same study area as the present study, where whales are known to migrate towards feeding grounds in the Ligurian Sea (Castellote *et al*., 2012; Gozalbes and Tomás, 2012). Interestingly, Dailey and Perrin (1973) also reported a decrease of infection levels of the same tetrabothriid cestodes with age in spotted (*Stenella attenuata*) and spinner (*S. longirostris*) dolphins in the Pacific.

A second hypothesis is that the dietary shift of western Mediterranean striped dolphins towards more neritic prey (Bosch de Castro, 2014; Aznar *et al*., 2015) could have minimized contacts between host and parasite. In fact, *B. vasculosum* have been reported mainly in the intestine of oceanic odontocetes, in Pacific (Pendergraph, 1971; Zam *et al*., 1971), Atlantic (Dailey and Perrin, 1973; Mignucci-Giannoni *et al*., 1998; Costa *et al*., 2000; Aguilar-Aguilar *et al*., 2001) and Mediterranean waters (Raga and Carbonell, 1985; Fernández *et al*., 2004). Accordingly, *B. vasculosum* could essentially be considered an oceanic species. Therefore, one could speculate that the shift of western Mediterranean striped dolphins from an oceanic habitat towards more neritic one could have affected the likelihood of parasite recruitment.

Interestingly, our results indicated significant negative effect of host body length on the abundance of tetrabothriid species. A previous study (Mateu *et al*., 2014, see Chapter 4) was unable to detect such an effect and attributed this failure to the fact that the sample was composed mostly on adult dolphins. The sample size for this study is much greater (128 vs. 52 in the previous study), and includes a wider range of body length (220-106cm compared to 213-163cm); both factors could have increased the power of tests to detect host effects. However, the tendency was negative, meaning that juvenile dolphins were more susceptible to infection and/or fed more on intermediate/paratenic hosts. In this context, Gómez-Campos *et al*. (2011) reported significant dietary differences of immature vs. adult striped dolphins; apparently the former had a more diverse diet than adults, as reported for the population from the northwest Pacific and the north Atlantic, where it was found that immature striped dolphins could consume some prey depleted in $\delta^{13}C$ that were not consumed by adults. The association between ontogenetic differences in both diet and parasite load can be a starting point to identify the intermediate/paratenic hosts for tetrabothriids. Similar results have been observed for other species of the genus *Stenella* (Dailey and Perrin, 1973).

In summary, this is the first parasitological study that investigates long-term trends in the abundance and composition of the intestinal helminth community of a cetacean species. Additionally, we have also compared dietary and helminth data to investigate the possible effects of a dietary shift on the composition of the intestinal helminth community of western Mediterranean waters. The results are not conclusive, but point to significant changes in the infection of some parasites, also stressing the need for using large host sample sizes and long-term datasets if one is to draw valid inferences about infection trends.

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CHAPTER VIII:

THE ROLE OF LANTERN FISH (MYCTOPHIDAE) IN THE LIFE-CYCLE OF CETACEAN PARASITES FROM WESTERN MEDITERRANEAN WATERS

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The role of lantern fish (Myctophidae) in the life-cycle of cetacean parasites from western Mediterranean waters

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ABSTRACT

Myctophids (lantern fish) and cephalopods play a key role in trophic webs from the continental slope and oceanic waters linking the zooplankton to top predators. Many cetaceans feed on both lantern fish and cephalopods, and such prey would thus be expected to bridge the trophic gap in the life-cycles of helminths infecting cetaceans. However, information on the life-cycles of most of these helminths is extremely scanty. We examined the parasite fauna of myctophids and cephalopods in two areas from the western Mediterranean where at least 21 helminth taxa from cetaceans have been reported and both cetacean diversity and abundance is high. A total of 1012 individuals of 8 lantern fish species, namely, *Ceratoscopelus maderensis*, *Lampanyctus crocodilus*, *Notoscopelus elongatus*, *Benthosema glaciale*, *Myctophum punctatum*, *Lobianchia dofleini*, *Diaphus holti* and *Hygophum benoiti*, and 792 individuals of 2 cephalopod species, *Alloteuthis media* and *Sepietta oweniana*, were collected from the Gulf of Valencia and Alboran Sea (Spanish Mediterranean) during 2010–2012 and examined for larval helminths. All these species have been reported as prey for at least some cetacean species in the area. Only five helminth taxa were found. The nematodes *Anisakis pegreffii* and *Anisakis physeteris* were detected in *N. elongatus* and *C. maderensis* (overall prevalence for *Anisakis*: 8.1% and 0.5%, respectively). Their prevalence in *N. elongatus* was significantly higher than that from the other three myctophid species with $n > 50$ individuals. A single individual of *Hysterothylacium* sp. was found in *N. elongatus* (prevalence: 0.5%) and Raphidascarididae gen. spp. in *N. elongatus* and *L. crocodilus* (prevalence: 20.3% and 0.7%, respectively). Juvenile didymozoid digeneans (Torticaecum type) were detected in *N. elongatus* and *L. crocodilus* (prevalence: 18.5% and 4.3%, respectively). Two unidentified cestode plerocercoids were collected from *N. elongatus.* Our study suggests, for the first time, that myctophids could play a role as paratenic hosts in the oceanic life-cycle of *A. pegreffii* and *A. physeteris* in the western Mediterranean. None of the other larvae identified at least to family level infect cetaceans, but some of them can be transmitted to large predatory fish. The extreme scarcity of such cetacean parasites in this, and previous parasitological surveys of mesopelagic fish and cephalopods is at odds with the key role of these preys in the diet of oceanic cetaceans.

1. Introduction

Mesopelagic fishes, especially of the family Myctophidae (lantern fish), account for a high abundance and biomass in the continental slope and oceanic waters (Olivar et al., 2012). These fish prey mainly on small copepods, euphausiids, amphipods and decapods (Williams et al., 2001; Klimpel et al., 2008) linking the zooplankton to top predators, such as large cephalopods, fish and cetaceans (Olivar et al., 2012). Cephalopods also play a key trophic role because they, depending on ontogenetic stage and size, feed on small crustaceans or mesopelagic fish (Quetglas et al., 2010) and are, in turn, common prey of several top predators (Clarke, 1996; Boyle and Rodhouse, 2005).

The diet of many cetaceans, especially of species exploiting offshore waters, is largely composed of mesopelagic fish and/or cephalopods (Clarke, 1996; Barlow et al., 2008). These prey could thus be expected to bridge the trophic gap in the life-cycles of parasites infecting cetaceans (Klimpel et al., 2008). However,

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evidence for this ecological role is very limited. Cetaceans harbour a relatively diverse, highly specific fauna of trophically-transmitted metazoan parasites (Raga et al., 2009). Surprisingly, there is little information on the life-cycle for the vast majority of these parasites. Natural intermediate or paratenic hosts have not been identified for any of the ca. 40 species of digeneans, or the ca. 30 species of nematodes of the families Pseudaliidae and Tetrameridae infecting cetaceans (Fernández, 1996; Raga et al., 2009; see Lehnert et al. 2010 for an exception). In the case of cestodes and acanthocephalans, data about intermediate and paratenic hosts is very limited (Hoberg, 1996, Klimpel et al., 2006, Aznar et al., 2007, Gregori et al., 2012). The identity of all the hosts involved in the cycle has been ascertained only for some nematode species of the genus *Anisakis* (e.g. Klimpel et al., 2004).

Of the seven odontocete species that regularly occur in Spanish Mediterranean waters, five species are in offshore waters, especially from the upper slope, and two species are mainly on the continental shelf (Raga and Pantoja, 2004; Gómez de Segura et al., 2006 and references therein). The striped dolphin, *Stenella coeruleoalba*, is by far the most abundant species, with a population size an order of magnitude larger than that of any other cetacean species (Gómez de Segura et al., 2006). Overall, these odontocete species harbor at least 21 trophically-transmitted metazoan parasite taxa in the area, including five digeneans, eight cestodes, seven nematodes and one acanthocephalan (Mateu et al., 2011, 2014; Quiñones et al., 2013, and references therein). Dietary data indicate that the striped dolphin has a mixed diet of mesopelagic fish and cephalopods in the area; Risso's dolphin, *Grampus griseus*, long-finned pilot whale, *Globicephala melas* and Cuvier's beaked whale, *Ziphius cavirostris* feed almost exclusively on offshore, pelagic cephalopods, and bottlenose dolphin, *Tursiops truncatus*, and common dolphin, *Delphinus delphis*, rely on inshore fish as main prey, although mesopelagic fish represent a sizeable portion of common dolphin's diet (Blanco et al., 1995, 2006, and references therein; C. Blanco, unpublished results). Accordingly, one would expect that mesopelagic fish and cephalopods play a significant role in the transmission of cetacean parasites in this area, but no study has yet addressed this issue.

In this paper we investigate the larval helminth fauna from eight species of mesopelagic fish from the family Myctophidae, and two species of benthopelagic cephalopods collected from the Spanish Mediterranean. All selected fish and cephalopods have been reported as prey of cetaceans in the area and were collected in localities where the diversity and abundance of cetaceans is particularly high. The goal is to identify intermediate and/or paratenic hosts for parasites of cetaceans in order to understand their ecological role in the life-cycle. This is the first study on the helminth fauna of myctophids in Mediterranean waters (Klimpel et al., 2009).

2. Materials and methods

2.1. Sample collection

Samples were collected in the Gulf of Valencia and the Alboran Sea (Fig. 1), which include areas proposed as Protected Areas of Mediterranean Importance due to the high cetacean diversity and abundance (Raga and Pantoja, 2004). Collecting at the Gulf of Valencia (39°26′28″N, 0°15′24″W) was carried out in November 2010 by the fishing trawler "PAUSEP" at depths between 400 m and 430 m. Collecting at the Alboran Sea was carried out during MEDITS surveys (Bertrand et al., 2002) in May 2011 and April/May 2012 on board of the research vessel "Cornide de Saavedra", using a GOC 73 bottom trawl sampler operating at depths 44–790 m.

A total of 1012 individuals of eight species of the family Myctophidae was collected and examined for larval helminths, i.e. *Ceratoscopelus maderensis* ($n=390$), *Lampanyctus crocodilus* ($n=295$), *Notoscopelus elongatus* ($n=222$), *Benthosema glaciale* ($n=70$), *Myctophum punctatum* ($n=14$), *Lobianchia dofleini* ($n=9$), *Diaphus holti* ($n=8$) and *Hygophum benoiti* ($n=4$). A total of 792 individuals of two cephalopod species, namely, *Alloteuthis media* ($n=670$) and *Sepietta oweniana* ($n=122$), from the Alboran Sea was also examined for larval helminths (Table 1). Immediately after capture, all fish and cephalopods were fixed and preserved in 70% ethanol on ship. In the laboratory, each individual was weighed and measured to the nearest millimetre. Data on total length, mantle length and weight are shown in Table 2. Gills, gonads, stomach and intestine, liver, kidney, heart and brain of the fish, and gills, gonads and digestive tract of the cephalopods, were placed individually in saline, torn apart and examined under a stereomicroscope (20–100 ×). Epiaxial and hypoaxial muscle of the fish, and mantle and (opened) siphon of the cephalopods, were pressed between two glasses and examined by candling under a stereomicroscope.

The collection of all helminth larvae was attempted except in the case of tiny nematodes, occurring in the stomach wall of fish, that were identified as belonging to the family Raphidascarididae (see Section 3). Specimens were collected, fixed and preserved in either 70% ethanol for morphological analysis, or absolute ethanol for molecular analysis. For identification, nematodes were cleared in lactophenol and, in the case of *Anisakis*, assigned to type I or II (*sensu* Berland, 1961). Juvenile digeneans of the family Didymozoidae were stained with aluminium carmine and prepared as permanent mounts using Canada Balsam following standard protocols.

2.2. Molecular analysis

Molecular identification was carried out in order to identify the larval stages of *Anisakis* spp. since morphological characters do not allow their recognition to species level. To identify *Anisakis* larvae the mitochondrial cytochrome oxidase subunit II (mtDNA *cox2*) gene was sequenced in seven specimens obtained from six individuals of *N. elongatus*. The total DNA was extracted from 2 mg of tissue from each single nematode by using cetyltrithylammonium bromide method (CTAB) and the mtDNA*cox2* gene was amplified using primers no. 210R and no. 211F (see Valentini et al., 2006 for further details on the standard protocol).

The small-subunit ribosomal DNA (SSU rRNA) of other tiny nematode larvae ($\leq 300\ \mu$m) found in the stomach wall of fish was sequenced according to Nadler et al. (2007). In this case, DNA from a single individual was successfully extracted using Chelex® and the SSU rRNA was amplified using primers Philonema F (5′ GCCTATAATGGTGAAACCGCGAAC 3′) and PhilPCRr (5′ CCGGTTCAAGCCACTGCGATTA 3′) (Černotíková et al., 2011) in a final volume of 30 μl. The PCR was carried out using the following conditions: initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 40 s, 50°C for 40 s, and 72°C for 2 min, with a final extension of 72°C for 10 min. PCR products were purified with Macherey-Nagel NucleoSpin Gel and PCR Clean-up. Sequencing was carried out in an ABI 3730 automated sequencer using an ABI BigDye Terminator v 3.1 kit. Internal primers for sequencing were WR800 (5′ GGTATCGTTTACGGTCAG 3′), Ameb 620F (5′ GCCAGCACCCGCGGTAATTCC 3′) WF400 (5′ GCGCAAATTACCCACTCT 3′) (Černotíková et al., 2011). Sequence identity was checked under a phylogenetic analysis using 113 taxa from the Ascaridida, Rhigonematida, Spirurida and Oxyurida used by Nadler et al. (2007), plus four previously published sequences of the Pseudaliidae (i.e. *Pseudalius inflexus* AY295816, *Torynurus convolutus* AY295818, *Stenurus minor* AY295817 and *Halocercus invaginatus* AY295808).

2.3. Statistical analysis

Ecological terms follow Bush et al. (1997). The 95% confidence interval (CI) for prevalence was calculated with Sterne's exact

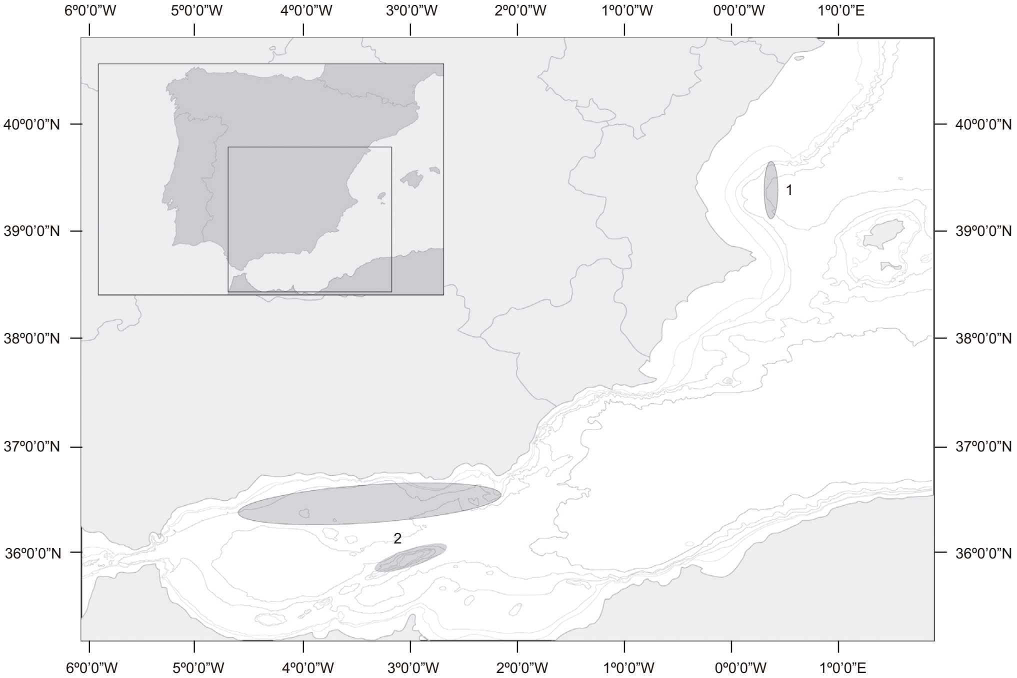

Fig. 1. Localities where myctophid and cephalopod species were sampled in the western Mediterranean waters: Gulf of Valencia (1) and Alboran Sea (2).

Table 1
Sampling details for eight species of myctophid fish and two cephalopod species collected from the western Mediterranean. GV: Gulf of Valencia; AS: Alboran Sea; *n*, number of specimens analyzed.

Area	Date	Species	*n*
Teleostei			
GV	November 2010	*Ceratoscopelus maderensis*	4
GV	November 2010	*Lampanyctus crocodilus*	1
GV	November 2010	*Notoscopelus elongatus*	221
GV	November 2010	*Benthosema glaciale*	26
AS	May 2011	*C. maderensis*	386
AS	May 2011–April/May 2012	*L. crocodilus*	294
AS	May 2011	*N. elongatus*	1
AS	May 2011	*B. glaciale*	44
AS	May 2011	*Myctophum punctatum*	14
AS	May 2011	*Lobianchia dofleini*	9
AS	May 2011	*Diaphus holti*	8
AS	May 2011	*Hygophum benoiti*	4
Cephalopoda			
AS	April/May 2012	*Alloteuthis media*	670
AS	April/May 2012	*Sepietta oweniana*	122

Table 2
Weight and total length (mantle length for cephalopods) of individuals of 8 myctophid species and 2 cephalopod species collected from the western Mediterranean.

	n	Weight (g) $\bar{x} \pm$ SD [range]	Total length (cm) $\bar{x} \pm$ SD [range]
Teleostei			
Ceratoscopelus maderensis	390	0.91 ± 0.23 [0.40–2.77]	5.89 ± 0.49 [4.60–8.10]
Lampanyctus crocodilus	295	12.22 ± 7.02 [1.64–37.37]	13.52 ± 2.49 [7.90–20.20]
Notoscopelus elongatus	222	12.63 ± 4.22 [1.41–26.61]	11.62 ± 1.49 [6.00–15.20]
Benthosema glaciale	70	0.58 ± 0.25 [0.21–1.30]	4.15 ± 0.65 [2.50–5.90]
Myctophum punctatum	14	1.09 ± 0.22 [0.79–1.52]	5.89 ± 0.45 [5.00–6.80]
Lobianchia dofleini	9	0.41 ± 0.10 [0.24–0.59]	4.04 ± 0.41 [3.40–4.50]
Diaphus holti	8	0.83 ± 0.48 [0.32–1.63]	4.73 ± 0.98 [3.40–6.40]
Hygophum benoiti	4	0.96 ± 0.25 [0.71–1.30]	5.38 ± 0.48 [4.70–5.80]
Cephalopoda			
Alloteuthis media	670	3.44 ± 2.31 [0.67–20.50]	4.78 ± 1.17 [2.20–8.90]
Sepietta oweniana	122	3.73 ± 1.50 [0.76–7.88]	2.15 ± 1.04 [1.00–12.50]

method and, for the mean intensity and mean abundance of each helminth taxa, with the bias-corrected and accelerated bootstrap method using 20,000 replications (Reiczigel and Rózsa, 2005).

One-tailed Spearman correlation tests were performed to investigate whether the abundance of *Anisakis* spp. increased with host length. Pair-wise Fisher tests were carried out to compare differences in prevalence of *Anisakis* spp. between fish species with $n > 50$ individuals, namely, *C. maderensis*, *L. crocodilus*, *N. elongatus* and *B. glaciale*. Probability values were corrected by the sequential Bonferroni procedure.

The free software Quantitative Parasitology v. 3 (Reiczigel and Rózsa, 2005) was used to set 95% CIs, and the statistical package SPSS v. 19 was used for the remaining analyses (SPSS Inc., Chicago, Illinois, USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Morphological identification

Helminth taxa were found in four of the eight myctophid species and in none of the cephalopod species analyzed. *Notoscopelus elongatus* was the host with the highest species richness ($n = 6$), followed by *C. maderensis* ($n = 2$), *B. glaciale* ($n = 1$) and *L. crocodilus* ($n = 1$) (Table 3).

A total of 21 larvae of *Anisakis* spp. was found in the body cavity of 18 of the 222 *N. elongatus* and two out of the 390 *C. maderensis* examined (Table 3). The range of intensity was 1-2 larvae per fish. Morphological identification was possible for 15 larvae collected

Table 3
Infection parameters for larvae of helminth species found in 4 species of the Myctophidae collected from the western Mediterranean. *P*, prevalence (%); MI, mean intensity; MA, mean abundance; *n*, number of hosts analyzed. Range of intensity is in square brackets and 95% confidence interval for parameters in parentheses.

	N. elongatus ($n=222$)			*C. maderensis* ($n=390$)			*B. glaciale* ($n=70$)			*L. crocodilus* ($n=295$)		
Helminth taxa	P (95% CI)	MA (95% CI)	MI (95% CI) [range]	P (95% CI)	MA (95% CI)	MI (95% CI) [range]	P (95% CI)	MA (95% CI)	MI (95% CI) [range]	P (95% CI)	MA (95% CI)	MI (95% CI) [range]
Nematoda												
Anisakis spp.**	8.1 (5.1–12.5)	0.09 (0.05–0.14)	1.17 (1.00–1.33) [1–2]	0.5 (0.1–1.9)	0.01 (0.00–0.01)	1.00* [1]	–	–	–	–	–	–
A. physeteris	4.1 (2.0–7.6)	0.05 (0.02–0.09)	1.33 (1.00–1.56) [1–2]	–	–	–	–	–	–	–	–	–
A. pegreffii	1.4 (0.4–4.0)	0.01 (0.00–0.03)	1.00* [1]	–	–	–	–	–	–	– –	–	–
Raphidascarididae gen. spp.***	20.3 (15.4–26.1)	?	?	–	–	– –	–	– –	–	0.7 (0.1–2.5)	?	–
Hysterothylacium sp.	0.5 (0.03–2.6)	0.00 (0.00–0.01)	1.00* [1]	–	–	–	–	– –	–	–	–	–
Cestoda												
Cestoda spp.	0.5 (0.03–2.6)	0.00 (0.00–0.01)	1.00* [1]	0.3 (0.02–1.5)	0.00 (0.00–0.01)	1.00* [1]	–	–	–	–	–	–
Digenea												
Didymozoidae (Torticaecum type)	18.5 (13.7–24.1)	0.40 (0.27–0.55)	2.17 (1.73–2.76) [1–8]	–	–	–	4.3 (1.2–11.9)		1.00* [1]	–	–	–

* 95% Confidence intervals could not be calculated due to small sample size.
** Includes all individuals found from the genus *Anisakis*.
*** Only presence was determined.

Table 4
Matrix of nominal probability values of pair-wise Fisher tests that compare differences in prevalence of *Anisakis* spp. between 4 fish species of the family Myctophidae from the western Mediterranean.

	Benthosema glaciale	*Ceratoscopelus maderensis*	*Lampanyctus crocodilus*	*Notoscopelus elongatus*
Benthosema glaciale	–	–	–	–
Ceratoscopelus maderensis	1	–	–	–
Lampanyctus crocodilus	1	0.509	–	–
Notoscopelus elongatus	0.009*	<0.001*	<0.001*	–

* Statistically significant after the sequential Bonferroni correction.

from *N. elongatus*, of which 3 and 12 were assigned to type I and II, respectively. Type I larvae were identified as *Anisakis pegreffii* and Type II as *Anisakis physeteris* (see Section 3.2). Pair-wise Fisher tests showed that *N. elongatus* was significantly more infected by *Anisakis* spp. than the other three myctophid species with $n>50$ individuals ($p \leq 0.009$ in all comparisons) (Table 4). Spearman correlation test showed no statistically significant relationship between the abundance of *Anisakis* spp. and host total length ($r_s < 0.095$, $p > 0.15$ in all tests). However, no *Anisakis* spp. were found infecting individuals of *C. maderensis* smaller than 6.5 cm or in individuals of *N. elongatus* smaller than 8 cm.

Very small larval nematodes (≤ 300 μm) were found encysted in the stomach wall, with cysts protruding into the stomach lumen. All specimens examined ($n=10$) superficially resembled *Ascarophis* spp., but their small size and lack of obvious diagnostic traits precluded reliable assignation even to family level. However, the molecular analysis indicated that these nematodes could be assigned to the family Raphidascarididae (see Section 3.2).

A single third-stage larva of *Hysterothylacium* sp. and two cestode pleroceroids with an invaginated scolex were collected from the body cavity of *N. elongatus* (Table 3).

A total of 89 juvenile digeneans of the family Didymozoidae was found in the intestine of 41 of 222 *N. elongatus*, and 3 of 70 *B. glaciale* (Table 3). Examination of 64 specimens from 31 individuals of *N. elongatus* allowed assignation to the juvenile type Torticaeum (*sensu* Pozdnyakov and Gibson, 2008). Specimens had a ventral sucker larger than the oral sucker, an intestinal bifurcation anterior to ventral sucker, a well-developed chambers in the caeca lumina, no "Drüsenmagen" and no gland-cells surrounding the oesophagus. The mean length ($\pm$S.D.) of worms ($n=3$) was 1.4 ± 0.07 mm (range: 1.34–1.47), and mean width was 1 ± 0.00 mm.

3.2. Molecular identification

Molecular identification was attempted for all taxa whose adults could potentially infect cetaceans (i.e., all except *Hysterothylacium* sp. and didymozoids). DNA extraction was attempted for the two cestode larvae but it was unsuccessful.

Sequences at the mtDNA *cox2* were obtained for two Type I and five Type II larvae of *Anisakis* larvae collected from *N. elongatus*. Sequences (629 bp) were compared with those from all *Anisakis* spp., which are deposited in GenBank. Sequences from the *Anisakis* Type I specimens collected were identical, and matched 100% those sequences of adults of *A. pegreffii* collected from the short-beaked common dolphin, *D. delphis*, in the NE Atlantic, and the bottlenose dolphins, *T. truncatus*, in the SE Atlantic (Valentini et al., 2006) (DQ116428.1). The specimens of *Anisakis* Type II from *N. elongatus* matched 100% those sequences of adults collected from the sperm whale, *Physeter macrocephalus*, in the Mediterranean Sea (Valentini et al., 2006) (DQ116432.1). Sequences are available in GenBank under accession numbers: KF972438 (*A. pegreffii*) and KF972439 (*A. physeteris*).

DNA from the ribosomal marker 18S could be obtained from a single specimen of the nematode encysted in the stomach wall from *N. elongatus*. The length of the PCR product was 1120 bp. BLAST showed a 99% of similarity of our sequence with sequences of species of the superfamily Ascaridoidea. The molecular comparison with ascaridoid species revealed a higher affinity with taxa from the Raphidascarididae. Sequences are available in GenBank under accession number: KF972437 (Raphidascarididae gen. sp.).

4. Discussion

The results of this study indicate that the larvae Type I and II found in myctophids are *A. pegreffii* and *A. physeteris*, respectively. We cannot rule out that other species of *Anisakis* occur in the sample since not all larvae collected were identified by molecular methods, (see, e.g., Farjallah et al., 2008; Serracca et al., 2013). *Anisakis pegreffii* is the most widespread species of *Anisakis* in the Mediterranean, dolphins being its main definitive hosts (Mattiucci and Nascetti, 2008). Larvae have been reported in a number of demersal and pelagic fish species from both the western and eastern basins (e.g. Farjallah et al., 2008; Mattiucci and Nascetti, 2008). However, there is only a previous record in myctophids, namely, in *M. punctatum* from the Mid-Atlantic Ridge (Klimpel et al., 2010). Our finding in *N. elongatus* would suggest that *A. pegreffii* can circulate through mesopelagic food webs using myctophids as paratenic hosts. Myctophids, including *N. elongatus* and *C. maderensis*, are a sizeable component in the diet of striped dolphins in the study area (C. Blanco, pers. comm.), and adults of *A. pegreffii* has been isolated from striped dolphins (Mattiucci et al., 2014; Mateu unpublished results). However, infection levels of *A. pegreffii* in *N. elongatus* are extremely low compared with those of other species of *Anisakis* infecting myctophids, i.e., *A. simplex* (sensu stricto) in *Notoscopelus kroyeri* and *M. punctatum* on the Mid-Atlantic Ridge (prevalence: 100%, see Klimpel et al., 2008). Infections of *A. pegreffii* are also low in oceanic cetaceans from the study area (Mateu, unpublished results), suggesting that either the life-cycle of *A. pegreffii* is more neritic or that its population is small in the study area.

Sperm whales (*Physeter macrocephalus*) are considered to be the main definitive hosts for *A. physeteris* (Mattiucci and Nascetti, 2008). High infections of stomach nematodes, presumably *A. physeteris*, have been observed in sperm whales worldwide (e.g. Evans and Hindell, 2004; Mazzariol et al., 2011). Sperm whales inhabit deep-water habitats and usually forage in depths between 500 and 1200 m, especially at the edge of the continental shelf (Whitehead, 2009). Larvae of *A. physeteris* have been reported in six fish and one cephalopod species typical from the upper slope (Mattiucci and Nascetti, 2008; Meloni et al., 2011). Sperm whales are considered to be teuthophagous, but fish also contribute to the diet of this species in some localities (Evans and Hindell, 2004 and references therein), raising the question of the relative role of cephalopods vs fish in the life-cycle of *A. physeteris* (Mattiucci and Nascetti, 2008). Our study reports, for the first time, *A. physeteris* in myctophids. *N. elongatus* likely acquires the nematode by consuming infected euphausiids or copepods (see Klimpel et al., 2004). However, sperm whales rarely ingest myctophids (Evans and Hindell, 2004), thus it is likely that *N. elongatus* helps bridging the trophic gap in the life-cycle of *A. physeteris* by being prey of fish or cephalopods that are consumed by sperm whales. For instance, sperm whales feed on squids, e.g. *Histioteuthis bonnellii* and *H. reversa* (Mazzariol et al., 2011), whose diet is largely based on myctophids (Quetglas et al., 2010).

Our study indicates that the prevalence of *Anisakis* spp. was significantly highest in *N. elongatus*. We are not aware of dietary particularities that make this species more prone to *Anisakis* infections (see Klimpel et al., 2010, and references therein). Furthermore, *N. elongatus* does not seem to accumulate more larvae because of its larger body size. Body size had no significant effect on infection levels in any host species, and other myctophids (i.e., *L. crocodilus* and *C. maderensis*) had similar body sizes as *N. elongatus* but lower parasite loads. However, the effect of sampling locality on infection levels should not be ignored: all individuals of *N. elongatus*, except one, were sampled in a different locality than those of *L. crocodilus* and *C. maderensis* (Table 1). Geographical factors should also be considered in accounting for the greater prevalence of *A. physeteris* vs *A. pegreffii* in *N. elongatus*. Although *A. pegreffii* is the dominant species in Mediterranean fishes, *A. physeteris* has proven to be more abundant in some localities (e.g. South of Valencia, see Mattiucci et al., 2004), perhaps because these localities are preferentially inhabited by sperm whales.

No anisakids were found in any of the two squid species analyzed in this study. Both *A. media* and *S. oweniana* are small in size and are largely zooplanktophagous (Zuev and Nesis, 1971; Bergström, 1985), and both factors could reduce the chances of infection within a host's short life span. Reports of infection of *Anisakis* in other cephalopods are not uncommon in the Mediterranean, but infection levels seem to vary significantly, depending on the study area. The prevalence of *Anisakis* larvae in the medium-sized squid *Illex coindetii* ranged from 1.7% to 4.8% in the Tyrrhenian Sea and the Ligurian Sea (Gestal et al., 1999; Serracca et al., 2013), but from 13.1% to 55.0% in the Adriatic Sea and Sardinia (Angelucci et al., 2011).

Two additional nematode taxa were found in this study, i.e., *Hysterothylacium* sp. and Raphidascarididae gen. spp. The possibility that the latter are also an undetermined species of *Hysterothylacium* need to be explored in the future. Species of *Hysterothylacium* use planktonic and benthic invertebrates as intermediate hosts, and various fish species as paratenic or definitive hosts (Klimpel and Rückert, 2005, and references therein). There is single previous record of *Hysterothylacium* in myctophids, namely, an unidentified species infecting *Notoscopelus resplendens* from off New South Wales (Pacific Ocean) (see Klimpel et al., 2009).

The taxonomic identity of juvenile specimens of the Didymozoidae is very difficult to ascertain since their morphology is rather uniform (Pozdnyakov and Gibson, 2008). Juveniles of the Torticaecum type have been reported from chaetognaths (Gómez del Prado-Rosas et al., 1999) and fish, including myctophids (Mordvinova, 2000). This study represents the first record of Torticaecum didymozoids in *N. elongatus* and in Mediterranean waters. Fish and squid have been reported as third intermediate or paratenic hosts of juvenile didymozoids, scombroid fish being the most common definitive hosts (Pozdnyakov and Gibson, 2008).

The two cestode larvae collected from *N. elongatus* and *C. maderensis* superficially resembled *Scolex pleuronectis*, a name that includes a heterogeneous array of plerocercoids of the order Tetraphyllidea infecting sharks and skates as adults, which have frequently been reported in myctophids worldwide (Klimpel et al., 2009).

In summary, from a potential pool of at least 21 taxa, we were able to detect only two trophically-transmitted parasites typical of cetaceans, namely, *A. pegreffii* and *A. physeteris*. The possibility that the plerocercoids we found belonged to previous stages of tetraphyllidean cestodes infecting cetaceans, i.e. *Monorygma grimaldii* and *Phyllobothrium delphini* (Aznar et al., 2007) or even cestode species of the Tetrabothriidae (Mateu et al., 2014) cannot be ruled out. Other larvae of helminths infecting cetaceans could have hardly been overlooked since larvae as small as 300 μm long were visually detected. However, some larvae of parasites infecting cetaceans could have missed in the sample if their infection levels in myctophids and cephalopods are very low in the study area. We tried to overcome this potential problem by selecting sampling areas with a high diversity of both cetacean and their parasites, but we lacked information about the minimum host sample size necessary needed to detect larvae with potentially low prevalence (e.g. Gregori et al., 2012). Finally, there is also the possibility that mesopelagic fish and cephalopods are actually not involved in the life-cycle of most helminths infecting cetaceans. In reviews of metazoan parasites from deep-sea fish, including myctophids (Klimpel et al., 2009), and cephalopods, including key squid species in the diet of teuthophagous cetaceans (Hochberg, 1983), only larvae from two taxa typical from cetaceans, namely *Anisakis* spp. and *Bolbosoma* spp., have been reported to date. Elucidating the life-cycle of helminths from cetaceans thus remains, for the most part, elusive.

5. Conclusions

Our study reports, for the first time, *A. physeteris* in myctophids and suggests that two anisakid species, namely, *A. pegreffii* and, particularly, *A. physeteris*, are the dominant taxa infecting a community of myctophids from western Mediterranean waters. Myctophids, particularly *N. elongatus*, are suggested to play a role in the mesopelagic life-cycle of both species. None of the other larvae identified at least to family level infect cetaceans, but some of them can be transmitted to large predatory fish. The failure to detect larvae of other trophically-transmitted parasites infecting cetaceans (except perhaps the cestode larvae) emphasizes the need for more parasitological research on cetacean prey.

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CHAPTER IX:

GEOGRAPHICAL VARIATION IN METAZOAN PARASITES OF THE DEEP-SEA FISH *BATHYPTEROIS MEDITARRANEUS* BAUCHOT, 1962 (OSTEICHTHYES: IPNOPIDAE) FROM THE WESTERN MEDITERRANEAN

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Geographical variation in metazoan parasites of the deep-sea fish *Bathypterois mediterraneus* Bauchot, 1962 (*Osteichthyes*: *Ipnopidae*) from the Western Mediterranean

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ABSTRACT

This study examines the parasite fauna of *Bathypterois mediterraneus*, the most common fish below 1500 m in Western Mediterranean waters. Samples were obtained during July 2010 from the continental slope of two different areas (off Catalonia and Balearic Islands) in three different bathymetric strata at depths between 1000 and 2200 m. The parasite fauna of *B. mediterraneus* included a narrow range of species: *Steringophorus* cf. *dorsolineatum*, *Scolex pleuronectis*, *Hysterothylacium aduncum*, *Anisakis* sp. larva 3 type II and *Sarcotretes* sp. *Steringophorus* cf. *dorsolineatum* and *H. aduncum* were the most predominant parasites. *H. aduncum* showed significant differences in abundance between depths of 2000–2200 m with 1000–1400 m and 1400–2000 m, irrespective of locality, whereas *S.* cf. *dorsolineatum* showed significant differences between the two localities at all depths except for 2000–2200 m. We suggest the possible usefulness of these two parasites as geographical indicators for discriminating discrete stocks of *B. mediterraneus* in Western Mediterranean waters.

1. Introduction

Due to obvious difficulties regarding the marine realm, information about the biology of deep-sea fauna is scarce and the available data is fragmentary (Klimpel et al., 2009). The genus *Bathypterois* Günther (*Osteichthyes*: *Ipnopidae*) is composed of 23 species, commonly named tripodfishes, distributed worldwide in temperate and tropical deep-sea waters (Sulak, 1984). Three species have been described in the Mediterranean Sea: *Bathypterois dubius* Vaillant, *Bathypterois mediterraneus* Bauchot and *Bathypterois grallator* (Goode and Bean) (Sulak, 1984). The Mediterranean tripodfish *B. mediterraneus*, an endemic species to the Mediterranean Sea, is distributed in the western and eastern basins (Sulak, 1977). This species was first reported in Western Mediterranean waters off the coast of Spain (Balearic sea) (Bauchot, 1962) where it is the most abundant fish species below 1500 m (Stefanescu et al., 1992a) and one of the Mediterranean teleosts found at largest depths (up to more of 3300 m according to D'Onghia et al. (2004)). Based on morphological evidences, Queró and Ribes (1999) suggested that *B. mediterraneus* is a junior synonym of *B. dubius*, the most common species of the genus in the Mediterranean Sea. However, no further studies have supported this synonymy. In any case, *B. mediterraneus* could represent the Mediterranean population of *B. dubius*. Although *B. mediterraneus* is one of the most abundant species in deep-sea Mediterranean communities, knowledge of many aspects of its biology is very limited and on many occasions incomplete. In the last two decades, a number of studies have concentrated on several aspects of the ecology of this species such as its bathymetric distribution (Geistdoerfer and Rannou, 1972; Stefanescu et al., 1992a; Cartes et al., 2004); growth and depth size trends (Morales-Nin, 1990; Stefanescu et al., 1992b; Morales-Nin et al., 1996; Moranta et al., 2004); aspects regarding its diet and feeding ecology (Carrassón and Matallanas, 1990, 1994, 2001; Polunin et al., 2001); and its reproductive biology (Fishelson and Galil, 2001; D'Onghia et al., 2004; Porcu et al., 2010). However, available data on the parasites infecting *B. mediterraneus* are scarce. As far as we are aware, there are no parasitological surveys of any of the twenty-three species of the genus *Bathypterois*, and only a few studies exist which have reported the presence of parasites in *B. mediterraneus* (Tortonese and Orsi, 1970), *Bathypterois ater* (Reimer, 1985) and *B. dubius* (Bray, 1995; Bray et al., 1999).

Parasite host specificity as well as their strict environmental requirements have been used to separate stocks and populations

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(Overstreet, 1997). Parasite fauna can provide information and serve as not only as natural markers for the identification of fish populations (Mackenzie, 1983; Williams et al., 1992) but also about other aspects of fish biology, such as dietary habits (Campbell et al., 1980; Palm et al., 1998) or environmental preferences (water temperature, salinity variations, etc.). Deep-sea environments are fragile ecosystems and an understanding of the structure and functioning of these ecosystems is necessary in order to preserve them. Knowledge of stock structure and degree of mixing among populations is crucial for the improvement of basic knowledge of the ecosystems' natural dynamics, especially in the deep-sea.

The aim of this study is to provide, for the first time, a parasitological survey of *B. mediterraneus* in the Western Mediterranean waters. The parasite component communities are analyzed from two different localities and three different depths, to evaluate the use of parasites as biological tags for stock discriminations in this species.

2. Materials and methods

In July 2010, 170 specimens of *B. mediterraneus* were captured from the continental slope in Western Mediterranean waters (Fig. 1), on board the research vessel "García del Cid" with an OTSB14 bottom trawl, a standard sampler for the study of deep-sea fauna (Merret and Marshall, 1981), in the framework of the Spanish oceanographic project "ANTROMARE" (CTM2009-08074-E). Samples were obtained from the continental slope of two different areas: the coast of Catalonia (off Barcelona) and the Balearic Islands (Table 1), natural abiotic parameters being similar in both localities, with minor variations than 0.05 in units of temperature (°C), salinity (pss) and oxygen (ml/l) (unpublished data of the ANTROMARE project).

Immediately after capture, all specimens were frozen at -20 °C on board. Once in the laboratory and after thawing, each animal was weighed and measured (standard length, to the nearest millimetre). Using a standardized protocol all organs were then taken apart, weighed and examined separately in a Petri dish with 0.9% saline under a stereomicroscope. Muscle tissues were also examined by transparency with transmitted light. All parasites were collected, fixed and preserved in 70% ethanol. For their identification, adult platyhelminths were stained with alum carmine, dehydrated in ethanol, cleared in dimethyl phthalate, and mounted in Canada balsam. In the case of nematodes, individuals were cleared with glycerol, whereas parasites of other phyla were examined on wet ethanol mounts.

To analyze the parasitic fauna of *B. mediterraneus*, data were grouped into two size categories of fish hosts: immature (size 1; standard length < 113 mm) and mature individuals (size 2; standard length ≥ 113 mm) (Carrassón and Matallanas, 2001). Data were also grouped according to locality (Barcelona and Balearic Islands) and depth of capture (three bathymetric strata: 1000–1400 m, 1400–2000 m, 2000–2200 m). Parasitological terms prevalence (P) and mean abundance (MA) were calculated according to Bush et al. (1997). Metazoan parasite diversity was calculated based on parasite abundance, using Brillouin's Index (PRIMER v6; Anderson et al. (2008)). Fish condition was assessed by the hepatosomatic index (HSI) calculated as liver weight (g)/ eviscerated body weight (g) $\times 100$ and the Fulton's condition factor (*K*) calculated as eviscerated body weight (g)/length $(\text{cm})^3 \times 100$. Mean abundances were log-transformed ($\log(x+1)$) prior to the General Linear Model (GLM) analyses. Differences in two fish condition indices (*K*, HSI), and in three infra-community descriptors (mean species richness, mean abundance and mean diversity) were tested to explore the effect of the "locality/depth" factors (five categories that will be henceforth referred to as Barcelona 1000–1400 m depth, Barcelona 1400–2000 m depth, Barcelona 2000–2200 m depth, Balearic Islands 1400–2000 m depth, Balearic Islands 2000–2200 m depth) and size (immature and mature) by GLM with post-hoc pairwise comparisons.

To obtain an overall perspective we used canonical correspondence analysis (CCA) to generate a biplot of the relationships

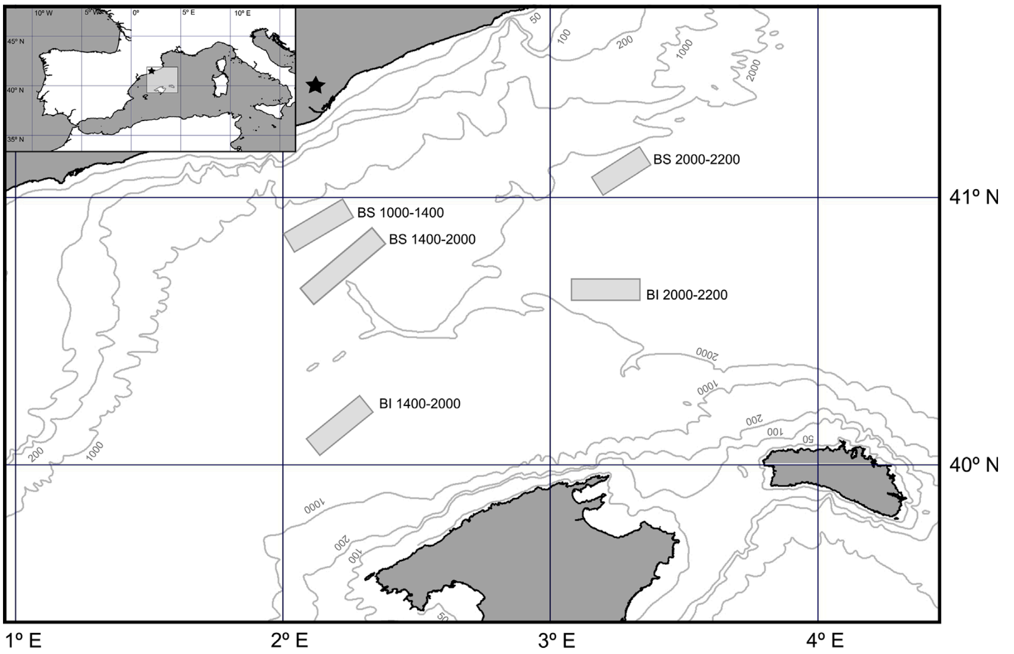

Fig. 1. Map of the study area between Barcelona and Balearic Islands. BS: Barcelona slope, BI: Balearic Islands slope.

Table 1
Sampling data for *Bathypterois mediterraneus* in the present study. Loc: locality, BS: Barcelona slope, BI: Balearic Islands, n=number of specimens sampled.

Station	Date	Depth (m) initial-final	Initial situation		Final situation		Loc	n
			Latitude (deg min, N)	Longitude (deg, min, E)	Latitude (deg min, N)	Longitude (deg, min, E)		
OTSB5	10/07/10	1275–1262	40°53.43	2°02.61	40°55.27	2°09.50	BS	9
OTSB6	10/07/10	1264–1352	40°54.75	2°07.43	40°52.94	2°00.56	BS	8
OTSB9	12/07/10	1764–1723	40°38.57	2°06.09	40°38.22	2°02.14	BS	26
OTSB10	12/07/10	1762–1812	40°31.74	2°01.28	40°29.87	2°06.08	BS	15
OTSB11	13/07/10	1629–1631	40°55.11	2°27.81	40°57.69	2°32.24	BS	5
OTSB13	16/07/10	2111–2003	40°41.20	3°07.66	40°°36.45	3°05.74	BI	19
OTSB14	16/07/10	2201–2186	40°38.17	3°24.71	40°37.01	3°30.92	BI	3
OTSB20	20/07/10	1631–1578	40°°07.86	2°10.51	40°09.2	2°13.90	BI	29
OTSB21	20/07/10	1486–1486	40°04.41	2°08.93	40°06.33	2°13.72	BI	20
OTSB24	22/07/10	2171–2223	41°05.46	3°22.20	41°03.17	3°11.29	BS	36

Table 2
Prevalence (p%) and mean abundance (MA ± standard deviation, SD) of the parasites of *B. mediterraneus* collected from the different locations and depths in Western Mediterranean waters. n: number of specimens studied. The same letters in the super-index of a row indicate lack of significant differences.

	Total		Barcelona slope						Balearic Islands			
	n=170		1000–1400 m n=17		1400–2000 m n=46		2000–2200 m n=36		1400–2000 m n=49		2000–2200 m n=22	
	p%	MA ± SD	p%	MA ± SD	p%	MA ± SD	p%	MA ± SD	p%	MA ± SD	p%	MA ± SD
Trematoda												
Steringophorus cf. *dorsolineatum*	47.06	0.79 ± 1.10	29.41	0.53 ± 1.01^{a}	43.48	0.59 ± 0.78^{a}	25.00	0.36 ± 0.68^{a}	71.43	1.37 ± 1.37^{b}	50.00	0.86 ± 1.21^{ab}
Cestoda												
Scolex pleuronectis	2.35	0.03 ± 0.20	5.88	0.06 ± 0.24^{a}	–	–	–	–	6.12	0.08 ± 0.34^{a}	–	–
Nematoda												
Hysterothylacium aduncum larva	34.71	1.03 ± 2.31	5.88	0.06 ± 0.24^{a}	21.74	0.26 ± 0.58^{a}	69.44	2.19 ± 2.56^{b}	16.33	0.41 ± 1.87^{a}	68.18	2.86 ± 3.80^{b}
Anisakis sp. larva Type II	1.18	0.01 ± 0.11	–	–	2.17	0.02 ± 0.15^{a}	–	–	–	–	4.55	0.05 ± 0.21^{a}
Crustacea												
Sarcotretes sp.	1.77	0.01 ± 0.11	–	–	–	–	–	–	6.12	0.04 ± 0.20	–	–

between the parasite abundance and site habitat variables (locality and depth) using individual fish as replicate samples (Ter Braak and Smilauer, 1998). To gain insight into the suggested differences shown by the CCA (see Section 3), GLM with post-hoc pairwise comparison and generalized linear model (GZM) (using negative binomial model) were performed to test the differences in abundance and prevalence, respectively, among the parasite populations for the five locality/depth categories.

3. Results

A total of five parasitic species were found to infect *B. mediterraneus* in the area studied: the fellodistomid digeneans *Steringophorus* cf. *dorsolineatum* (Olsson, 1868) (n=135); the tetraphyllidean larval cestodes reported under the collective name *Scolex pleuronectis* Müller, 1788 (n=4); two larval nematodes, the raphidascarid *Hysterothylacium aduncum* (Rudolphi, 1802) (n=175) and the anisakid *Anisakis* sp. larva 3 type II (n=2); and the pennellid copepod *Sarcotretes* sp. (n=2) (Table 2).

The most prevalent and abundant species was the digenean *S.* cf. *dorsolineatum* (overall p=47.06%, MA=0.79) (Table 2) which was found as adult, mostly in the intestine, although it was occasionally recovered from the stomach and the pyloric caeca. The third larval stage (L3) of the raphidascarid nematode *H. aduncum* was also frequently found (overall p=34.71%, MA=1.03) encysted mainly in the mesentery of the intestine and occasionally in that of the stomach. The other nematode species, the anisakid *Anisakis* sp. larva 3 type II (*sensu* Berland, 1961) was found encysted in the abdominal cavity and the mesenteries, with low overall prevalence (1.18%) and mean abundance (0.01) (Table 2). The four tetraphyllidean plerocercoids were found only in the intestine. Finally, the two female specimens of the copepod *Sarcotretes* sp. were found attached to the head or the dorsal part of two *B. mediterraneus*. These specimens had about 30 mm long, with medium sized proboscis, and neck slightly shorter than trunk.

There were no significant differences for either parasitological data or condition indices between mature and immature individuals (GLM, $p > 0.05$ in all cases). Interactions between host size and locality/depth for all parasitological (mean species richness, total mean abundance, mean diversity and MA of each taxon) and condition indices (K, HSI) were not detected (GLM, $p > 0.05$ in all cases). GLM with post-hoc pairwise comparison showed significant differences between locality/depth in mean species richness (GLM, $F_{4,165}=5.26$, $p < 0.001$), mean abundance (GLM, $F_{4,165}=9.66$, $p < 0.001$), K ($F_{4,164}=7.29$, $p < 0.0001$) and HSI ($F_{4,155}=14.65$, $p < 0.0001$). Mean species richness was higher in the Balearic Island samples at a depth of 2000–2200 m, decreasing in the Barcelona site and towards shallower waters; whereas for mean abundance, the maximum values were shown in both localities at a depth of 2000–2200 m (Table 3). Diversity was low in all cases and no significant trends were found (Table 3).

The condition factor (K) was significantly higher in the samples from off Barcelona at 1000–2000 m compared with those from the Balearic Islands site at 2000–2200 m, and decreased as the depths increased, whereas the HSI that also decreased with depth was significantly lower off Barcelona than off Balearic Islands (Table 3).

We grouped the five parasite species by means of ordination (CCA) (Fig. 2). The first axis of the CCA explained 86.9% of the variance in the

Table 3
Mean and standard deviation of parasitological (mean species richness, mean abundance, mean diversity) and condition (condition factor: *K*, hepatosomatic: HSI) indices, for each locality/depth and for the total samples available. The same letters in the super-index of a row indicate lack of significant differences.

		Barcelona slope			Balearic Islands	
	Total $n=170$ $\bar{x} \pm$ sd	1000–1400 m $n=17$ $\bar{x} \pm$ sd	1400–2000 m $n=46$ $\bar{x} \pm$ sd	2000–2200 m $n=36$ $\bar{x} \pm$ sd	1400–2000 m $n=49$ $\bar{x} \pm$ sd	2000–2200 m $n=22$ $\bar{x} \pm$ sd
Mean species richness	0.87 ± 0.69	0.41 ± 0.62^{a}	0.67 ± 0.70^{ab}	0.94 ± 0.58^{bc}	1.00 ± 0.71^{bc}	1.23 ± 0.612^{c}
Mean abundance	1.90 ± 2.5	0.64 ± 1.17^{a}	0.87 ± 0.96^{a}	2.56 ± 2.59^{bc}	1.9 ± 2.33^{b}	3.77 ± 4.02^{c}
Mean diversity	0.09 ± 0.16	0.06 ± 0.14^{a}	0.08 ± 0.15^{a}	0.07 ± 0.16^{a}	0.10 ± 0.16^{a}	0.12 ± 0.2^{a}
Condition factor (*K*)	0.65 ± 0.09	0.73 ± 0.07^{a}	0.67 ± 0.08^{b}	0.63 ± 0.09^{bc}	0.64 ± 0.09^{bc}	0.60 ± 0.08^{c}
Hepatosomatic index (HIS)	1.56 ± 0.81	1.29 ± 0.37^{ab}	0.62 ± 0.46^{a}	0.74 ± 0.24^{a}	2.86 ± 2.51^{c}	1.87 ± 1.84^{b}

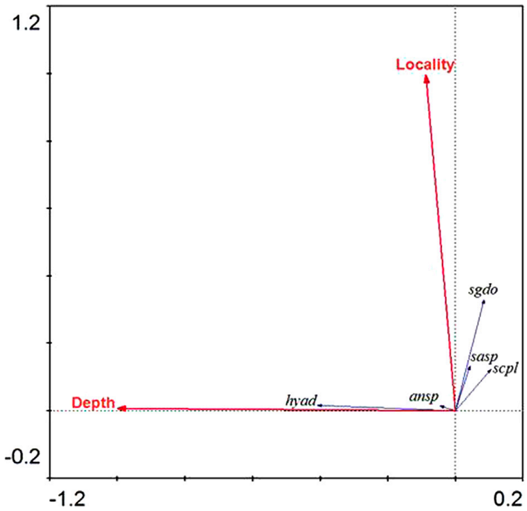

Fig. 2. Representation of parasite abundance and localities/depths affecting parasite community structure on the first two axes of the canonical correspondence analysis (CCA). Abbreviations of parasites: ansp: *Anisakis* sp. type II, hyad: *Hysterothylacium aduncum*, sasp: *Sarcotretes* sp., scpl: *Scolex pleuronectis*, sgdo: *Steringophorus* cf. *dorsolineatum*.

parasite-environment relationship. The first axis was mostly related to depth, with negative values associated with the greatest depths due mainly to the abundance of *H. aduncum*, but also of *Anisakis* sp. type II. *H. aduncum*, was the only parasite species to show a significant difference in abundance between 2000 and 2200 m and the remaining depths in both localities (GLM, $F_{4,165}=10.66$, $p<0.0001$) (Table 2). Significant differences were also found in the prevalence of *H. aduncum* among depths (GZM, $\chi^2_2=35.37$, $p<0.001$). The second axis of the CCA was related to locality, due mainly to the abundance of *S.* cf. *dorsolineatum* in Balearic Islands fish. Significant differences in the abundance and prevalence of *S.* cf. *dorsolineatum* were observed between localities (GLM, $F_{4,165}=6.00$, $p<0.0001$; GZM, $\chi^2_2=11.30$, $p<0.001$; respectively), both being higher on the Balearic Islands slope (Table 2). None of the other parasite species reported in *B. mediterraneus* showed significant differences either in abundance or in prevalence between localities or depths (GLM for MA, GZM for *p*, $p>$ 0.05 in all cases).

4. Discussion

This is the first comprehensive parasitological study of *B. mediterraneus*, a principal component of the fish community in the deep Western Mediterranean and the first on a species of the genus *Bathypterois*. The only previous report on parasites of *B. mediterraneus* is the finding of two copepod individuals (incorrectly identified as Lernaeenidae gen. sp.) which possibly belong to the same genus of the pennellid reported herein according to measurements and photographs (Tortonese and Orsi, 1970). The fauna of parasites infecting *B. mediterraneus* is relatively poor, consisting of four helminth species and a single crustacean species. The digenean *S.* cf. *dorsolineatum* and the larval nematode *H. aduncum* are apparently predominant in this area, while the other three species (*S. pleuronectis*, *Anisakis* sp. Type II, and *Sarcotretes* sp.) are rare (prevalence < 3% in the entire dataset). All these parasite species represent new host records. *Sarcotretes* sp. appears to be a new species according to the different body length, proportions and cephalic traits (see species key in Uyeno et al. (2012)). The specimens of *S.* cf. *dorsolineatum* in *B. mediterraneus* are morphologically similar to *S. dorsolineatum*, a parasite of *B. dubius* in the Atlantic Ocean, although molecular studies would be required in this case to confirm the species identity. The other three parasites are generalists, reported in many fish species worldwide (Gibson et al., 2005). This poor parasite fauna, with several generalist species, differs from the parasite faunas in other deep-sea demersal fish, such as e.g. macrourids, which show greater parasite richness together with more host-specific parasite species (Walter et al., 2002; Klimpel et al., 2008; Palm and Klimpel, 2008; Kellermanns et al., 2009). Probably, the diet of *B. mediterraneus* based on benthopelagic planktonic calanoid copepods (Carrassón and Matallanas, 2001) contributed to the observation of patterns in parasite richness and composition more characteristic for meso- and bathy-pelagic fish hosts (Klimpel et al., 2006, 2010). Our results do not agree with the suggestion by Klimpel et al. (2006) indicating that higher biomass in the deep-sea benthic environment increases the availability of potential intermediate hosts, resulting in increased parasite diversity in demersal fish. Notwithstanding, the parasitic abundance in *B. mediterraneus* increased with depth. The significant lower values of fish condition indexes (*K* and HSI) found at depth of 2000–2200 m could be related to the higher rates of parasite infection observed (Lloret et al., 2012). *Steringophorus* cf. *dorsolineatum* was the most predominant taxon in *B. mediterraneus* in the NW Mediterranean Sea. The digenean life-cycle is complex, including invertebrates and/or fish as intermediate hosts and, in the case of deep-sea parasites, mostly teleosts as final host (Bray, 2002). Bray et al. (1999) indicated that *Steringophorus* spp. are probably transmitted to the final host (*Bathypterois* spp. among other fish) by mean of a wide range of second intermediate hosts. It is a known fact that *Steringophorus furciger* develops within the bivalve *Nuculana minuta* (Køie, 1979) the commonest mollusc in the deep-sea (Gage and Tyler, 1991), which are not part of the diet of *B. mediterraneus. Steringophorus dorsolineatum* only parasitises species of *Bathypterois* and, therefore, certain invertebrates, other than mollusks, part of the diet of these fish and living in the deep waters, must act as intermediate host. The significantly higher abundance of *S.* cf. *dorsolineatum* on the slope of the Balearic Islands compared to the abundance found on the continental slope could be due to biological (e.g. availability of intermediate hosts) or environmental differences between both areas. Mysids are the only taxon with higher presence in the diet of *B. mediterraneus* from the Balearic Islands slope found at 1400–2000 m depth, compared to the fish off Barcelona at the same depth range (Cartes, pers. comm.), and so therefore they may act in this case as intermediate hosts for *S.* cf. *dorsolineatum*. Since natural abiotic parameters have been shown to be very similar in both localities (unpublished data of the ANTROMARE project), environmental factors such as pollution, diversity, etc. require further investigations. *H. aduncum* was frequently found and showed increasing prevalence and abundance with depth. On the continental shelf and the deep slope certain planktonic and benthic invertebrates (calanoid and harpacticoid copepods, amphipods, isopods, mysids and shrimps) are described as first intermediate hosts of these nematodes in Atlantic waters (Køie, 1993; Marcogliese, 1996; Jackson et al., 1997; Klimpel and Rückert, 2005). However, the higher abundance of *H. aduncum* at 2000–2200 m depth does not correspond to a dietary preference for calanoid copepods, since the ingestion of these copepods seems to decrease with depth (its dietary importance being 15% lower at 2000–2200 m than at 1000–1400 m). The only prey taxon that increases significantly with depth in the diet of *B. mediterraneus* are cumaceans (increasing their index of relative dietary importance (IRI) from15.8 at 1000–1400 m to 1214.7 at the maximum depth of 2200 m) (Carrassón and Matallanas, 2001). These data suggest that cumaceans may act as paratenic hosts for *H. aduncum*. This species was found in more than eight fish species from deep Mediterranean waters (unpublished data of the ANTROMARE project,and is also the most prevalent and least host-specific nematode species in the North Atlantic (Køie, 1993). *H. aduncum* can be found from the neritic zone to deep-sea and are able to infect teleosts from different genus (Køie, 1993; Marcogliese, 1996; Balbuena et al., 1998; Klimpel and Rückert, 2005; Kleinertz et al., 2012) than may act as paratenic or final hosts depending on the size of the L3 in the first paratenic host (González, 1998). When in the first host, the size of the L3 is between 2 and 3 mm long (as calanoid copepods and cumaceans) they remain as third stage larvae in the intestine of the fish (Køie, 1993). In this sense, as *H. aduncum* specimens have always been found at the L3 stage in *B. mediterraneus*, this fish species acts as a paratenic host which allows the proper growth of the larvae prior to molting to the next stage.

The unidentified tetraphyllidean plerocercoids (*S. pleuronectis*) are very commonly found parasitizing all kinds of predatory oceanic and deep-sea fish (Klimpel et al., 2001). *B. mediterraneus*, where *S. pleuronectis* is rare, joins the list of second intermediate hosts. These plerocercoids belong to a complex of tetraphyllidean species, morphologically undistinguishable at the larval stage, for which fishes act as second intermediate hosts, and mature within the stomach or spiral valve of elasmobranchs (Khalil et al., 1994). In the case of *S. pleuronectis* in *B. mediterraneus* the adult could be found within bathyal sharks, as *Centroscymnus coelolepis*, which predate *B. mediterraneus* within these Mediterranean waters (Carrassón et al., 1992).

Regional environmental conditions at sampled localities are frequently discussed in order to explain differences in parasite abundance for the same host species (MacKenzie and Abaunza, 1998; Kleinertz et al., 2012). As previously commented, no significant differences were observed in this study, either in temperature, salinity, oxygen concentration or turbidity between the different stations sampled. Other factors must therefore be responsible for the differences in the infestation patterns observed in *B. mediterraneus* in different depth strata and localities. Probably, the availability of different intermediate and/or paratenic hosts, as already mentioned, and depth distributions contribute to these differences.

Parasites are frequently used as biological tags for stock discriminations of marine fish (MacKenzie and Abaunza, 1998; Moore et al., 2003; Oliva and González, 2004). They are especially appropriate for studies of deep-sea species for which artificial tags can either be used with difficulty or not at all (MacKenzie and Abaunza, 1998). In this sense, significant differences in population descriptors and in infracommunity indices between both localities for *S.* cf. *dorsolineatum* and among depths in *H. aduncum* may suggest a potential role for discriminating discrete stocks of *B. mediterraneus. Steringophorus* cf. *dorsolineatum* could be used to differentiate animals between the continental slope and the insular slope, whereas *H. aduncum* could characterize the maximum depth of 2000–2220 m regardless of the locality. Moreover, parasite fauna can provide important taxonomic information in this case. Parasite copepods found in *B. mediterraneus* are different from those previously reported in the closest fish species, *B. dubius,* considered as a possible synonym (Queró and Ribes, 1999). The apparently specific parasite *Sarcotretes* sp. show diagnostics differences with the *S. eristaliformis* cited in *B. dubius* (Yamaguti, 1963). Although the sampling effort conducted for the studies of parasites in *B. dubius* were probably not the same as that performed in this study, the existence of two different species of *Sarcotretes* in both fish must be considered in order to separate both host species or, at least, to determine a degree of isolation between populations.

5. Conclusion

B. mediterraneus from Western Mediterranean waters shows a low richness of parasites, with five parasitic species, all new host records. This poor parasitic fauna with several generalist species is more common in meso- and bathy-pelagic fish hosts than in demersal fish. The significant differences found in population descriptors and in infracommunity indices shown by *S.* cf. *dorsolineatum* from both localities and *H. aduncum* among depths could prove the potential of these two parasitic taxa for discriminating discrete stocks of *B. mediterraneus*, and could be used as biological tags for geographical and bathymetric discrimination in Western Mediterranean waters. In addition to stock discrimination, the parasite fauna can provide important taxonomic information for the host genus *Bathypterois*. The different species of *Sarcotretes* found in *B. mediterraneus*, with respect to those described in *B. dubius*, would suggest the separation of both host species, or at least, determine a degree of isolation between populations.

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HELMINTHS FROM THE D
STRIPED DOLPHIN (*STEN*
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CHAPTER X:
CONCLUSIONS

In the present study, the liver and pancreas (n=103), the stomach (n=47) and the intestine (n=128) of striped dolphins, *Stenella coeruleoalba*, from western Mediterranean waters were analyzed for helminth parasites. The general goal was to report, for the first time, on the helminth fauna of this cetacean based on a suitable host sample size, to investigate determinants of helminth specificity and community structure, and to analyze and interpret potential long-term changes in helminth faunas. Additionally, 1012 individuals of eight lantern fish species (*Ceratoscopelus maderensis*, *Lampanyctus crocodilus*, *Notoscopelus elongatus*, *Benthosema glaciale*, *Myctophum punctatum*, *Lobianchia dofleini*, *Diaphus holti* and *Hygophum benoiti*), 170 individuals of the Mediterranean spiderfish, *Bathypterois mediterraneus*, and 792 individuals of two cephalopod species (*Alloteuthis media* and *Sepietta oweniana*), from the same area, were also analyzed for helminths. The primary goal was to find and describe infective stages of the helminths infecting striped dolphins and other oceanic cetaceans, as well as to explore the role of these fish and cephalopods in the life-cycle of cetacean parasites. The following conclusions are drawn from this study:

9.1. The helminth community of the digestive tract of the striped dolphin in western Mediterranean waters was composed of (i) two brachycladiid digeneans infecting hepato-pancreatic ducts, i.e., *Brachycladium atlanticum* and *Oschmarinella rochebruni*; (ii) one heterophyid digenean, *Pholeter gastrophilus*, and at least one anisakid nematode, *Anisakis pegreffii* in the stomach and (iii) three tetrabothriid cestodes, i.e., *Tetrabothrius forsteri*, *Trigonocotyle globicephalae* and *Strobilocephalus triangularis*, and immature individuals of the polymorphid acanthocephalan *Bolbosoma vasculosum* in the intestine. All taxa reported are specific to cetaceans, an outcome that was predicted in advance based on the operation, in the oceanic realm, of contact or compatibility filters derived from the phylogenetic distance between cetaceans and other sympatric vertebrates.

9.2. Intestinal helminth infracommunities were depauperate and largely unpredictable. Both traits are expected in cetaceans and other large oceanic vertebrates because infective stages of trophically-transmitted helminths are highly “diluted” in the oceanic realm and, therefore, the probability of transmission to the definitive host is low. Low recruitment rates have been related to the lack of structure in the intestinal helminth communities of other oceanic cetaceans.

9.3. There were not significant effects of host body length, age or sex on the abundance of any of the helminth species, or on infracommunity descriptors (total abundance, species richness and Brillouin’s diversity index), of intestinal helminth communities when the analysis was based on a sample composed mostly of adult individuals (n= 53). This result would reinforce the idea that the probability of transmission to the definitive host is low and, therefore, any potential host effect is weak. However, a re-analysis based on a larger sample size (n= 128) which included more juvenile individuals, revealed significant effects of host body length (or age) on community structure. In particular, the abundance of tetrabothriid cestodes tended to decrease with host body length. There are significant age-related changes in the diet of western Mediterranean striped dolphins that could account for this pattern. The influence of ontogenetic effects on infection levels of tetrabothriids has been reported in other species of the genus *Stenella*.

9.4. *Oschmarinella rochebruni* were found in striped dolphins with high prevalence (61.2%). However, this parasite was not found in the liver and pancrease of sympatric Risso's dolphins, bottlenose dolphins, common dolphins and long-finned pilot whales. Also, transient immature worms were not found along the intestine. These data suggests that possibly the contact filter (i.e., dietary habits) define specificity of *O. rochebruni* in striped dolphins.

9.5. *Brachycladium atlanticum* was found in striped dolphins with moderate prevalence (39.8%), but was also found in 1 out of 8 common dolphins analyzed (prevalence: 12.5%). In the striped dolphin, host individual exerted significant effects upon the morphology and fecundity of *B. atlanticum*, which, apparently, were not related to crowding effects. This finding call for a careful re-examination of the use of morphometric traits in taxonomic studies of digeneans from cetaceans. Individuals of *B. atlanticum* from the common dolphin were dwarf compared to those collected from the striped dolphin, and were also less fecund as an associated allometric effect. This suggests that the compatibility filter could also shape the specificity of *B. atlanticum* to striped dolphins. Nevertheless, additional morphological data from worms collected from other cetaceans are necessary to confirm this hypothesis.

9.6. The stomach digenean *Pholeter gastrophilus* occurred with high prevalence (over 85%), especially in the fundic chamber, whereas nematodes identified as *Anisakis pegreffii* occurred in less of 15% of dolphins, mainly in the pyloric stomach. Infection levels of *A. pegreffii* in striped dolphins from the western Mediterranean are surprisingly low compared with species of *Anisakis* in cetaceans from other regions. Apparently, this is not a species-specific effect because low infection levels of *Anisakis* have also been reported in other cetaceans and fish from the western Mediterranean, suggesting that there are low population densities of these nematodes in the region.

9.7. Long-term (1990-2010) trend analysis of the abundance and composition of the intestinal helminth community of the striped dolphin indicated that only the abundance of *B. atlanticum* changed significantly over the years. In particular, infection levels were highest in 1990, and the parasite seldom appeared in subsequent years. This suggests that 1990 was an exceptional year or that the subsequent decrease of infection levels reflect a change in host's diet upon prey species that does not harbor the acanthocephalan. The second hypothesis is consistent with data indicating a significant dietary shift of striped dolphins in the studied period. However, we failed to find a significant relationship between host diet and helminth community structure. The question remains whether we are unable to detect clear long-term changes because only heavily parasitized animals were analyzed (i.e., a non-random sample) or because there were no actual changes to be detected. This is the first time a long-term analysis has been carried out for parasites of cetaceans.

9.8. A total of 1012 individuals of eight myctophid species (*Ceratoscopelus maderensis*, *Lampanyctus crocodilus*, *Notoscopelus elongatus*, *Benthosema glaciale*, *Myctophum punctatum*, *Lobianchia dofleini*, *Diaphus holti* and *Hygophum benoiti*) were analyzed for helminths. The nematodes *Anisakis pegreffii* and *Anisakis physeteris* were only detected in *N. elongatus* and *C. maderensis* with low prevalence, and prevalence in *N. elongatus* was significantly higher than that from the other three myctophid species with n>50 individuals. A single individual of *Hysterothylacium* sp. was found in *N. elongatus* and Raphidascarididae gen. spp. in *N. elongatus* and *L. crocodilus*. Two unidentified cestode plerocercoids were collected from *N. elongatus*. This study provides the first parasitological study of myctophids in the Mediterranean. Only *Anisakis* spp. are parasites infecting cetaceans.

9.9. The finding of *A. pegreffii* in *N. elongatus* would support the hypothesis that *A. pegreffii* circulates through mesopelagic food webs using myctophids as paratenic hosts. Myctophids, including *N. elongatus* and *C. maderensis*, are a sizeable component in the diet of striped dolphins in the study area and *A. pegreffii* has been isolated from striped dolphins. Myctophids could also play a significant role in the life-cycle of *A. physeteris*, but it is unclear to what extent they are directly consumed by the main definitive host, the sperm whale, *Physeter macrocephalus*, or are consumed by cephalopods that are, in turn, the main prey of sperm whales.

9.10. In spite of the large sample of hosts analyzed, no helminth parasites were found in the cephalopods *Alloteuthis media* and *Sepietta oweniana*.

9.11. A total of five parasitic species were found infecting the Mediterranean spiderfish *Bathypterois mediterraneus* in the area studied: the digenean *Steringophorus* cf. *dorsolineatum*; tetraphyllidean larval cestodes reported under the collective name *Scolex pleuronectis*; two larval nematodes, the raphidascarid *Hysterothylacium aduncum* and the anisakid *Anisakis* sp. larva 3 type II; and the pennellid copepod *Sarcotretes* sp. The finding of all helminth species represents new host records. Only, *Anisakis* sp. is a parasite infecting cetaceans.

9.12. *Hysterothylacium aduncum* showed significant differences in abundance between depths of 2000-2200m with 1000-1400m and 1400-2000m, irrespective of locality, whereas *Steringophorus cf. dorsolineatum* showed significant differences between the two localities at all depths except for 2000-2200m. We suggest the possible usefulness of these two parasites as geographical indicators for discriminating discrete stocks of *B. mediterraneus* in western Mediterranean waters.

9.13. From a potential pool of at least 21 taxa reported in cetaceans in the region, only infective stages of *Anisakis* species were detected in mesopelagic and bathydemersal fish. The failure to find additional taxa is hardly attributable to detection bias. It is possible that some larvae of parasites infecting cetaceans could have been missed in the sample if their infection levels in mesopelagic and bathydemersal fish, and cephalopods, are very low in the study area. Alternatively, the selected species could not harbor further infective stages in helminths infecting cetaceans. Elucidating the life-cycle of helminths from cetaceans thus remains, for the most part, elusive. This study stresses the need for more parasitological research on cetacean prey.

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APPENDIX 1

DIGESTIVE TRACT OF THE
NELLA COERULEOALBA)
TERRANEAN: INFECTION
LONG-TERM SCALE

Appendix 1. Biological data of striped dolphins, *Stenella coeruleoalba*, from western Mediterranean waters stranded between 1990 and 2010 and analyzed for intestinal parasites (Chapters 4 and 7).

Collection date	Stranding locality	Total length (cm)	Sex
11/07/1990	Benicasim (V)	194	Female
15/08/1990	Cullera (V)	200	Male
16/08/1990	Foios (V)	185	Female
18/08/1990	El Perelló (V)	180	Male
21/08/1990	Santa Pola (V)	213	Male
23/08/1990	La Manga (M)	192	Male
24/08/1990	El Saler (V)	201	Male
24/08/1990	Pinedo (V)	184	Male
29/08/1990	Santa Pola (V)	182	Male
29/08/1990	Cullera (V)	200	Female
29/08/1990	La Manga (M)	204	Male
30/08/1990	Altea (V)	195	Female
01/09/1990	Almazora (V)	213	Male
01/09/1990	Alboraya (V)	194	Female
01/09/1990	Santa Pola (V)	206	Female
01/09/1990	Peñíscola (V)	194	Female
02/09/1990	Guardamar del Segura (V)	173	Female
02/09/1990	Alboraya (V)	195	Female
02/09/1990	La Manga (M)	201	Male
08/09/1990	La Mata (V)	192	Female
08/09/1990	Vergel (V)	195	Male
09/09/1990	Pinedo (V)	192	Female
09/09/1990	Cullera (V)	210	Female
10/09/1990	Puzol (V)	202	Male
10/09/1990	San Juan (V)	183	Female
11/09/1990	El Saler (V)	204	Male
14/09/1990	Valencia (V)	193	Male
18/09/1990	Gandía (V)	203	Male
18/09/1990	Vergel (V)	189	Female
19/09/1990	Alicante (V)	198	Female
22/09/1990	El Portús (M)	206	Male
21/09/1990	Nules (V)	207	Male
22/09/1990	Alcocéber (V)	184	Male
23/09/1990	Oropesa (V)	190	Female
26/09/1990	Delta del Ebro (C)	163	Female
28/09/1990	Masnou (C)	208	Male
29/09/1990	Palamós (C)	200	Male
29/09/1990	Guardamar del Segura (V)	179	Male

30/09/1990	Palamós (C)	200	Female
30/09/1990	Benicarló (V)	191	Female
01/10/1990	Grao de Castellón (V)	204	Male
01/10/1990	Amposta (C)	190	Male
01/10/1990	Amposta (C)	210	Male
01/10/1990	Sant Feliu (C)	204	Female
01/10/1990	Palamós (C)	187	Female
16/10/1990	Mataró (C)	193	Female
17/10/1990	L'Estartit (C)	210	Male
22/10/1990	Selva (C)	211	Female
22/10/1990	Cadaqués (C)	172	Female
03/12/1990	Guardamar del Segura (V)	200	Male
07/12/1990	Benisa (V)	187	Female
29/12/1990	Benicasim (V)	194	Male
20/03/1991	Torrevieja (V)	191	Female
21/03/1991	Daimuz (V)	204	Male
03/04/1991	Oliva (V)	193	Female
20/02/1992	Sueca (V)	199	Female
22/03/1992	Pinedo (V)	196	Male
04/05/1992	Cullera (V)	197	Female
15/06/1992	Vergel (V)	195	Male
11/08/1992	Puerto de Sagunto (V)	208	Male
15/02/1993	Oliva (V)	179	Female
26/11/1993	Almazora (V)	210	Male
10/05/1994	San Juan (V)	161	Male
08/07/1994	Cullera (V)	206	Female
27/02/1995	Vergel (V)	192	Female
01/03/1995	Tavernes de la Valldigna (V)	162	Male
07/12/1995	Vergel (V)	183	Female
28/06/1996	Cullera (V)	157	Male
07/10/1996	El Campello (V)	173	Male
30/01/1997	Oliva (V)	198	Female
08/03/1997	Denia (V)	188	Female
04/04/1997	Grao de Castellón (V)	170	Female
06/02/1998	Benicasim (V)	132	Male
05/10/1998	Torreblanca (V)	211	Female
15/10/1998	Oropesa (V)	158	Male
22/11/1998	Gandía (V)	184	Male
01/09/1999	Nules (V)	155	Male
07/1999	Murcia (M)	203	-
08/02/2000	Gandía (V)	206	Female
27/03/2000	Cullera (V)	106	Female
06/04/2001	Grao de Castellón (V)	212	Male

15/12/2001	Torrevieja (V)	154	Female
17/02/2002	San Juan (V)	150	Male
18/07/2002	Valencia (V)	205	Female
23/02/2003	Benidorm (V)	179	Female
07/06/2003	Oropesa(V)	186	Female
08/06/2003	Pilar de la Horadada (V)	164	Male
09/06/2003	El Saler (V)	156	Male
07/12/2003	El Saler (V)	153	Female
30/01/2004	Jávea (V)	155	Male
16/12/2004	Jávea (V)	195	Male
02/08/2005	Denia (V)	190	Male
18/10/2005	Torreblanca (V)	161	Female
06/11/2006	Tavernes de la Valldigna (V)	208	Female
12/06/2007	Pilar de la Horadada (V)	203	Male
09/07/2007	Oliva (V)	192	Female
12/08/2007	Elche (V)	187	Male
19/08/2007	Port Saplaya (V)	155	Female
20/08/2007	Oliva (V)	183	Male
24/08/2007	Denia (V)	147	Male
25/08/2007	Alcocébre (V)	203	Male
30/08/2007	Piles (V)	165	Male
09/09/2007	Oliva(V)	129	Male
13/09/2007	Nules (V)	194	Male
21/09/2007	El Saler (V)	156	Female
28/10/2007	Jávea (V)	200	Male
24/11/2007	Denia (V)	198	Female
28/11/2007	Oliva (V)	145	Male
16/02/2008	Xeraco (V)	123	Male
19/02/2008	El Saler (V)	175	Male
21/02/2008	El Saler (V)	138	Female
27/02/2008	Peñíscola (V)	208	Male
21/08/2008	Calpe (V)	166	Male
26/08/2008	Benidorm (V)	161	Female
31/12/2008	Calpe (V)	195	Male
24/02/2009	Denia (V)	192	Male
26/02/2009	Guardamar del Segura (V)	195	Female
13/03/2009	Santa Pola (V)	158	Male
14/03/2009	Moncófar (V)	180	Female
15/03/2009	Alboraya (V)	199	Female
21/03/2009	Elche (V)	160	Female
12/05/2009	Piles (V)	174	Female
12/07/2009	Cullera (V)	153	Male
30/08/2009	Guardamar del Segura (V)	198	Male

14/12/2009	Campello (V)	192	Female
26/01/2010	Santa Pola (V)	220	Male
27/01/2010	Cullera (V)	195	Female
16/02/2010	Sueca (V)	198	Female

HELMINTHS FROM THE D STRIPED DOLPHIN (*STEN* IN THE WESTERN MEDIT PATTERNS WITHIN A

APPENDIX 2

DIGESTIVE TRACT OF THE
NELLA COERULEOALBA)
ERRANEAN: INFECTION
LONG-TERM SCALE

Appendix 2. Biological data of stranded cetaceans from western Mediterranean waters analyzed for hepato-pancreatic parasites. M: Murcia; V: Valencian Community; C: Catalonia (Chapter 5).

Species	**Collection date**	**Stranding locality**	**Total length (cm)**	**Sex**
Stenella coeruleoalba	15/08/1990	Blanes (C)	198	Female
Stenella coeruleoalba	23/08/1990	La Manga (M)	192	Male
Stenella coeruleoalba	28/08/1990	Perellonet (V)	160	Male
Stenella coeruleoalba	29/08/1990	La Manga (M)	204	Male
Stenella coeruleoalba	29/08/1990	Cullera (V)	200	Male
Stenella coeruleoalba	29/08/1900	Santa Pola (V)	182	Male
Stenella coeruleoalba	30/08/1990	Altea (V)	195	Female
Stenella coeruleoalba	30/08/1990	Perellonet (V)	202	Male
Stenella coeruleoalba	01/09/1990	Almazora (V)	213	Male
Stenella coeruleoalba	01/09/1990	Peñíscola (V)	194	Female
Stenella coeruleoalba	01/09/1990	Santa Pola (V)	206	Female
Stenella coeruleoalba	02/09/1990	La Manga (M)	201	Male
Stenella coeruleoalba	02/09/1990	Alboraya (V)	195	Female
Stenella coeruleoalba	02/09/1990	Guardamar del Segura (V)	173	Female
Stenella coeruleoalba	07/09/1990	Piles (V)	158	Female
Stenella coeruleoalba	07/09/1990	Roda de Bará (C)	177	Male
Stenella coeruleoalba	08/09/1990	La Mata (V)	192	Female
Stenella coeruleoalba	08/09/1990	Vergel (V)	195	Male
Stenella coeruleoalba	09/09/1990	Pinedo (V)	192	Female
Stenella coeruleoalba	09/09/1990	Cullera (V)	210	Female
Stenella coeruleoalba	09/09/1990	Torrevieja (V)	201	Male

Stenella coeruleoalba	10/09/1990	Puzol (V)	202	Male
Stenella coeruleoalba	10/09/1990	San Juan (V)	183	Female
Stenella coeruleoalba	11/09/1990	El Saler (V)	204	Male
Stenella coeruleoalba	11/09/1990	Port de la Selva (C)	193	Male
Stenella coeruleoalba	13/09/1990	Vilaseca (C)	194	Male
Stenella coeruleoalba	14/09/1990	Malvarrosa (V)	193	Male
Stenella coeruleoalba	18/09/1990	Gandía (V)	203	Male
Stenella coeruleoalba	18/09/1990	Vergel (V)	189	Female
Stenella coeruleoalba	19/09/1990	Alicante (V)	198	Female
Stenella coeruleoalba	21/09/1990	Nules (V)	207	Male
Stenella coeruleoalba	22/09/1990	El Portús (M)	206	Male
Stenella coeruleoalba	22/09/1990	Alcocéber (V)	184	Male
Stenella coeruleoalba	23/09/1990	Oropesa (V)	190	Female
Stenella coeruleoalba	26/09/1990	Delta del Ebro (C)	163	Female
Stenella coeruleoalba	28/09/1990	Masnou (C)	208	Male
Stenella coeruleoalba	29/09/1990	Guardamar del Segura (V)	179	Male
Stenella coeruleoalba	29/09/1990	Palamós (C)	200	Male
Stenella coeruleoalba	30/09/1990	Benicarló (V)	191	Female
Stenella coeruleoalba	30/09/1990	Sant Pere Pescador (C)	198	Female
Stenella coeruleoalba	30/09/1990	Palamós (C)	200	Female
Stenella coeruleoalba	01/10/1990	Grao (V)	204	Male
Stenella coeruleoalba	01/10/1990	Amposta (C)	190	Male
Stenella coeruleoalba	01/10/1990	Amposta (C)	210	Male
Stenella coeruleoalba	01/10/1990	Sant Feliu (C)	204	Female
Stenella coeruleoalba	01/10/1990	Palamós (C)	187	Female

Stenella coeruleoalba	01/10/1990	Castell Ampu (C)	166	Female
Stenella coeruleoalba	04/10/1990	Castelldefels (C)	206	Male
Stenella coeruleoalba	07/10/1990	Pilar de la Horadada (V)	206	Male
Stenella coeruleoalba	15/10/1990	Guardamar (V)	202	Female
Stenella coeruleoalba	15/10/1990	L’Ampolla (C)	200	Female
Stenella coeruleoalba	16/10/1990	Mataró (C)	193	Female
Stenella coeruleoalba	17/10/1990	Moraira (V)	199	Female
Stenella coeruleoalba	17/10/1990	L’Estartit (C)	210	Male
Stenella coeruleoalba	17/10/1990	L’Estartit (C)	210	Male
Stenella coeruleoalba	22/10/1990	Selva (C)	211	Male
Stenella coeruleoalba	22/10/1990	Cadaqués (C)	172	Female
Stenella coeruleoalba	03/12/1990	Guardamar del Segura (V)	200	Male
Stenella coeruleoalba	07/12/1990	Benisa (V)	187	Female
Stenella coeruleoalba	19/12/1990	Masnou (C)	177	Male
Stenella coeruleoalba	29/12/1990	Benicasim (V)	194	Male
Stenella coeruleoalba	02/03/1991	Cullera (V)	170	Male
Stenella coeruleoalba	20/03/1991	Torrevieja (V)	191	Female
Stenella coeruleoalba	21/03/1991	Daimuz (V)	204	Male
Stenella coeruleoalba	23/11/1993	Almazora (V)	210	Male
Stenella coeruleoalba	07/08/1994	Cullera (V)	206	Female
Stenella coeruleoalba	27/02/1995	Vergel (V)	192	Female
Stenella coeruleoalba	03/01/1996	Benidorm (V)	192	Male
Stenella coeruleoalba	15/02/1996	Denia (V)	193	Female
Stenella coeruleoalba	30/01/1997	Oliva (V)	198	Female
Stenella coeruleoalba	08/03/1997	Denia (V)	188	Female

Stenella coeruleoalba	14/02/1998	Cabo de las Huertas (V)	196	Female
Stenella coeruleoalba	22/11/1998	Gandía (V)	184	Male
Stenella coeruleoalba	22/01/2002	Campello (V)	155	Male
Stenella coeruleoalba	17/02/2002	San Juan (V)	150	Male
Stenella coeruleoalba	18/07/2002	Valencia (V)	205	Female
Stenella coeruleoalba	10/04/2003	Calpe (V)	197	Female
Stenella coeruleoalba	09/06/2003	El Saler (V)	156	Male
Stenella coeruleoalba	25/05/2004	Sueca (V)	199	Male
Stenella coeruleoalba	15/12/2004	El Perelló (V)	179	Female
Stenella coeruleoalba	16/12/2004	Jávea (V)	195	Male
Stenella coeruleoalba	09/04/2007	Daimuz (V)	110	Male
Stenella coeruleoalba	06/08/2007	Elche (V)	157	Male
Stenella coeruleoalba	12/08/2007	Elche (V)	187	Male
Stenella coeruleoalba	19/08/2007	Port Saplaya (V)	155	Female
Stenella coeruleoalba	20/08/2007	Oliva (V)	183	Male
Stenella coeruleoalba	24/08/2007	Denia (V)	147	Male
Stenella coeruleoalba	25/08/2007	Alcocébre (V)	203	Male
Stenella coeruleoalba	30/08/2007	Piles (V)	165	Male
Stenella coeruleoalba	13/09/2007	Nules (V)	194	Male
Stenella coeruleoalba	21/09/2007	El Saler (V)	156	Female
Stenella coeruleoalba	24/11/2007	Denia (V)	198	Female
Stenella coeruleoalba	28/11/2007	Oliva (V)	145	Male
Stenella coeruleoalba	16/02/2008	Xeraco (V)	123	Male
Stenella coeruleoalba	19/02/2008	El Saler (V)	175	Male
Stenella coeruleoalba	21/02/2008	El Saler (V)	138	Female

Stenella coeruleoalba	27/02/2008	Peñíscola (V)	208	Male
Stenella coeruleoalba	01/03/2008	Nules (V)	194	Female
Stenella coeruleoalba	04/04/2008	El Saler (V)	190	Male
Stenella coeruleoalba	29/07/2008	Port Saplaya (V)	186	Female
Stenella coeruleoalba	21/08/2008	Calpe (V)	166	Male
Stenella coeruleoalba	26/08/2008	Benidorm (V)	161	Female
Stenella coeruleoalba	31/12/2008	Calpe (V)	195	Male
Grampus griseus	04/09/1989	Oropesa (V)	170	Female
Grampus griseus	02/04/1990	Moncófar (V)	305	Male
Grampus griseus	05/09/1990	Gandía (V)	170	Female
Grampus griseus	14/02/1992	Pobla de Farnals (V)	272	Male
Grampus griseus	02/06/1993	Vergel (V)	210	Female
Grampus griseus	31/01/1995	Gandía (V)	295	Male
Grampus griseus	06/06/1995	Almazora (V)	230	Female
Grampus griseus	21/01/1997	Gandía (V)	305	Female
Grampus griseus	19/11/1998	Oliva (V)	289	Female
Grampus griseus	20/03/1999	Orihuela (V)	290	Female
Grampus griseus	12/05/1999	Pobla de Farnals (V)	308	Male
Grampus griseus	25/01/2002	Denia (V)	305	Female
Grampus griseus	20/03/2002	Vinaroz (V)	260	Male
Grampus griseus	13/04/2002	Puerto de Sagunto (V)	292	Female
Grampus griseus	05/10/2002	Torrevieja (V)	173	Male
Grampus griseus	03/01/2003	Alboraya (V)	285	Male
Grampus griseus	18/04/2006	Benidorm (V)	208	Female
Grampus griseus	15/11/2007	Elche (V)	305	Male

Tursiops truncatus	08/11/1990	Benidorm (V)	203	Female
Tursiops truncatus	26/01/1991	El Saler (V)	289	Female
Tursiops truncatus	12/06/1992	Castellón (V)	259	Female
Tursiops truncatus	22/08/1992	Santa Pola(V)	219	Male
Tursiops truncatus	27/04/1994	Benidorm (V)	269	Male
Tursiops truncatus	10/05/1994	Moraira (V)	294	Male
Tursiops truncatus	12/10/1995	Alcoceber (V)	245	Male
Tursiops truncatus	22/06/2001	Vinaroz (V)	223	Female
Tursiops truncatus	02/02/2002	Benisa (V)	279	Female
Tursiops truncatus	24/03/2003	El Puig (V)	250	Female
Tursiops truncatus	30/11/2003	Orihuela (V)	216	Female
Tursiops truncatus	22/04/2006	Santa Pola (V)	324	Male
Tursiops truncatus	18/12/2006	Alcalá de Chivert (V)	232	Male
Tursiops truncatus	27/03/2007	Campello (V)	196	Male
Delphinus delphis	03/06/1995	El Saler (V)	174	Female
Delphinus delphis	22/07/1997	Columbretes (V)	102	Female
Delphinus delphis	22/07/1997	Columbretes (V)	189	Female
Delphinus delphis	26/04/2000	Xeraco (V)	201	Female
Delphinus delphis	23/10/2001	Santa Pola (V)	189	Female
Delphinus delphis	27/02/2003	Oropesa (V)	161	Male
Delphinus delphis	16/09/2003	El Perelló (V)	106	Male
Delphinus delphis	23/08/2006	Chilches (V)	192	Female
Globicephala melas	08/06/1982	San Juan (V)	388	Female
Globicephala melas	31/09/1982	San Juan (V)	375	Female
Globicephala melas	02/10/1982	Xeraco (V)	393	Female

Globicephala melas	17/03/1987	Cullera (V)	455	Male
Globicephala melas	07/1988	-	-	-

HELMINTHS FROM THE D STRIPED DOLPHIN (*STEN* IN THE WESTERN MEDIT PATTERNS WITHIN A

APPENDIX 3

DIGESTIVE TRACT OF THE
NELLA COERULEOALBA)
TERRANEAN: INFECTION
LONG-TERM SCALE

Appendix 3. Biological data of striped dolphins, *Stenella coeruleoalba*, from western Mediterranean waters stranded in 1990 analyzed for stomach parasites. M: Murcia; V: Valencian Community; C: Catalonia (Chapter 6).

Collection date	Stranding locality	Total length (cm)	Sex	Age (years)
23/08/1990	La Manga (M)	192	Male	23
29/08/1990	Sta. Pola (V)	182	Male	10
29/08/1990	Cullera (V)	200	Female	-
29/08/1990	La Manga (M)	204	Male	12
30/08/1990	Altea (V)	195	Female	22
30/08/1990	El Perellonet (V)	202	Male	15
01/09/1990	Almazora (V)	213	Male	-
01/09/1990	Peñíscola (V)	194	Female	15
01/09/1990	Sta. Pola (V)	206	Female	27
02/09/1990	Alboraya (V)	195	Female	20
02/09/1990	Guardamar del Segura (V)	173	Female	4
02/09/1990	La Manga (M)	201	Male	12
07/09/1990	Piles (V)	158	Female	5
08/09/1990	Vergel (V)	195	Male	-
09/09/1990	Pinedo (V)	192	Female	11
09/09/1990	Cullera (V)	210	Female	-
10/09/1990	Puzol (V)	202	Male	-
10/09/1990	San Juan (V)	183	Female	10
11/09/1990	El Saler (V)	204	Male	16
14/09/1990	Malvarrosa (V)	193	Male	-
18/09/1990	Gandía (V)	203	Male	-
18/09/1990	Vergel (V)	189	Female	23
19/09/1990	Playa del Cocó (V)	198	Female	-
21/09/1990	Nules (V)	207	Male	19
22/09/1990	Alcocéber (V)	184	Male	19
22/09/1990	El Portús (M)	206	Male	20
23/09/1990	Oropesa (V)	190	Female	15
28/09/1990	Masnou (C)	208	Male	25
29/09/1990	Palamós	200	Male	12

30/09/1990	Guardamar del Segura (V)	179	Male	15
30/09/1990	Benicarló (V)	191	Male	14
30/09/1990	Palamós (C)	200	Female	23
01/10/1990	Benicasim (V)	204	Male	28
01/10/1990	Amposta (C)	190	Male	18
01/10/1990	Amposta (C)	210	Male	25
01/10/1990	St. Feliu de Guixols (C)	204	Female	16
01/10/1990	Palamós (C)	187	Female	19
01/10/1990	Barcelona (C)	166	Female	2
15/10/1990	L'Ampolla (C)	200	Female	18
16/10/1990	Mataró (C)	193	Female	12
03/12/1990	Guardamar del Segura (V)	200	Male	26
19/12/1990	Masnou (C)	177	Male	7
02/03/1991	Cullera (V)	170	Male	-
1990	Barcelona (C)	-	Male	-
1990	Barcelona (C)	-	Male	13
1990	Barcelona (C)	-	Female	7
1990	Barcelona (C)	-	-	-

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