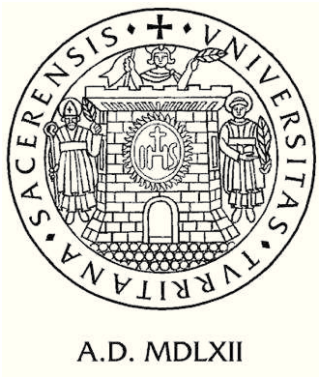

UNIVERSITÀ DEGLI STUDI DI SASSARI

SCUOLA DI DOTTORATO IN

RIPRODUZIONE, PRODUZIONE, BENESSERE ANIMALE E SICUREZZA DEGLI ALIMENTI DI ORIGINE ANIMALE

Direttore: Prof. Giovanni Garippa

INDIRIZZO: **Produzione e Sicurezza degli Alimenti di Origine Animale (XXV CICLO)**

Coordinatore: Prof. Enrico De Santis

GILL METAZOAN PARASITES OF TUNAS (SCOMBRIDAE: THUNNINI) FROM THE WESTERN MEDITERRANEAN SEA: SYSTEMATICS, ASSEMBLAGES AND USE AS BIOLOGICAL TAGS

Docente Guida

Chiar.mo Prof. Giovanni Garippa

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Tesi di dottorato di

Salvatore Mele

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Salvatore Mele. Gill metazoan parasites of tunas (Scombridae: Thunnini) from the western Mediterranean Sea: systematics, assemblages and use as biological tags. Tesi di dottorato in Riproduzione, Produzione, Benessere Animale e Sicurezza degli Alimenti di Origine Animale. Università degli studi di Sassari.

Corrigenda

Page XV, 2nd paragraph, 2nd line, instead of "a total of 334 fish specimens" should read "a total of 356 fish specimens".

Page XV, 3rd paragraph, 2nd line, instead of "and 13 records" should read "and 14 new host records".

Page XXI, 2nd paragraph, 3rd line, instead of "esaminati 334 esemplari" should read "esaminati 356 esemplari".

Page 41, 1st paragraph, 2nd line, instead of "a total of 334 fish specimens" should read "a total of 356 fish specimens".

Page 124, 1st paragraph, 3rd line, instead of "with 11, five here reported for the first time in this area: *Caligus coryphaenae*, *Capsala magronum*, *Capsala paucispinosa*, *Didymosulcus* sp. 2, *P. appendiculatus* (*T. thynnus*" should read "with 11, three here reported for the first time in this area: *Capsala magronum*, *Capsala paucispinosa*, *Didymosulcus* sp. 2 (*T. thynnus*".

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A mia madre

A mio padre

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No hay placer más complejo que el pensamiento y a él nos entregábamos.
Jorge Luis Borges, *El Aleph*, 1957

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ABSTRACT

This PhD thesis describes the metazoan gill parasites of five tuna species (Scombridae: Thunnini) from the western Mediterranean Sea, and analyses their assemblages, comparing the results with the existing data from the Mediterranean Sea, the Atlantic Ocean and other seas. It is the first study that makes a comparative analysis of the gill parasites of tunas, and it is the most comprehensive data collection on the gill parasites of the Atlantic and Mediterranean tunas. The final goal of this work is to provide practical information to evaluate the usefulness of the gill parasites of tunas as biological tags for studies on host biology, ecology and migrations. It is particularly useful for fish as tunas, because the biological samplings are often limited by their big size and high commercial value, and gills are (relatively) easy to store and examine and represent a waste of the slaughter process of the big tunas.

The five species of the Mediterranean tunas: *Auxis rochei*, *Euthynnus alletteratus*, *Katsuwonus pelamis*, *Thunnus alalunga*, *Thunnus thynnus*, were considered for the study. Between 2006 and 2011, a total of 334 fish specimens were sampled from seven localities of the western Mediterranean Sea: Gibraltar Strait, 9 *A. rochei* and 22 *E. alletteratus*; Alboran Sea, 31 *K. pelamis*; Algerian Sea, 63 *A. rochei* and 105 *E. alletteratus*; Gulf of Valencia, 29 *E. alletteratus*; Balearic Sea, 4 *K. pelamis* and 30 *T. alalunga*; Sardinia Sea, 49 *T. thynnus*; Tyrrhenian Sea, 4 *T. thynnus*. In addition, 10 *T. thynnus* from the Levantine Sea (eastern Mediterranean Sea) were analysed. The samples of *A. rochei*, *E. alletteratus*, *K. pelamis* and *T. alalunga* were collected during routine samplings of the Spanish government projects (GPM-3 and GPM-4) from the big game competition and from the traditional Spainish tuna traps *almadrabas*, those of *T. thynnus* during the sampling of the International Commission for the Conservation of Atlantic Tunas from the Italian and Turkish scientific fishing and from the Italian tuna traps *tonnare*.

Overall 39 parasite species were found in the gills of the five western Mediterranean tunas, adding 30 new records for this area and 13 new host records: six in *A. rochei* (*Allopseudaxine macrova*, *Caligus bonito*, *Churavera triangula*, *Didymozoon auxis*, *Hexostoma auxisi* and Nematobothriinae gen. sp. 1); four in *E. alletteratus* (*Didymozoon* sp., Didymozoinii gen. sp., Nematobothriinae gen. sp. 2, Nematobothriinae gen. sp. 3); two in *K. pelamis* (*D. reniformis* and *Koellikeria* sp.); two in *T. alalunga* (*Capsala paucispinosa* and *Didymozoon pretiosus*). The dominant parasite group were the didymozoid trematodes (25 species), followed by monogeneans (8 species), copepods (5 species) and isopods (1 species).

The analysis of the parasite assemblages of the five tuna species at the infra- and

component community levels allowed to obtain detailed information on their parasitofauna, and to evaluate possible correlations between the levels of infection and the host size, as well as the characteristics of the parasite assemblages according to age, year of sampling and locality.

The parasites of the gills and head of the bullet tuna *A. rochei* were studied in the Algerian Sea (33 spm in May 2008 and 30 in May 2011) and the Gibraltar Strait (9 in May 2008). The parasite assemblages of the Algerian Sea included seven species, of which five located in the gills: three monogeneans (*Allopseudaxine macrova*, *Churavera triangula*, *Hexostoma auxisi*), one didymozoid (*Didymozoon auxis*), and one copepod (*Caligus bonito*); one in the eye (Nematobothriinae gen. sp. 1); and one in the nasal sinuses (*Unicolax mycterobius*). In the Gibraltar Strait, just three of these parasites were found: *A. macrova*, *D. auxis* and *U. mycterobius*. The parasite assemblages of *A. rochei* from the Algerian Sea were analysed according to host size and year, no differences were found between sampling years. The prevalence and mean abundance of *A. macrova* and *D. auxis* of the large fish were higher in than those of the small ones, suggesting that large fish migrated from the tropical areas of the Atlantic Ocean, where these parasites infect other host species with similar levels of infection.

The parasites of the head of the little tunny *E. alletteratus* were studied in the Algerian Sea (83 spm in June 2008, 22 in June 2009 and 20 in June 2011), the Gibraltar Strait (9 in June 2008) and the Gulf of Valencia (6 in September 2010, 23 in October 2011). The parasite assemblages included 13 species, eight from the gills: two monogeneans (*Capsala manteri*, *Hexostoma thunninae*), four didymozoids (*Didymocystis* sp. 1, *Didymocystis* sp. 2, Didymozoinae gen. sp., *Didymozoon* sp.), and two copepods (*Caligus bonito* and *Pseudocycnus appendiculatus*); and five from other areas of the head (*Ceratocolax euthynni*, *Melanocystis* sp., Nematobothriinae gen. sp. 2, Nematobothriinae gen. sp. 3 and *Unicolax collateralis*). In the Gulf of Valencia, Didymozoinii gen. sp., *C. euthynni* and Nematobothrinae gen. sp. 3 were not found, and in the Gibraltar Strait, *Didymocystis* sp. 2, *Didymozoon* sp., *H. thunninae*, *Melanocystis* sp., *P. appendiculatus* and *U. collateralis* were the only species recorded.

The parasite assemblages of the Algerian Sea were analysed according to sampling year, and the didymozoids had higher prevalence in 2011 than in 2009 and 2008, and in 2009 than in 2008. These differences between sampling years could indicate a migration of fish from different localities in different years, and the higher infections of didymozoids in 2011 suggest that this host group could have migrated from some area of the Mediterranean Sea with high abundance of intermediate hosts infected by didymozoid stages.

Comparing the samples of the Algerian Sea and the Gulf of Valencia of 2011, the infection of three didymozoid (*Didymozoon* sp., *Melanocystis* sp. Nematobothriinae gen. sp. 2) and the

monogenean *H. thunninae* were higher in the large fish of the Algerian Sea than in small ones of the Gulf of Valencia. These differences of didymozoid infections can be attributable to the different feeding strategy of these groups, but also to the feeding grounds; moreover, the occurrence of the ectoparasites can be influenced by the host length and the environmental conditions.

The whole parasite assemblage of the western Mediterranean little tunny was also compared with that of the south-western Atlantic one, finding significant differences of prevalence for several parasites (*A. macrova*, *C. bonito*, *C. magronum*, *C. pelamydis*, *Didymocystis* sp. 2, *Didymozoon* sp., *H. keokeo*, *H. lintoni*, *H. thunninae, L. multisacculatum*, *Melanocystis* sp., *M. ventrosicula* and Nematobothriinae sp. 2), suggesting that the fish populations from these areas are well separated.

The parasites of the gills of the skipjack tuna *K. pelamis* were studied in the Alboran (31 spm) and Balearic Sea (4 spm) in July 2008. The parasite assemblages included nine species: height didymozoids (*Atalostrophion* cf. *biovarium*, *Didymocylindrus filiformis*, *Didymocylindrus simplex*, *Didymocystis reniformis*, *Didymoproblema fusiforme*, *Didymozoon longicolle*, *Koellikeria* sp., *Lobatozoum multisacculatum*), and one copepod (*Caligus bonito*). *Koellikeria* sp. and *L. multisacculatum* were not recorded in the fish from the Balearic Sea. The results were compared between localities and with published data from the Atlantic Ocean (central-eastern, central-western and south-western Atlantic Ocean). Two species had higher prevalence (*D. simplex* and *D. longicolle*) and two lower prevalence (*A. macrova* and Capsalidae gen. sp.) in the Alboran Sea than in the south-western Atlantic Ocean, suggesting that these host populations are separated. The presence in the western Mediterranean hosts of *D. fusiforme* and *L. multisacculatum*, two didymozoids typical of various species of the tropical Atlantic tunas never recorded in other Mediterranean hosts, indicate a migration of the skipjack tuna from the tropical Atlantic areas to the Mediterranean Sea.

The parasites of the gills of the albacore *T. alalunga* were studied in 30 specimens from the Balearic Sea in July 2008. The parasite assemblage included 9 species: one capsalid (*Capsala paucispinosa*); six didymozoids (*Didymosulcus aahi*, *Didymosulcus dimidiatus*, *Didymozoon longicolle*, *Didymozoon pretiosus*, *Nematobothrium latum* and *Wedlia bipartita*); two crustaceans (*Pseudocycnus appendiculatus* and *Rocinela* sp.). The levels of infection of each parasite species were calculated according to host size, dividing the hosts into three groups (small, medium and large); no differences were found between the size groups, suggesting that the different size classes of albacore share the habitat, the feeding grounds and the food items. Most of the parasites found in the Balearic Sea are not reported in the north Atlantic albacore (*D.*

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aahi, *D. dimidiatus*, *D. longicolle* and *Rocinela* sp.), comfirming the separation of these two populations.

The parasites of the gills of the Atlantic bluefin tuna *T. thynnus* were studied in the Levantine Sea (10 spm in May-June 2007), Sardinia Sea (30 in May-June 2006 and 19 in May-June 2007) and Tyrrhenian Sea (4 in September 2007). The parasite assemblages of the tunas from the Sardinia Sea included 11 species: four monogeneans (*Capsala magronum*, *Capsala onchidiocotyle*, *Capsala paucispinosa* and *Hexostoma thynni*), five didymozoids (*Didymocystis reniformis*, *Didymosulcus* sp. 2, *Didymosulcus wedli*, *Didymozoon pretiosus* and *Wedlia bipartita*), and two copepods (*Caligus coryphaenae* and *Pseudocycnus appendiculatus*); four didymozoids were found in the fish from the Levantine Sea (*Didymosulcus* sp. 2, *Didymosulcus wedli*, *Didymozoon pretiosus* and *Wedlia bipartita*); and one didymozoid (*Didymosulcus* sp. 2) in the fish from the Tyrrhenian Sea.

Dividing the hosts into four groups (small, medium and large and extra large), the analysis of the prevalence and mean abundance showed differences according to host size: the levels of infection of *Didymosulcus* sp. 2 were higher in the small tunas of the Tyrrhenian Sea than in all the other groups; the prevalence of *D. reniformis* was higher in the medium size hosts of the Adriatic Sea (data from literature) and the Atlantic Ocean (data from literature) than in the large fish of the western Mediterranean Sea; the mean abundance of *D. wedli* was higher in the large fish of the Sardinia Sea than in the small Tyrrhenian fish. The differences of the levels of infection of didymozoids between the small and large fish can be due to different feeding strategies, but also to differences of habitat and feeding areas.

The analysis of the data according to locality showed that generally (apart *Didymosulcus* sp. 2, *Koellikerioides apicalis*, *Wedlia bipartita*) the prevalences of the parasites of the Atlantic tunas were higher than those of the medium and small hosts from the Mediterranean Sea, and they were similar to those of the extra large Mediterranean tunas. In a general point of view, the parasite assemblages of the small tunas from the Tyrrhenian Sea and medium tunas the Adriatic Sea, Levantine Sea and north-eastern Atlantic Sea form discrete units according to the localities, being the Atlantic group richer than those of the Mediterranean Sea. The Mediterranean extra large tunas showed a parasite assemblage similar to that of the medium Atlantic fish, suggesting their migration from the Atlantic area, while the large tunas of the Sardinia Sea had a parasite assemblage intermediate between the medium tunas of the Atlantic Ocean and those of the medium size Mediterranean fish. This can be due to a mix of specimens poorly infected of the Mediterranean areas with other heavy infected coming from to the Atlantic Ocean.

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The comparative analysis of the parasite assemblages of the five tuna species, based on the present and published data of the Atlantic and Mediterranean Area, showed that sixteen parasites are shared between these hosts. Eight of them are shared only in the Atlantic Ocean (*A. macrova*, *C. magronum*, *C. onchidiocotyle*, *C. coryphaenae*, *Caligus pelamydis* and *Caligus productus, Euryphorous brachypterus, L. multisacculatum*), three only in the western Mediterranean Sea (*C. paucispinosa*, *D. longicolle*, *D. pretiosus*), and five in both areas (*D. reniformis*, *W. bipartita*, *C. bonito*, *P. appendiculatus*). Several species of the first group are not present in the same host in the Mediterranean Sea (*C. pelamydis* and *C. productus*), or they are found only in some category of them (size class, locality); these occurrences, *i.e. A. macrova* in the large specimens of *A. rochei*, *C. magronum* and *C. coryphaenae* in the large specimens of *T. thynnus*, *Lobatozoum multisacculatum* in *K. pelamis*, suggest that these hosts spent a part of their life in the Atlantic Ocean.

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RIASSUNTO

I PARASSITI METAZOI DELLE BRANCHIE DEI TONNI (SCOMBRIDAE: THUNNINI) DEL MAR MEDITERRANEO OCCIDENTALE: SISTEMATICA, ASSOCIAZIONI E UTILIZZO COME MARCHE BIOLOGICHE. La presente tesi di dottorato descrive i parassiti metazoi delle branchie delle cinque specie di tonno (Scombridae: Thunnini) presenti nel Mar Mediterraneo occidentale, e ne analizza le associazioni, confrontando i risultati ottenuti con i dati esistenti in letteratura sui i tonni del Mar Mediterraneo e dell'Oceano Atlantico. È il primo studio comparativo sulla parassitofauna branchiale delle diverse specie di tonno, ed è la più completa raccolta di dati sui parassiti branchiali dei tunnidi dell'Oceano Atlantico e del Mar Mediterraneo. L'obiettivo finale della presente ricerca è quello di stabilire la validità dei parassiti delle branchie dei tonni come marche biologiche per studi sulla biologia, ecologia e le migrazioni di questi grandi pelagici. Lo studio delle marche biologiche è, infatti, particolarmente utile per pesci come i tonni, poiché i campionamenti biologici sono spesso limitati a causa delle loro notevoli dimensioni e dall'alto valore commerciale che possono raggiungere gli esemplari di queste specie; lo studio dei parassiti branchiali presenta numerosi vantaggi in considerazione del fatto che le branchie sono (relativamente) facili da conservare ed esaminare, e rappresentano uno dei pochi scarti del processo di lavorazione dei tonni.

Nel presente studio sono state prese in considerazione le cinque specie di tonni del Mar Mediterraneo: *Auxis rochei*, *Euthynnus alletteratus*, *Katsuwonus pelamis*, *Thunnus alalunga*, *Thunnus thynnus*. Tra il 2006 e il 2011, sono stati complessivamente esaminati 334 esemplari provenienti da sette località del Mar Mediterraneo occidentale: Golfo di Valencia, 29 *E. alletteratus*; Mar di Alboran, 31 *K. pelamis*; Mar di Algeria, 63 *A. rochei* e 105 *E. alletteratus*; Mar delle Baleari, 4 *K. pelamis* e 30 *T. alalunga*; Mar di Sardegna, 49 *T. thynnus*; Mar Tirreno, 4 *T. thynnus*; Stretto di Gibilterra, 9 *A. rochei* e 22 *E. alletteratus*. Inoltre, sono stati esaminati 10 *T. thynnus* provenienti dal Mar di Levante (Mar Mediterraneo orientale). I parassiti branchiali di *A. rochei*, *E. alletteratus*, *K. pelamis* e *T. alalunga* sono stati raccolti durante i campionamenti di routine nell'ambito di progetti di ricerca del Governo spagnolo (GPM-3 e GPM-4), nelle gare di pesca sportiva e nelle tonnare spagnole (*almadrabas*), quelli di *T. thynnus* durante i campionamenti della Commissione Internazionale per la Conservazione dei Tonni dell'Atlantico, nelle campagne di pesca scientifica italiane e turche, e nelle tonnare sarde.

Nelle branchie delle cinque specie di tonni del Mar Mediterraneo occidentale sono state repertate complessivamente 39 specie parassitarie; per 30 delle quali si tratta di nuove segnalazioni per questa area e 14 per questi ospiti: sei in *A. rochei* (*Allopseudaxine macrova*,

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Caligus bonito, *Churavera triangula*, *Didymozoon auxis*, *Hexostoma auxisi* e Nematobothriinae gen. sp. 1), quattro in *E. alletteratus* (*Didymozoon* sp, Didymozoinii gen. sp., Nematobothriinae gen. sp. 2, Nematobothriinae gen. sp. 3), due in *K. pelamis* (*D. reniformis* e *Koellikeria* sp.), due in *T. alalunga* (*Capsala paucispinosa* e *Didymozoon pretiosus*). Il gruppo di parassiti risultato dominante è quello dei trematodi didimozoidi (25 specie), seguiti dai monogenei (8 specie), copepodi (5 specie) e isopodi (1 specie).

L'analisi delle associazioni dei parassiti delle cinque specie di tonno a livello di *infracommunity* e *component community* ha permesso di ottenere informazioni dettagliate sulla fauna parassitaria branchiale di questi pelagici, e valutare possibili correlazioni tra i livelli di infestazione e taglia dell'ospite, così come le caratteristiche delle associazioni secondo la classe di taglia dell'ospite, anno e località di campionamento.

La ricerca di parassiti delle branchie e della testa del tombarello *A. rochei* è stata compiuta su esemplari provenienti dal Mar di Algeria (33 esemplari catturati a maggio del 2008 e 30 a maggio del 2011) e dallo Stretto di Gibilterra (9 esemplari pescati a maggio del 2008). Nei tonni del Mar di Algeria sono state repertate sette specie, di cui cinque nelle branchie: tre monogenei (*A. macrova*, *C. triangula*, *Hexostoma auxisi*), un didimozoide (*Didymozoon auxis*), e un copepode (*C. bonito*); una nella cavità oculare (Nematobothriinae gen. sp. 1), ed una dei seni nasali (*Unicolax mycterobius*). In quelli pescati nello Stretto di Gibilterra sono state invece riscontrate le seguenti tre specie: *A. macrova*, *D. auxis* e *U. mycterobius*.

Le associazioni dei parassiti di *A. rochei* del Mar di Algeria sono state analizzate in base alla taglia dell'ospite e l'anno di campionamento. Non sono state riscontrate differenze tra gli anni di campionamento. La prevalenza e l'abbondanza media di *A. macrova* e *D. auxis* dei pesci di grande taglia sono risultate più elevate di quelle di taglia inferiore; ciò suggerisce che i pesci di maggiori dimensioni possano essere migrati dalle zone tropicali dell'Oceano Atlantico, dove questi parassiti infestano anche altre specie ospite con simili livelli di infestazione.

La ricerca di parassiti delle branchie e della testa del tonnetto alletterato *E. alletteratus* è stata eseguita su esemplari provenienti dal Golfo di Valencia (6 esemplari catturati a settembre del 2010 e 23 a ottobre del 2011), dal Mar di Algeria (83 pescati a giugno del 2008, 22 a giugno del 2009 e 20 a giugno del 2011), e dallo Stretto di Gibilterra (9 esemplari catturati a giugno del 2008). La fauna parassitaria è risultata composta da 13 specie, otto delle quali sono state repertate nelle branchie: due monogenei (*Capsala manteri*, *Hexostoma thunninae*), quattro didimozoidi (*Didymocystis* sp. 1, *Didymocystis* sp. 2, Didymozoinae gen. sp., *Didymozoon* sp.), e due copepodi (*C. bonito* e *Pseudocycnus appendiculatus*); e cinque sono state riscontrate in altre localizzazioni nella testa (*Ceratocolax euthynni*, *Melanocystis* sp., Nematobothriinae gen. sp. 2,

Nematobothriinae gen. sp. 3 e *Unicolax collateralis*). Tutte le specie sono state trovate nei tonnetti alletterati del Mar di Algeria. Nei tonnetti alletterati del Golfo di Valencia non è stata evidenziata la presenza di *C. euthynni*, Didymozoinii gen. sp. e Nematobothrinae gen. sp. 3, mentre nei tonnetti dello Stretto di Gibilterra sono state riscontrate *Didymocystis* sp. 2, *Didymozoon* sp., *H. thunninae*, *Melanocystis* sp., *P. appendiculatus* ed *U. collateralis*.

Le associazioni dei parassiti del Mar di Algeria sono state analizzate secondo l'anno di campionamento: i didimozoidi hanno evidenziato una prevalenza più elevata nel 2011 rispetto al 2009 e al 2008, e nel 2009 rispetto al 2008. Tali differenze tra anni di campionamento potrebbero indicare una migrazione di pesci provenienti da varie località nei diversi anni; i valori elevati dell'infestazione da didimozoidi riscontrati nei tonni del 2011 suggeriscono che questi potrebbero essere migrati nel Mar di Algeria da un'area del Mar Mediterraneo dove i tonni si siano potuti infestare alimentandosi di ospiti intermedi abbondantemente infestati dalle fasi larvali dei didimozoidi.

Confrontando i dati relativi alle comunità parassitarie del Golfo di Valencia e del Mar di Algeria del 2011, è possibile rilevare che l'infestazione di tre didimozoidi (*Didymozoon* sp., *Melanocystis* sp., Nematobothriinae gen. sp. 2) e del monogeneo *H. thunninae* è più elevata nei tonni di grandi dimensioni del Mar di Algeria rispetto a quelli di piccole dimensioni del Golfo di Valencia. La differenza d'infestazione dei didimozoidi tra i giovani tonnetti del Golfo di Valencia e gli adulti del Mar di Algeria può essere attribuibile alla diversa strategia di alimentazione dei gruppi, ma anche a diverse zone di alimentazione; inoltre, la presenza di ectoparassiti può essere influenzata dalla taglia dell'ospite e dalle condizioni ambientali.

Il confronto fra le associazioni parassitarie del tonnetto alletterato del Mediterraneo occidentale e dell'Atlantico sud-occidentale, ha evidenziato differenze significative di prevalenza per diversi parassiti (*A. macrova*, *C. bonito*, *C. magronum*, *C. pelamydis*, *Didymocystis* sp. 2, *Didymozoon* sp., *H. keokeo*, *H. lintoni*, H. *thunninae*, *L. multisacculatum*, *Melanocystis* sp., *M. ventrosicula* e Nematobothriinae sp. 2), indicando che le popolazioni di tonnetto alletterato di queste aree sono ben separate.

La ricerca di parassiti delle branchie del tonnetto striato *K. pelamis* è stata effettuata su esemplari provenienti dal Mar di Alboran (31 esemplari) e dal Mar delle Baleari (4 esemplari) nel luglio 2008. La fauna parassitaria branchiale è risultata composta da nove specie di parassiti: otto didimozoidi (*Atalostrophion* cf. *biovarium*, *Didymocylindrus filiformis*, *Didymocylindrus simplex*, *D. reniformis*, *Didymoproblema fusiforme*, *Didymozoon longicolle*, *Koellikeria* sp, *Lobatozoum multisacculatum*), ed un copepode (*C. bonito*). *Koellikeria* sp. e *L. multisacculatum* non sono stati riscontrati nei tonni del Mar delle Baleari. I risultati sono stati confrontati tra

località e con i dati in precedenza pubblicati riguardanti il tonnetto striato dell'Oceano Atlantico (Oceano Atlantico centro-orientale, centro-occidentale e sud-occidentale). Il riscontro di una prevalenza più elevata di *D. simplex* e *D. longicolle* e di una prevalenza inferiore di *A. macrova* e Capsalidae gen. sp. nei tonnetti striati del Mar di Alboran rispetto a quelli dell'Oceano Atlantico sud-occidentale, indicano che queste popolazioni di ospiti sono separate. La presenza di *D. fusiforme* e *L. multisacculatum* nei tonnetti striati del Mar Mediterraneo occidentale, due didimozoidi tipici di varie specie di tonni tropicali atlantici, mai registrati in altri ospiti del Mediterraneo, suggerisce una migrazione del tonnetto striato dalle zone tropicali dell'Oceano Atlantico al Mar Mediterraneo.

La ricerca di parassiti delle branchie del tonno alalunga *T. alalunga* è stata effettuata su 30 esemplari catturati nel Mar delle Baleari nel luglio 2008. La fauna parassitaria branchiale è risultata costituita da nove specie: un capsalide (*C. paucispinosa*), sei didimozoidi (*Didymosulcus aahi*, *Didymosulcus dimidiatus*, *D. longicolle*, *Didymozoon pretiosus*, *Nematobothrium latum* e *Wedlia bipartita*), due crostacei (*Pseudocycnus appendiculatus* e *Rocinela* sp.). I livelli d'infestazione di ogni specie parassitaria sono stati calcolati secondo la taglia dell'ospite, dividendo gli ospiti in tre gruppi di taglia (piccola, media e grande). Non sono state riscontrate differenze tra i gruppi di taglia, e questo fatto suggerisce che le diverse classi di taglia abbiano un comportano alimentare simile e condividano le aree di alimentazione. La maggior parte dei parassiti presenti nel tonno alalunga del Mar delle Baleari non è stata evidenziata nel tonno alalunga dell'Oceano Atlantico settentrionale (*D. aahi*, *D. dimidiatus*, *D. longicolle* e *Rocinela* sp.), confermando la separazione di queste due popolazioni di ospiti.

La ricerca di parassiti delle branchie del tonno rosso dell'Atlantico *T. thynnus* è stata effettuata su esemplari catturati nel Mar di Levante (10 esemplari pescati a maggio-giugno del 2007), Mar di Sardegna (30 catturati a maggio-giugno del 2006 e 19 a maggio-giugno del 2007) e Mar Tirreno (4 pescati a settembre del 2007). L'associazione parassitaria dei tonni del Mar di Sardegna è composta da 11 specie: quattro monogenei (*Capsala magronum*, *Capsala onchidiocotyle*, *C. paucispinosa* e *Hexostoma thynni*), cinque didimozoidi (*D. reniformis*, *Didymosulcus* sp. 2, *Didymosulcus wedli*, *Didymozoon pretiosus* e *Wedlia bipartita*), e due copepodi (*C. coryphaenae* e *Pseudocycnus appendiculatus*); nel tonno rosso del Mar di Levante sono stati repertati quattro didimozoidi (*Didymosulcus* sp. 2, *D. wedli*, *D. pretiosus* e *W. bipartita*); mentre un solo didimozoide (*Didymosulcus* sp. 2) è stato riscontrato nel tonno rosso del Mar Tirreno.

Dividendo gli ospiti in quattro gruppi di taglia (piccola, media, grande ed extra-grande), l'analisi della prevalenza e dell'abbondanza media ha evidenziato differenze in base alla taglia: i

livelli d'infestazione di *Didymosulcus* sp. 2 sono stati superiori nel gruppo dei piccoli tonni del Mar Tirreno rispetto a tutti gli altri gruppi; una maggiore prevalenza di *D. reniformis* è stata riscontrata negli ospiti di medie dimensioni del Mare Adriatico (dati di letteratura) e dell'Oceano Atlantico (dati di letteratura) piuttosto che negli ospiti di grande taglia del Mar Mediterraneo occidentale; una maggiore abbondanza media di *D. wedli* è stata evidenziata nei tonni di grande taglia del Mar di Sardegna piuttosto che in quelli di piccola taglia del Mar Tirreno. La differenza dei livelli d'infestazione dei didimozoidi tra gli ospiti di piccole e grandi dimensioni può essere dovuta alla diversa strategia alimentare adottata dai due gruppi, ma anche a differenze di habitat e aree di alimentazione.

L'analisi dei dati per località ha mostrato come in generale (a parte *Didymosulcus* sp. 2, *Koellikerioides apicalis*, *W. bipartita*) le prevalenze dei parassiti del tonno rosso di medie dimensioni dell'Atlantico siano superiori a quelle degli ospiti di medie e piccole dimensioni del Mediterraneo, e siano simili a quelle dei tonni extra-grandi del Mar Mediterraneo. In particolare le associazioni di parassiti dei tonni piccoli del Mar Tirreno e dei tonni di media taglia del Mar Adriatico, Mar di Levante e dell'Oceano Atlantico nord-orientale costituiscono unità distinte secondo le diverse località, essendo il gruppo atlantico più ricco di parassiti dei gruppi mediterranei. I tonni extra-grandi del Mar di Sardegna hanno un'associazione parassitaria simile a quella dei pesci di media taglia dell'Oceano Atlantico, suggerendo la loro migrazione dall'area atlantica, mentre i tonni di grande taglia del Mar di Sardegna hanno un'associazione di parassiti con caratteristiche intermedie tra quelle dei tonni di media taglia dell'Oceano Atlantico e quelle dei tonni di medie dimensioni del Mar Mediterraneo. Questo fatto potrebbe essere dovuto a un mescolamento di esemplari scarsamente infestati provenienti da aree del Mar Mediterraneo con altri altamente infestati provenienti dall'Oceano Atlantico.

L'analisi comparativa delle associazioni di parassiti delle cinque specie di tonno, sulla base dei risultati della presente tesi e dei dati bibliografici riguardanti le aree dell'Oceano Atlantico e del Mar Mediterraneo, ha rilevato 16 specie di parassiti condivisi tra questi ospiti. Otto di queste sono condivise esclusivamente nell'Oceano Atlantico (*A. macrova*, *C. magronum*, *C. onchidiocotyle*, *C. coryphaenae*, *Caligus pelamydis* e *Caligus productus*, *Euryphorous brachypterus*, *L. multisacculatum*), tre solo nel Mediterraneo occidentale (*C. paucispinosa*, *D. longicolle*, *D. pretiosus*), e cinque in entrambi i mari (*D. reniformis*, *W. bipartita*, *C. bonito*, *P. appendiculatus*). Diverse specie del primo gruppo non sono presenti nello stesso ospite nel Mar Mediterraneo (*C. pelamydis* e *C. productus*), oppure si trovano solo in alcune categorie di ospiti (classe di taglia, specie, località): *A. macrova* è presente solo nei grandi esemplari di *A. rochei*; *C. coryphaenae* e *C. magronum* nei grandi esemplari di *T. thynnus*; *L. multisacculatum* in *K.*

pelamis; suggerendo che questi ospiti abbiano trascorso una parte della loro vita nell'Oceano Atlantico.

CHAPTER 1. INTRODUCTION

The tunas (Osteichthyes: Scombridae: Thunnini) are fascinating organisms of the pelagic habitat widely present in the collective imaginary and myths of the antique cultures (Fig. 1.0.1) (Di Natale, 2012). The tuna fishery is the most ancient industrial activity in the fishery sector, since the tuna traps along the Mediterranean coasts have been operating from the Greek and Phoenician times (2000 BC) (Di Natale, 2012). It represented an important economic activity for the ancient western Mediterranean civilizations, such as Romans, Normands established in Sicily and Southern Italy (XII Century A.C) and the Aragon and Castilla kingdoms (XIV-XVII Centuries A.C). Nowadays tunas are the most valuable fishery resource exploited in the high seas (Majkowski *et al.*, 2011).

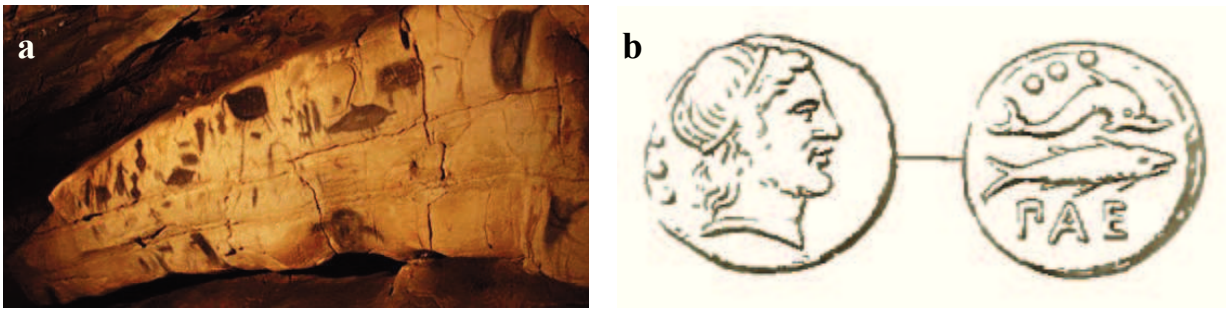

Figure 1.0.1. a, the engravings and drawings of tuna and dolphins on the rocky walls of the Genovese Cave on the Isle of Levanzo (Egadi Islands, Italy), XCII Century BC (http://reidsitaly.com). **b**, inscription on coin of the IV Century BC (Garrucci, 1854, Table CXXII, n. 26).

Seven species of tunas, the so called principal market tuna species, support the major part of the tuna market in terms of quantity and economic value (Catarci, 2004): the skipjack tuna *Katsuwonus pelamis* (Linnaeus, 1758); the albacore *Thunnus alalunga* (Bonnaterre, 1788); the yellowfin tuna *Thunnus albacares* (Bonnaterre, 1788); the southern bluefin tuna *Thunnus maccoyii* (Castelnau, 1872); the bigeye tuna *Thunnus obesus* (Lowe, 1839); the Pacific bluefin

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tuna *Thunnus orientalis* (Temminck and Schlegel, 1844); and the Atlantic bluefin tuna *Thunnus thynnus* (Linnaeus, 1758).

Among them, the skipjack tuna fishery is the most important in terms of volume of catches, and although the bluefin tunas contribute relatively little in terms of landings, they are among the most valuable fish in the sea, and a single fish can reach up to 100000 US $ in the Japan markets (a single specimen of *T. orientalis*, weighting 269 kg, was sold for 736000 US $ at the Tokyo Tsukiji fish market in 2012) (Majkowski *et al.*, 2011; BBC, 2012).

All tuna species are investigated and managed by regional fishery organizations (RFMOs). There are five main tuna fishery management organizations: the Commission for the Conservation of Southern Bluefin Tuna (CCSBT), the Indian Ocean Tuna Commission (IOTC), the Inter-American Tropical Tuna Commission (IATTC), the International Commission for the Conservation of Atlantic Tunas (ICCAT), and the Western and Central Pacific Fisheries Commission (WCPFC). The tuna fisheries of the Mediterranean Sea are managed by the General Fisheries Commission for the Mediterranean of the FAO (GFCM) and the ICCAT. The biological and ecological data on the principal market tunas are generally better known than those for other species of tuna (Majkowski *et al.*, 2011). However, even for these species, there are considerable uncertainties in the basic biological knowledge.

Tunas are opportunistic predators, occupying the top position in the pelagic food web (Block and Stevens, 2001). Several anatomical and physiological features allow these fish to be widespread worldwide (Brill, 1996): the counter current heat exchangers enables them to be regional endothermic (Graham and Dickson, 2004), allowing to penetrate cooler waters thus expanding their thermal niche in both latitude and depth (Collette and Block, 2001); the peculiar gill structure allows the ram ventilation, that joined with a higher gill surface area, ventilation volume, hematocrit and mean corpuscular haemoglobin concentration than in other fish species, supply the great oxygen demand required to maintain the high metabolism of the red muscles (Brill and Bushnell, 2001); the typical thunniform swimming compensates the need of fish to stay in continuous movement to ventilate and maintain the hydrostatic equilibrium (Graham and Dickinsons, 2004).

The migratory nature of these organisms, the difficulties to obtain the samples due to their high commercial value, the global nature of fisheries and their complex management, which involve several countries, make difficult the scientific and political management of these resources. For instance, the status of the populations of the small tunas (*Auxis rochei* (Risso, 1810), *Euthynnus alletteratus* (Rafinesque, 1810), *K. pelamis* and *T. alalunga*) in the Mediterranean Sea is highly uncertain or unknown (Di Natale, 2009). Various methods can be

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used to investigate the structure of the fish populations, including the study of parasites (Cadrin *et al*., 2005). Parasites are being used from almost a century as biological tags to provide information on various aspects of host biology (Mackenzie and Abaunza, 2005), and fish parasites can be used to enhance the knowledge of many aspects, such as phylogeny, biogeography, seasonal migration, age determination, length and growth rates, life history, schooling, and other forms of behaviour and ecology (Williams *et al*., 1992).

1.1 THE SYSTEMATICS OF TUNAS (SCOMBRIDAE: THUNNINI)

The studies of the parasites of fish are directly related with the proper identification of the host species (Bychowsky, 1957). In fact, many parasites are host-specific or shared between related hosts in a limited area of their distributions (Job, 1966; Gibson and Bray, 1986; Lim, 1998; Sasal *et al*., 1999; Oliva *et al*., 2008; Châari *et al*., 2011). Several taxonomic revisions have resulted in changes in the taxonomic position and nomenclature of tunas, leading to citations of the same fish species under several scientific and commercial synonyms (Collette and Nauen, 1983; Klawe, 1998). Therefore, here is provided a brief description of the classification followed in this work, associated with the principal anatomical/morphological characteristics.

Tunas include 15 species grouped into five genera (Fig. 1.1.1) (Collette *et al.*, 2001). They are metazoans, with an ossified structure of the bones, fin rays, and the global structure like the perch fish (genus *Perca*) which allow including them in the superclass Osteichthyes, superorder Acanthopterygii, order Perciformes. The presence of finlets posterior to the dorsal and anal fins, of a pair of caudal keels on the middle of the caudal peduncle and of grooves and depressions to conceal the first dorsal fin, the pectoral fin and the pelvic fins when swimming rapidly, characterise the family Scombridae. The presence of counter current heat exchangers composed of small arterial and venous vessels (*i.e.* Kishinoue's *retia mirabilia* in the red muscles and accessories *retia* in the brain and viscera) distinguishes the tunas (tribe Thunnini) from any other bony fish.

According to the distribution of the countercurrent heat exchangers, tunas are divided into two groups. One includes the species of the genera *Allothunnus*, *Auxis*, *Euthynnus* and *Katsuwonus* and the species of the subgenus *Thunnus* (*Neothunnus*) Kishinouye, 1923: *Thunnus* (*Neothunnus*) *albacares*, the blackfin tuna *Thunnus* (*Neothunnus*) *atlanticus* (Lesson, 1831), and the longtail tuna *Thunnus* (*Neothunnus*) *tonggol* (Bleeker, 1851). These species have a developed central and lateral *retia mirabilia* in the red muscles. The other group includes the species of the subgenus *Thunnus* (*Thunnus*) South, 1845: *Thunnus* (*Thunnus*) *alalunga*, *Thunnus* (*Thunnus*)

maccoyii, *Thunnus* (*Thunnus*) *obesus*, *Thunnus* (*Thunnus*) *orientalis* and *Thunnus* (*Thunnus*) *thynnus*). These species have a well developed lateral *retia mirabilia* in the red muscles and a *retia* in the viscera and the head (Collette *et al.*, 2001).

The species of the genus *Allothunnus* differs from all other tunas in its high number of gill rakers (70-80). It is a monophyletic genus with only one species, the slender tuna *Allothunnus fallai* Serventy, 1948 (Graham and Dickson, 2001).

The species of the genus *Auxis* differ from those of the genera *Euthynnus*, *Katsuwonus* and *Thunnus* in lacking the prominent frontoparietal foramina and the suture between the first vertebra and the skull, in having a separate origin of the dorsal and ventral branches of the cutaneous artery (Godsil, 1954) and for the presence of a single huge interpelvic process, which is about as long as the pelvic fins. There are two species of *Auxis* (Collette and Aadland, 1996): the narrow-corselet *A. thazard* (Lacepede, 1800) and the wide-corselet *A. rochei*. The number of scales of the corselet under the second dorsal fin (1-5 in *A. thazard*, 6 or more in *A. rochei*) and the number of gill rakers (37-43 in *A. thazard*, 40-47 in *A. rochei*) differentiate these species (Collette and Nauen, 1983).

The species of the genus *Euthynnus* differ from those of the genus *Katsuwonus* in having a lower number of gill rakers (29-40 *vs* 53-63) and vertebrae (37-39 *vs* 41) and several dark spots between the pelvic and pectoral fins. They differ from the species of the genus *Thunnus* in lacking a swim bladder (as in the species of *Auxis* and *Katsuwonus* and in *T. tongool*) and in having a lower number of pectoral fin rays and a larger length of the left lobe of the liver.

There are three species of *Euthynnus*: the kawakawa *Euthynnus affinis* Cantor, 1849; the Atlantic little tunny *Euthynnus alletteratus*; and the black skipjack tuna *Euthynnus lineatus* Kishinouye, 1920 (Collette *et al*., 2001). The kawakawa has a lower number of gill rakers (29-33) and of vertebrae (37) than the other *Euthynnus* species (33-39 gill rakers in *E. lineatus*, 37-40 in *E. alletteratus*, 39 vertebrae in both species). The absence of the vomerine teeth allows differentiating *E. alletteratus*.

The species of the genus *Katsuwonus* is differentiated by the higher number of vertebrae (41) than in other tunas (37-40), and by a higher number of gill rakers (53-63) than in most of tunas, except *A. fallai* (70-80). It is monotypic with only one species, the skipjack tuna *K. pelamis*.

Salvatore Mele. Gill metazoan parasites of tunas (Scombridae: Thunnini) from the western Mediterranean Sea: systematics, assemblages and use as biological tags. Tesi di dottorato in Riproduzione, Produzione, Benessere Animale e Sicurezza degli Alimenti di Origine Animale. Università degli studi di Sassari.

Kingdom Animalia
Phylum Chordata
Subphylum Vertebrata
Superclass Osteichthyes
Class Actinopterygii
Subclass Neopterygii
Infraclass Teleostei
Superorder Acanthopterygii
Order Perciformes
Suborder Scombroidei
Family Scombridae
Subfamily Scombrinae
Tribe Thunnini
Genus *Allothunnus*
Species *Allothunnus fallai* Serventy, 1948
Genus *Auxis*
Species *Auxis rochei* (Risso, 1810)
Species *Auxis thazard* (Lacepede, 1800)
Genus *Euthynnus*
Species *Euthynnus affinis* Cantor, 1849
Species *Euthynnus alletteratus* Rafinesque, 1810
Species *Euthynnus lineatus* Kishinouye, 1920
Genus *Katsuwonus*
Species *Katsuwonus pelamis* (Linnaeus, 1758)
Genus *Thunnus*
Species *Thunnus alalunga* (Bonnaterre, 1788)
Species *Thunnus albacares* (Bonnaterre, 1788)
Species *Thunnus atlanticus* (Lesson, 1831)
Species *Thunnus maccoyii* (Castelnau, 1872)
Species *Thunnus obesus* (Lowe, 1839)
Species *Thunnus orientalis* (Temminck *et* Schlegel, 1844)
Species *Thunnus tonggol* (Bleeker, 1851)
Species *Thunnus thynnus* (Linnaeus, 1758)

Figure 1.1.1. Classification of the tribe Thunnini (Collette *et al*., 2001).

The species of the genus *Thunnus* have more pectoral fin rays (30-36) than any other species of Thunnini (23-27), the first vertebra completely sutured to the skull, and the intestine has an additional loop not present in the other genera. The liver in the species of subgenus *Neothunnus* has no striations on the ventral surface of lobes, and the right one is the largest, while the liver in the species of subgenus *Thunnus* has striations on the ventral surface of lobes, the central one being the largest (Collette and Nauen, 1983). The absence of a swim bladder distinguishes *T. tonggol* from the other species of the subgenus *Neothunnus* (Collette *et al.*, 2001). The lower number of gill rakers of *T. atlanticus* (19-25) than those of *T. albacares* (26-34) differentiates these species. *Thunnus alalunga* is the only species of the genus with spleen located on the right side. It has much longer pectoral fins than any other species of the subgenus *Thunnus* (more than 31% of fork length, FL), although the fish smaller than 50 cm have proportionately smaller pectoral fins than *T. obesus*. The latter species has the deepest body, the larger eye diameter within the genus, the lowest number of gill rakers (as *T. alalunga*), and the

longer tail of kidney (reaching the 11-14 vertebra) within the subgenus (Godsil, 1954). The three bluefin tunas (*T maccoyii*, *T. orientalis* and *T. thynnus*) share most of the morphometric and meristic characteristics. They have very short pectoral fins and more gill rakers (3l-43) than any other species of the subgenus *Thunnus*. According to Collette and Nauen (1983) *T. maccoyii* differs from the other bluefin tunas in the colour of the median caudal keel (yellow *vs* dark) and in the first ventrally directed parapophysis (on ninth *vs* on eighth vertebra). Gibbs and Collette (1967) indicated that the number of gill rakers is the only morphological character useful to distinguish *T. orientalis* (mean number of gill rakers 35.9, range 24-43) and *T. thynnus* (mean 38.9, range 32-40).

1.2. THE HABITAT OF TUNAS IN THE WESTERN MEDITERRANEAN SEA

The western Mediterranean Sea is a semi enclosed sea (Font, 1985), encircled by the Provençal region on the north, by the Italian peninsula on the east, by the island of Sicily and the African continent on the south, and by the Iberian Peninsula on the west (Fig. 1.2.1). The easternmost part of the western Mediterranean Sea is in continuity with the central Mediterranean Sea through the Messina Strait and the strait of Sicily, and the westernmost part is connected with the Atlantic Ocean trough the Gibraltar Strait (IHO, 1954).

According to the bottom topography, characterised by the presence of large islands as Corsica and Sardinia, as well as the Balearic archipelago, it is composed by three main sub-basins: the Alboran Sea, the Tyrrhenian Sea and the Balearic basin, which in its turn is divided by the Balearic promontory into the Liguro-Provençal and the Algerian sub-basins (Carminati *et al.*, 2012).

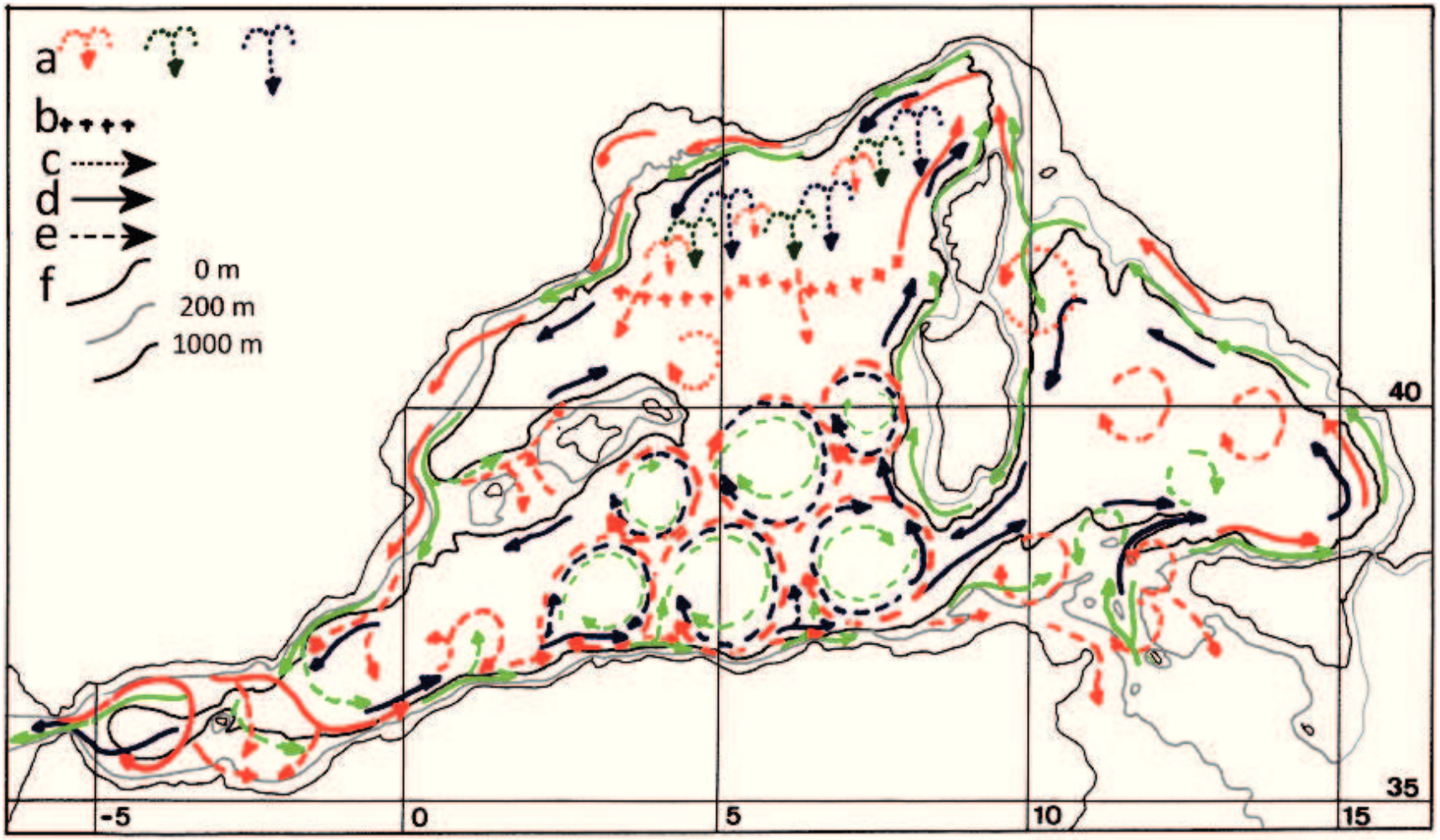

Figure1.2.1. Map of the western Mediterranean Sea. Lines represent the principal currents: **a**, winter mesoscale currents; **b**, North Balearic Front; **c**, wind-induced mesoscale eddies; **d**, currents with a more or less steady path; **e**, mesoscale currents thorough the year; **f**, isobaths. Key to colours: red, Atlantic Water; orange, Modified Atlantic Water; light green, Levantine Intermediate Water; dark green, Western Mediterranean Intermediate Water; blue, Western Mediterranean Deep Water. Modified from Millot (1999).

The western Mediterranean Sea has a negative water balance due to the loss of water through evaporation and outflow through the Sicilian Strait (Hokpins, 1985). Hence, the sea level of the western Mediterranean Sea is maintained by the exchanges across the Strait of Gibraltar (Cruzado, 1985). This inflow of less saline waters conditions the general circulation of the superficial water masses. In the Alboran Sea the inflowing surface Atlantic Waters produce two permanent eddies (*i.e.* water currents which flow in a roughly circular motion), which generate an oceanographic front located from Almeria (Spain) to Oran (Algeria) (Fig 1.2.1, 1.2.2) (Patarnello *et al*., 2007). During its flowing to east, the Atlantic Waters mix with the superficial waters of the Alboran Sea (Font, 1998). The resulting Modified Atlantic Waters follow the Algerian coast forming the Algerian Current. Flowing eastward, the Algerian Current becomes unstable and produces the so called "Algerian eddies" that migrate to the north into the Algerian sub-basin (Elhmaidi *et al*., 2010). The Algerian Current flows through the Sardinia Channel and splits into two branches at the Strait of Sicily: one enters in the Tyrrhenian Sea and the other goes to the eastern Mediterranean Sea (Hopkins, 1985).

At intermediate depths, between 200-500 m, there is the warm and salty Levantine Intermediate Water formed in the eastern Mediterranean basin (Millot, 1999).

The deeper waters are occupied by the Western Mediterranean Deep Waters, originating in the Liguro-Provençal region, due to the persistent and intense blowing of the cold winds Mistral and Tramontana over the Ligurian Sea and the Gulf of Lion, which increase the salinity and decrease the water surface temperature, and hence increase their density, producing their sinking to the deeper layers (Flos, 1985).

Due to similar but less intense cooling and evaporation processes occurring in the same area, the surface waters become the denser Western Intermediate Waters, which are placed between Levantine Intermediate Waters and the surface water masses. Depending on the volume of these Western Intermediate Waters originated each year, they can block the passage of Levantine Intermediate Waters through the Balearic channel. All these Mediterranean intermediate or deep water masses move southward from the Liguro-Provençal subasin to the Alboran Sea, and finally cross the Gibraltar Strait to reach the Atlantic Ocean, passing below the lighter Atlantic Waters (Robinson *et al*., 2001).

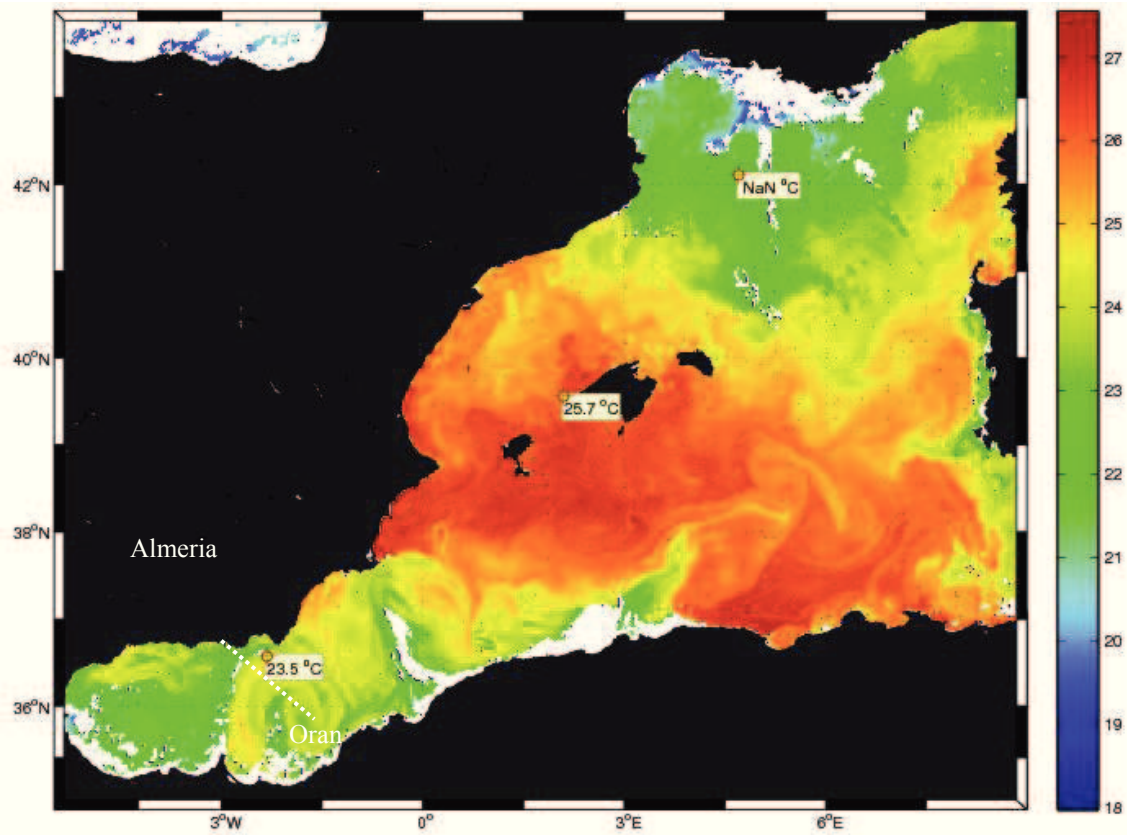

Figure1.2.2. Sea surface from satellite data and oceanographic buoys in the western Mediterranean Sea at 19 July 2012 (courtesy of Diego Alvarez from SOCIB, Palma, Illes Balears, Spain, www.socib.es).

Another important hydrographic process that occurs in the Western Mediterranean Sea is the formation of a stable thermocline during the warm season, which can reach up to 60 m in depth, separating the warm surface layers (up to 28º C in some areas) from colder (around 13º C) deeper waters. In the autumn the decrease of insolation and the autumnal storms break the thermocline (Flos, 1985).

1.3. DISTRIBUTION AND MIGRATIONS OF TUNAS IN THE WESTERN MEDITERRANEAN SEA

Tunas are worldwide distributed between the latitudes of ~70° N and ~45° S (McKenzie, 1964; Mather *et al*., 1995). Their geographical range and vertical distribution are strictly related to the water temperature, oxygen content and turbidity (Brill *et al*., 2005; Boyce *et al*., 2008). According to their habitat preferences tunas are divided into two groups: (i) living mainly in tropical waters (species of the genera *Auxis*, *Euthynnus*, *Katsuwonus* and the subgenus *Neothunnus*), and (ii) living in temperate waters (*Allothunnus* spp. and species of the subgenus *Thunnus*) (Fig. 1.3.1). The tropical species generally live in the surface layers (above the

termocline, McKenzie, 1964) of the equatorial and subtropical oceans, characterised by low salinity, high turbidity (due to the primary production), and high oxygen concentration (Brill *et al*., 2005). *Allothunnus* spp. and the four species of the subgenus *Thunnus* (*Thunnus*) (*Thunnus thynnus, Thunnus orientalis, Thunnus maccoyii* and *Thunnus alalunga*) inhabit the cold productive waters at high latitudes (Collette *et al.*, 2001), while *Thunnus* (*Thunnus*) *obesus* inhabits the deep waters as well as the surface layers of the equatorial areas (Brill *et al*., 2005). All tunas, with the exception of *Allothunnus*, spawn in warm waters. The tropical tunas spawn throughout the year in the equatorial region, and seasonally in the tropical areas, while the cool-water *Thunnus* spp. migrate seasonally from the feeding grounds to spawn in warm waters.

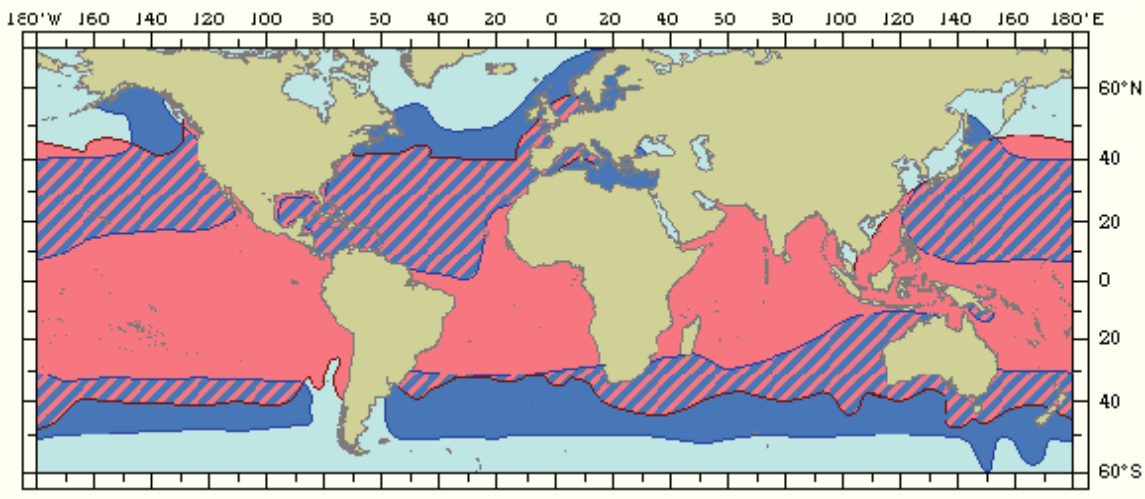

Figure 1.3.1. Geographical distribution of temperate (dark blue) and tropical (red) tunas (Majkowski, 2010). Light blue areas are not included in the geographical range of tunas; stripped areas represent temperate and tropical tuna overlaps**.**

There are five species of tunas in the western Mediterranean Sea: *Auxis rochei*, *Euthynnus alletteratus*, *Katsuwonus pelamis*, *T. alalunga* and *T. thynnus* (Alemany *et al*., 2010) (Fig. 1.3.2). Most of these species, except *K. pelamis*, can be found in this sea through the whole year, but the knowledge of their patterns of distribution and migration is scarce (Di Natale, 2009; Majkowski *et al.*, 2011). *Katsuwonus pelamis* is present in the western Mediterranean Sea in the summer (Macías *et al*., 2010) where it can also spawn (Alemany *et al*., 2010), but its movements into the Mediterranean Sea have not been investigated. However, it is likely that it could move northward from the tropical areas of the Atlantic Ocean following the seasonal variation of the 18° C isotherm (Cayre and Farrugio, 1986) and it could enter in the western Mediterranean Sea when this isotherm rises up the Gibraltar Strait.

Figure 1.3.2. The tuna species sampled: **a**, *Auxis rochei*; **b**, *Euthynnus alletteratus*; **c**, *Katsuwonus pelamis*; **d**, *Thunnus alalunga*; **e**, *Thunnus thynnus*.

All the other species are resident in the western Mediterranean Sea, although migrations outside of the Mediterranean Sea are reported for *T. alalunga* (Arrizabalaga *et al*., 2003), well documented for *T. thynnus* (Rooker *et al*., 2007) and only suggested for *A. rochei* (Sabatés and Recasens, 2001). Spawning of the resident species takes place in the summer, mainly around the Balearic Islands and Sicily (Piccinetti *et al.*, 1996; Tsuji *et al.*, 1997; Alemany *et al*., 2006, 2010). The minimum surface temperatures for spawning seems to be slightly different between species: the larvae of *A. rochei* are found at the lower temperature values (from 19º C), followed by *T. thynnus* (20.5° C), and those of the remaining species when water temperature is over 23º C (Alemany *et al*., 2010). During this period spawners swim in large schools and spend most of the time above the thermocline. The information on the movements of adults from the western Mediterranean Sea is mainly limited to the Atlantic bluefin tuna (Cort *et al.*, 2010; Sorell, 2011; Tudela *et al.*, 2011). The migrations of this species depend on fish age and size, and occur in association with reproduction and feeding (Cort *et al.*, 2011). Thus, after spawning almost all

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adults start a trophic migration from the western Mediterranean Sea to the Atlantic feeding grounds (Rooker *et al*., 2007). Nevertheless, some large specimens remain in this basin, staying in some cases within the spawning areas, where they can be found in deeper layers (*golfitani* tunas) (Mather *et al*., 1995), or often migrating within the western Mediterranean Sea to more productive areas, such as the Aeolian and Tuscan archipelagos, the Gulf of Lion and crossing the Strait of Messina toward the central Mediterranean Sea (Tudela *et al.*, 2011). Juveniles make shorter migrations than adults but with a similar pattern. They are more resident and spend more time within the western Mediterranean Sea, particularly in the productive areas (Gulf of Lion and Ligurian Sea) (De la Serna *et al.*, 2011; Sorell, 2011; Tudela *et al*., 2011). Nevertheless, many juveniles leave the Mediterranean Sea looking for richer grounds in the Atlantic Ocean (Mather *et al*., 2005; Royer *et al*., 2005). The western Mediterranean Sea is also a region of travelling of tunas that migrate from the Atlantic Ocean to the central and eastern Mediterranean Sea (Mather *et al*., 2005).

1.4. FISHERY AND FISHERY MANAGEMENT PROBLEMS OF TUNAS

The tuna fisheries are among the most important in the world (Fig. 1.4.1) (5.2 million t in 2010, 27% of total fish captures). The landings of the five species considered in this thesis (*Auxis rochei*, *Euthynnus alletteratus*, *Katsuwonus pelamis*, *Thunnus alalunga*, and *Thunnus thynnus*) represent the 61% of the total tuna catch. In 2010 the landing of these species in the Mediterranean is 25318 t, representing the 9% of the total fish landing in this area (Fig. 1.4.2) (FAO, 2011). Among them, the highest volume is that of *A. rochei* (9829 t), representing the 39% of the tuna catches from the Mediterranean Sea and the 3% of the total catches of *Auxis* spp. worldwide (Fig. 1.4.3).

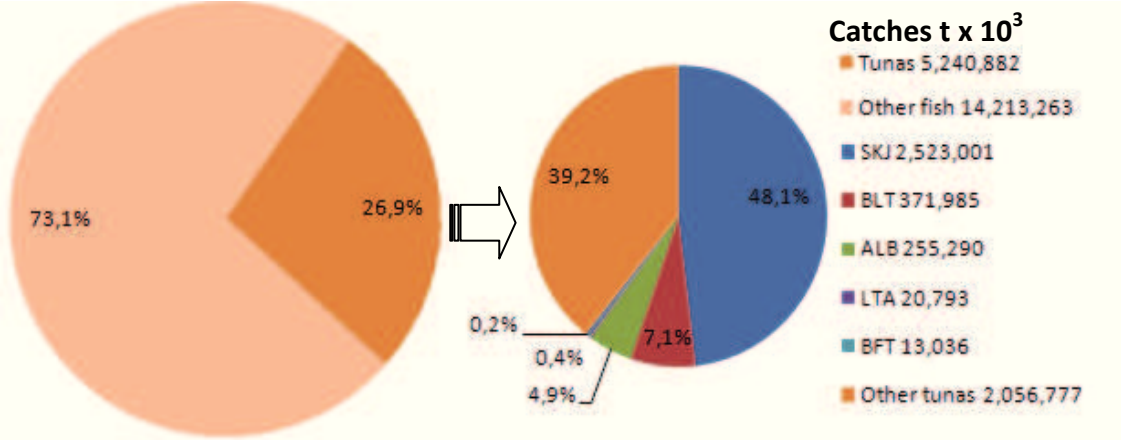

Figure 1.4.1. Global tuna catches (FAO, 2011). Abbreviations: ALB, *Thunnus alalunga*; BFT, *Thunnus thynnus*; BLT, *Auxis rochei*; LTA, *Euthynnus alletteratus*; SKJ, *Katsuwonus pelamis*.

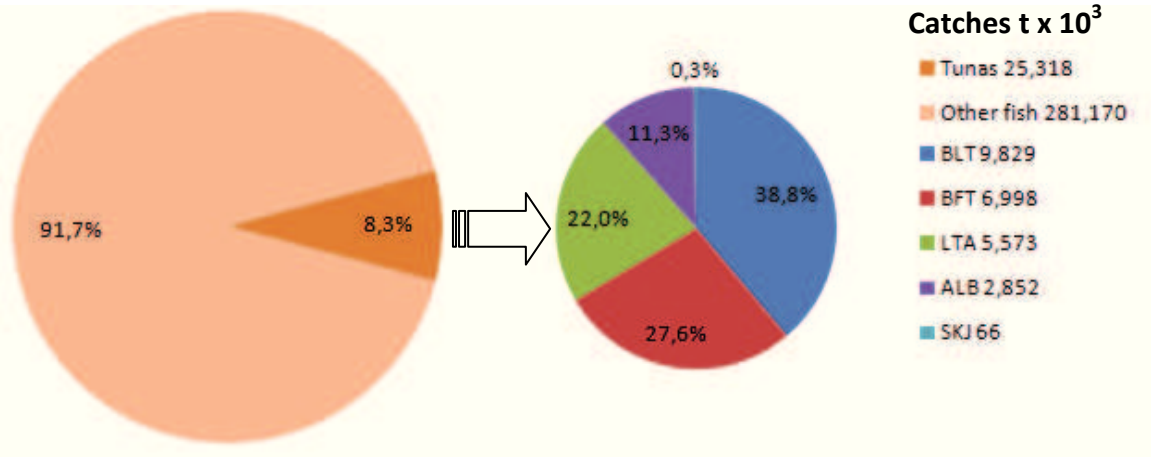

Figure 1.4.2. Mediterranean tuna catches (FAO, 2011). Abbreviations: ALB, *Thunnus alalunga*; BFT, *Thunnus thynnus*; BLT, *Auxis rochei*; LTA, *Euthynnus alletteratus*; SKJ, *Katsuwonus pelamis*.

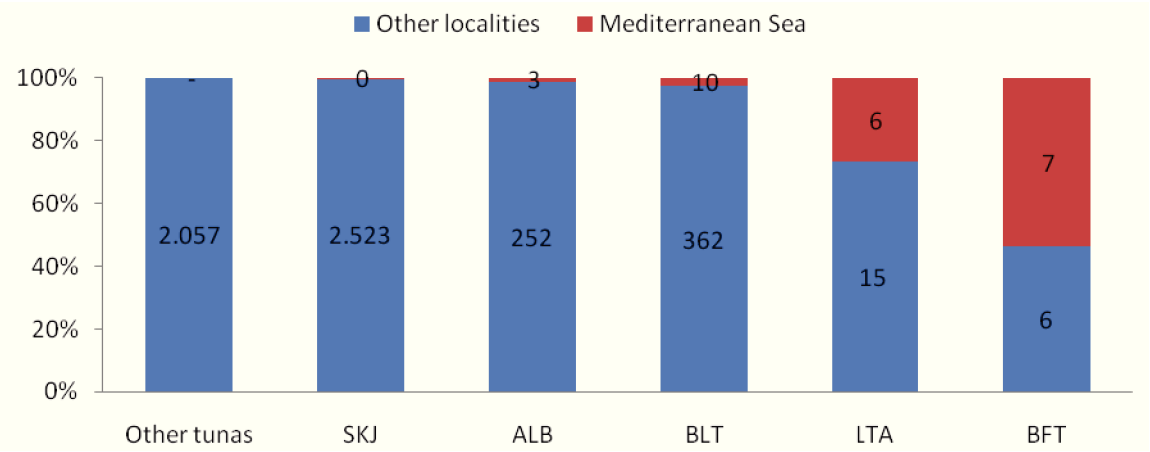

Figure 1.4.3. Global tuna catches (t x 10^6) (FAO, 2011). Abbreviations: ALB, *Thunnus alalunga*; BFT, *Thunnus thynnus*; BLT, *Auxis rochei*; LTA, *Euthynnus alletteratus*; SKJ, *Katsuwonus pelamis*.

In order of weight, it is followed by the fishery of *T. thynnus* (6998 t, 54% of the total catch of this species), which captures are limited by a total allowable catch determined by the ICCAT. The Mediterranean landings of *E. alletteratus* are 5573 t, 27% of the total volume of catches of this species, those of *T. alalunga* 2852 t (1% of total catch), and those of *K. pelamis* 66 t, which represent a negligible percentage of the global catches, since it is the third species in the ranking of total marine fish landings worldwide (Fig. 1.4.1-3) (FAO, 2011).

Several fishing gears are used in tuna fisheries: troll lines, drifting longlines, purse seines and traps (Di Natale, 2009). The oldest gear is the tuna trap (Di Natale, 2012), first developed by the Phoenicians and improved by the Romans, and that in its present form is being used from the Middle Age (Fig 1.4.3). The tuna traps have shown a broad spreading along the Africa, Sardinia and Iberia coasts from the XIV Century, during the expansion of the Aragon and Castilla

kingdoms in the Mediterranean Sea. This has been the dominant gear until the half of the XX Century, thereafter gradually disappearing because it is less competitive than others (Addis *et al.*, 2009).

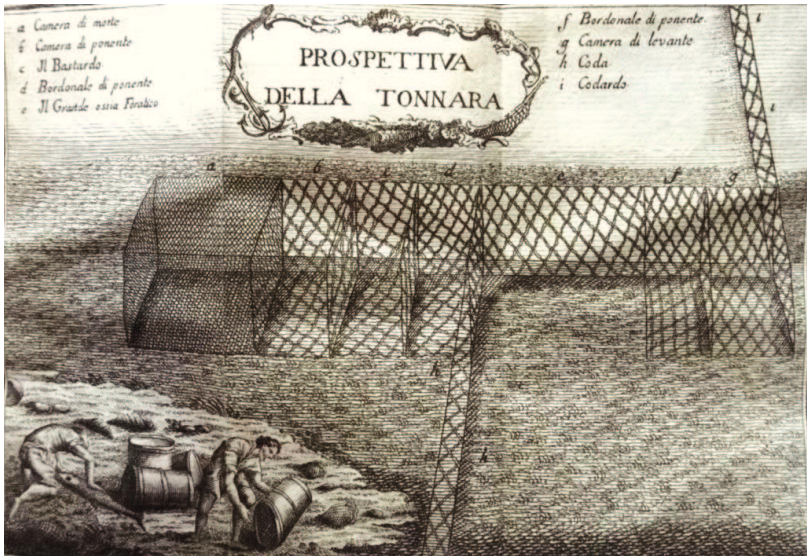

Figure 1.4.4. Plan of a tuna trap (*tonnara*) (Cetti, 1777).

The target species of the tuna trap is the bluefin tuna, but it is also used to catch small tunas (bullet tuna, Atlantic little tunny, skipjack tuna). To date, the number of gears deployed and the total catches of the tuna trap are increasing in the Moroccan and Iberian coasts of the Atlantic Ocean adjacent to the Gibraltar Strait, while they are decreasing along the Mediterranean coasts, replaced by other gears (Abib Idriss, 2009; Addis *et al*., 2009; Fontaneau, 2012). Hooks and line gears are also used from ancient times.

The introduction and improvement of new fishing gears, such as purse seine and longline, allowed increasing the fishing effort on tunas since World War II (Catarci, 2004). This fact concerned particularly *T. thynnus*, with a significant increase of the total catches in the last decades, mainly in the Mediterranean Sea, where this species has the most important spawning area and fishing grounds (Mathers *et al*., 2005). This caused a deterioration of the spawning stock biomass from the 1970s, that led to a full exploitation of the commercial stock (ICCAT, 2011). In order to respond to this situation, in 2007 the GFCM and ICCAT coordinated a multiannual plan of control, to allow the recovery of the stock, which strengthened the measures to protect this species, established limitations of fishing capacity and lowered the TACs for each country (Rec. 08-05 and transposed in regulation EC No. 1559/2007). The first results after the application of these measures seem to indicate a gradual recovery of the resource (ICCAT, 2011, 2012). Nowadays, the bluefin tuna fishery changes rapidly, due the improvement of the tuna

farming for fattening. In fact, most of the purse seine catches in the Mediterranean Sea are transferred to fattening cages rather than sold directly (ICCAT, 2011).

The albacore fishery supports the third largest fish canning industry, after those of the skipjack and yellowfin tunas (Catarci, 2004). The albacore for canning is generally caught by trolling in the Atlantic and Pacific Oceans. In the Mediterranean area the albacore is mostly caught for direct consumption by longlines and sport fishing (Di Natale, 2009). The Mediterranean stock represents a separate unit from the northern Atlantic one. Until now, it is considered underexploited although the real status of the stock is unknown (Majkowski *et al*., 2011).

The status of the stocks of the Atlantic little tunny, bullet tuna and skipjack tuna in the Mediterranean is generally unknown, although it is acknowledged that a significant number of fishermen depend on the catches of these species and that their economic value is high (Di Natale, 2009).

1.5. THE PARASITES OF TUNAS OF THE WESTERN MEDITERRANEAN SEA

Parasitism is one of the most common, if not the most common, way of life on Earth (Rhode, 2002), and it may be considered as the most successful life form on Earth. Parasitism is a close association between two organisms, one of which, the parasite, depends on the other, the host, deriving some benefit from it without necessarily damaging it (Rohde, 2005).

The general characteristics of the parasite groups infecting tunas are described in this chapter, whereas a detailed analysis of the gill and head parasites of tunas are reported in the chapters specific to each host species.

The commercial importance of tunas played a significant role to promote studies on its parasites worldwide, particularly in those countries where this fishery is well developed, such as France, Italy and Japan. The first studies on the parasites of tunas date from the XIX Century, and they started independently in each region: Ariola (1902), Brian (1906), Dollfus (1926, 1952), Legendre (1940), Dawes (1947) and Palombi (1949) in the Mediterranean Sea and Atlantic Ocean; and Goto (1894), Ishii (1935) and Yamaguti (1958, 1970) in the western Pacific Ocean. This fact caused some taxonomic confusion, which in many cases is still not resolved (Nikolaeva, 1985).

Most of the studies on tuna parasites deal with metazoan parasites, but also protozoans have been reported (Munday *et al*., 2003).

Kingdom Protozoa

The Protozoa comprise unicellular eukaryotic organisms which exist as structurally and functionally independent individual cells (Fig. 1.5.1) (O'Donoghue 2005).

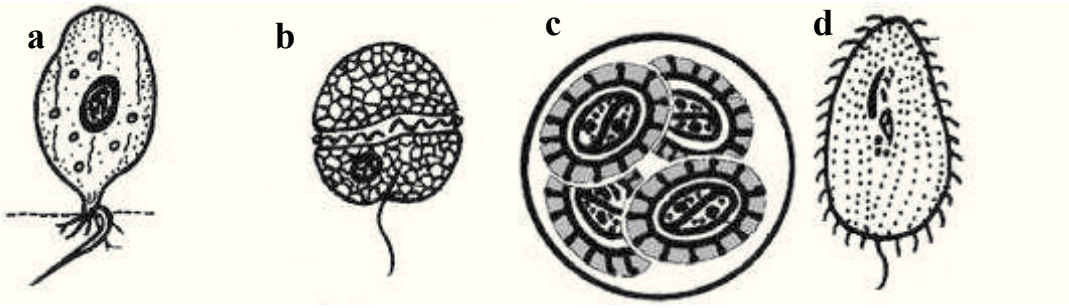

Figure 1.5.1. Protozoan parasites (after O'Donoghue, 2005): **a-b**, Myzozoa; **c**, Apicomplexa; **d**, Ciliophora.

Some protozoan species are parasites of tunas, such as: the ciliate *Uronema nigricans* (Müller, 1786) Florentin, 1901 (Ciliophora: Uronematidae) in the brain of *Thunus maccoyii* (Munday *et al.*, 1997); the apicomplexan *Goussia auxidis* (Dogiel, 1948) (Apicomplexa: Calyptosporidae) in the liver and spleen of tunas from the south Pacific Ocean (*Allothunnus fallai*, *Katsuwonus pelamis*, *Thunnus alalunga*, *Thunnus albacares*) (Jones, 1990); the myozoan *Ichthyodinium chabelardi* Hollande *et* Cachon, 1953 (Myzozoa: Dinophysiaceae) in the yolk-sac of the larvae of *T. albacares* (Yuasa *et al.*, 2007). To date, no protozoan infections are reported in tunas from the Mediterranean Sea.

Kingdom Fungi

The Fungi comprises eukaryotic organisms provided of cell wall and reproducing with spores. Almost all the species are saprophytes or symbiotic, but some live as parasites on plants or animals. Several species are parasites of marine fish. A microsporidian parasite (Microsporidia) was reported in the cells of the red muscle of *Thunnus maccoyii* (Zhang *et al.*, 2010) (Fig. 1.5.2).

In tunas, no adult cestodes have been described, whereas the larval stages of the eucestode orders Bothriocephalidea, Tetraphyllidea and Trypanorhyncha have been reported worldwide (Gibson *et al*. 2005). In the Mediterranean Sea, Mladineo *et al.* (2008) described plerocercoid larvae of *Hepatoxylon trichiuri* Holten, 1802 (Trypanorhyncha: Sphyriocephalidae) in the stomach mucosa of *Thunnus thynnus* from the Adriatic Sea.

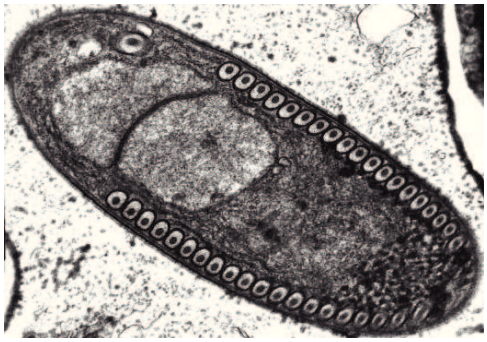

Figure 1.5.2. Microscopridian (after Capella-Gutiérrez *et al*., 2012).

Kingdom Metazoa

Metazoans are eukaryotic, heterotrophic and multicellular organisms, lacking rigid cell walls and motile structures, if only at certain life stages. This kingdom include endoparasites, organisms infecting the viscera and tissues (myxozoans, platyhelminths, acanthocephalans, nematodes), and ectoparasites, infecting the body surface (crustaceans and platyhelminths). This kingdom includes most of the parasite species of tunas (Munday *et al*., 2003; Mladineo *et al*., 2008).

Phylum Myxozoa

This phylum comprises more than 1350 species of microscopic obligate endoparasites characterised by polar capsules, which are complex intracellular structures with an inverted tubule that, through eversion, is used for host attachment (Evans *et al.,* 2010) (Fig. 1.5.3).

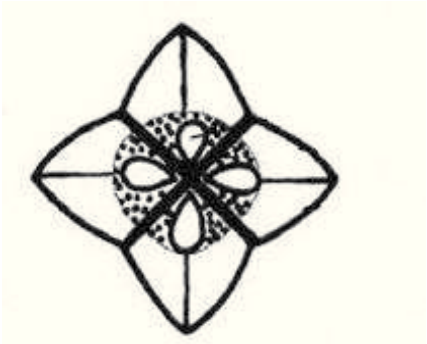

Figure 1.5.3. Myxosporidian (after Lom, 2005).

Some myxozoans of the family Kudoidae (Myxozoa: Myxosporea) have been reported in the muscle of tunas (Munday *et al*., 2003). Mladineo and Bocina (2006) described the first report of *Ceratomyxa thunni* (Myxozoa: Ceratomyxidae) in the gall bladder of *T. thynnus* from the Adriatic Sea.

Phylum Platyhelminthes

The phylum Platyhelminthes is the major sub-division of the kingdom Metazoa (Kearn, 2004). They are dorso-ventrally flattened, acoelomatic and hermaphroditic worms with an incomplete digestive tract (Matthews, 1998).

Platyhelminthes includes four classes: Cestoda, Digenea and Monogenea, that are exclusively parasitic, and Turbellaria that are mainly free-living with a few ectoparasitic representatives. The flatworms are by far the richest group infecting tunas (Bychowsky, 1957; Yamaguti, 1970; Gibson *et al.*, 2005).

Cestoda

Cestodes are heteroxenous parasites lacking digestive system. They are divided into two major subgroups: the Cestodaria, monozoic species with two orders; and the Eucestoda (tapeworms), polyzoic species divided into 11 orders. Eucestoda have the body organised into two portions: the scolex, with the organ of adhesion; and the strobila, consisting of a linear series of compartments (proglottids), each of which has one or more sets of reproductive organs (Fig. 1.5.4) (Rohde, 2005). Adults live in the digestive tract of the vertebrate definitive host (Caira and Reyda, 2005), whereas the larval stages infect the tissues of arthropods and vertebrates.

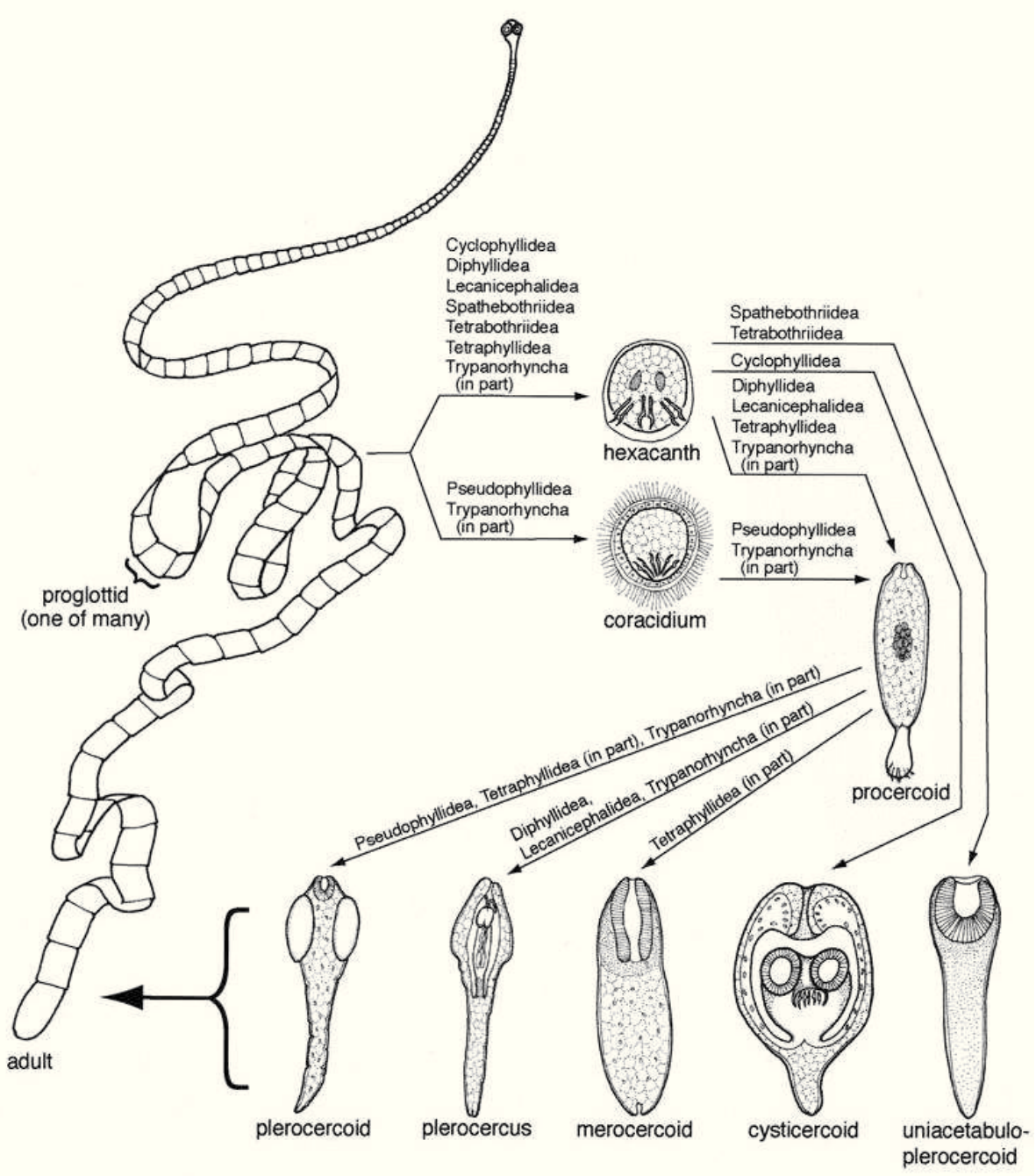

Figure 1.5.4. Adult and larval stages of eucestoda (after Cayra and Reida, 2005).

Monogenea

Monogenea is a class of monoxenous platyhelminths (Hayward, 2005). They are divided into two groups according to the structure of the posterior adhesive organ (opisthaptor): Monopisthocotylea and Poliopisthocotylea (Hayward, 2005).

Monopisthocotylea

Monopisthocotyleans are characterised by a single opisthaptor, usually with shape of a disc, or also sucker-like (Fig. 1.5.5). Most of the Monopisthocotylea are ectoparasites, although

some have secondarily adopted an endoparasitic way of life, infecting the gut, heart, muscle, circulatory and urogenital systems of amphibians, chelonids and fish (Euzet and Combs, 1998).

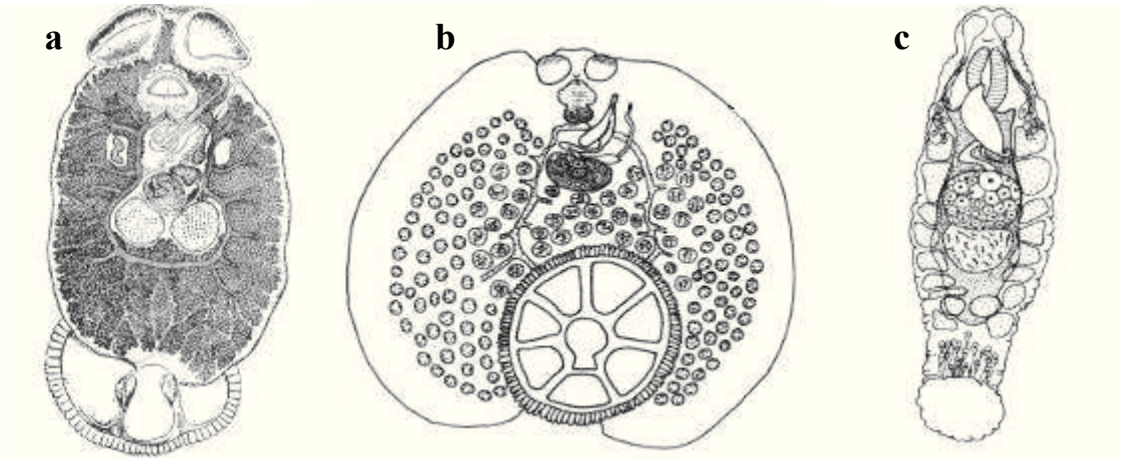

Figure 1.5.5. Monopisthocotylean monogeneans: **a-b**, Capsalidae (after Bychowsky, 1957; Whittington, 2005); **c**, Udonellidae (after Whittington, 2005).

Two families have been reported on tunas: Capsalidae and Udonellidae. In the Mediterranean Sea, three species of capsalids have been found on the gills of *T. thynnus*, *Capsala interrupta* (Monticelli, 1891), *Capsala laevis* (Verrill, 1874) and *C. onchidiocotyle* (Setti, 1899) (Palombi, 1949; Mladineo *et al.*, 2008).

Polyopisthocotylea

The opisthaptor of the polyopisthocotyleans has a higher grade of organization than monopisthocotyleans, comprising several muscular, cup-like suckers or clamp-like organs set on a disk or cotylophore, or on the ventral surface of the body (Fig. 1.5.6) (Whittington, 2005). The number and arrangement of these structures is used to distinguish various families. The members of this order are, with few exceptions, gill parasites.

Three families have been reported on the gills of tunas: Axinidae, Gastrocotylidae and Hexostomidae. Only three species of hexostomids have been reported on the gills of tunas from the Mediterranean Sea: *Hexostoma auxisi* on *Auxis* sp., *Hexostoma thunnina* on *E. alletteratus*, and *Hexostoma thynni* on *T. thynnus*.

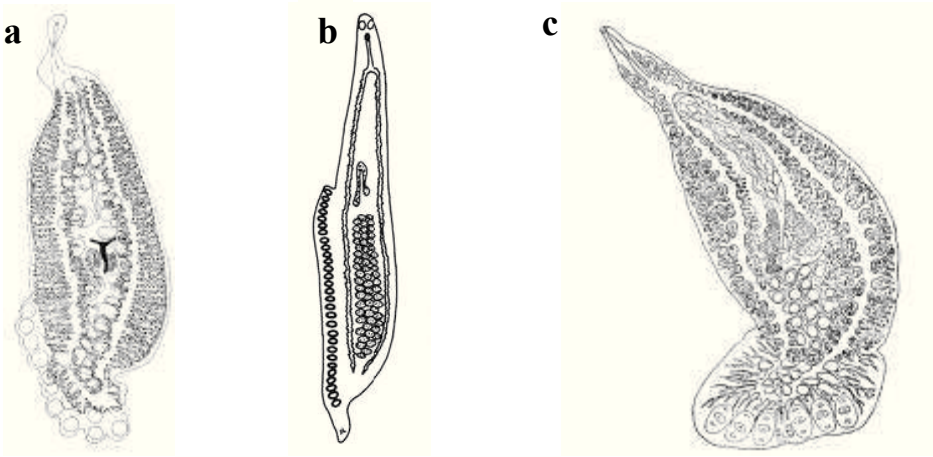

Figure 1.5.6. Polyophisthocotylean monogeneans: **a**, Axinidae (after Rohde and Roubal, 1980); **b**, Gastrocotylidae (after Hayward, 2005); **c**, Hexostomidae (after Bychowsky, 1957).

Trematoda

The class of trematodes comprises two subclasses, the Aspidogastrea (monoxenic) and the Digenea (heteroxenic and heterogenetic). The members of the first group have a ventral organ of attachment composed by several loculi or suckers whereas the species of the second have a ventral or postero-ventral sucker, sometimes absent (Fig. 1.5.7) (Bray *et al*., 2008).

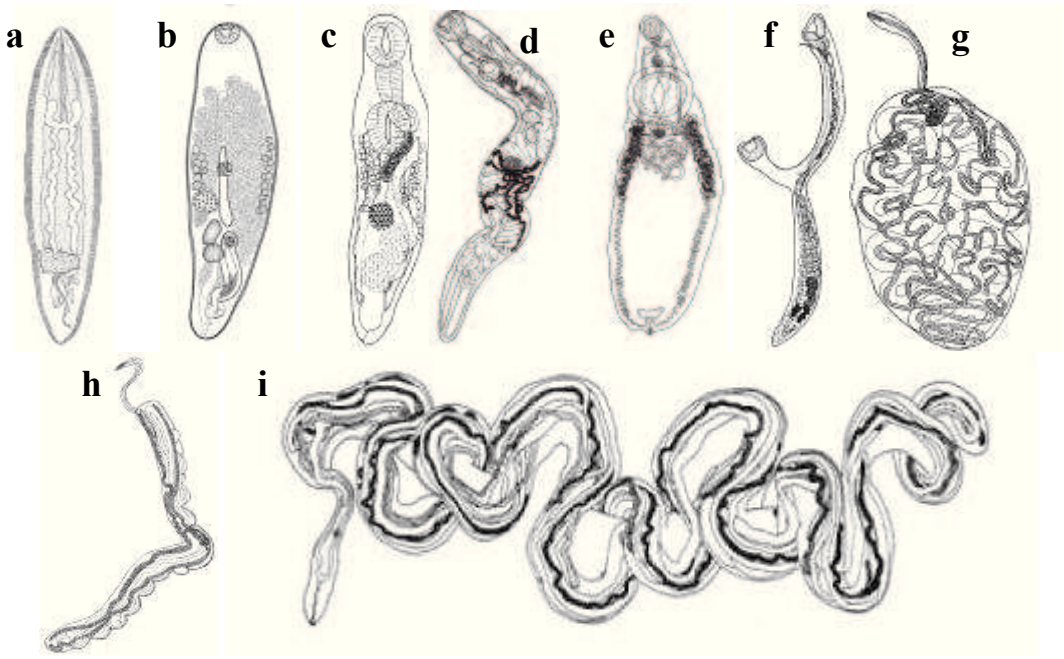

Figure 1.5.7. Digenean trematodes: **a**, Aporocotylidae (after Cribb, 2005); **b**, Bucephalidae (after Cribb, 2005); **c**, Fellodistomatidae (after Bray *et al*., 2008); **d**, Hemiuridae (after Bray *et al*., 2008); **e**, Hirudinellidae (after Bray *et al*., 2008); **f**, Syncoeliidae (after Bray *et al*., 2008); **g-h** Didymozoinae (after Madhavi, 1982); **i**, Nematobothriinae (after Cribb, 2005).

The adult digeneans are primarily parasites of the gut, but they also occur free or incapsulated in the tissues of the vertebrates (Cribb, 2005).

According to two-hosts or three-hosts life cycle, the immature stages can be found in the tissues of one or more intermediate hosts. In the two-host life cycles, the first intermediate host is a mollusc (rarely an annelid), where it replicates asexually producing sporocysts and rediae before to generate the cercaria (infective stage of definitive host). In the three-host life cycle, the cercaria infects a second intermediate host or, perhaps, a paratenic host that can be a fish, crustacean, cnidarian and mollusc.

Several families of digeneans infect tunas: Aporocotylidae, Bucephalidae, Didymozoidae, Fellodistomatidae, Hemiuridae, Hirudinellidae and Syncoeliidae (Silas, 1962; Gibson *et al*., 2005; Bray *et al*., 2008). Concerning the species occurring in the Mediterranean Sea, a didymozoid species (tissues flukes) (*Didymozoon auxis* Taschenberg, 1879) has been reported in the gills of *Auxis* sp. (Dollfus, 1926; Silas, 1962); an aporocotilid (blood fluke) in the heart of *T. thynnus* (Mladineo *et al*., 2008; Ruiz de Ybañez *et al*., 2010); a bucephalid, *Rhipidocotyle pentagonum* (Ozaki, 1924), and a hemiurid, *Stherrurus imocavus* Loss 1907, in the lumen of the gut of the same host (Silas, 1962). In addition, several species of didymozoids have been found encapsulated in the gills of *T. thynnus*: *Didymocylindrus filiformis* Ishii, 1935, *Didymocystis reniformis* Ariola, 1902, *Didymosulcus wedli* (Ariola, 1902), *Didymozoon longicolle* Ishii, 1935, *Didymozoon pretiosus* Ariola, 1902, *Kollikerioides apicalis* Yamaguti, 1970, *Wedlia bipartita* Ariola, 1902 (Ariola, 1902; Mariniello *et al*., 2000; Mladineo *et al*., 2008) and in the wall of the gut: *Koellikerioides intestinalis* (Yamaguti, 1970), *Koellikerioides internogastricus* Yamaguti, 1970, *Coeliodidymocystis abdominalis* (Yamaguti, 1938), *Didymocystoides oesophagicola* Yamaguti, 1970, *Oesophagocystis dissimilis* (Yamaguti, 1938); mouth cavity: *Didymosulcus palati* (Yamaguti 1970); operculum: *Didymocystoides bifasciatus* (Yamaguti, 1970) and skin of *T. thynnus*: *Didymocystoides pectoralis* Yamaguti, 1970 and *Platocystis alalongae* Yamaguti, 1938) (Mladineo *et al*., 2008).

Phylum Nematoda

The phylum Nematoda (round worms) comprises 256 families and more than 40000 species, and is one of the largest and most successful groups of the animal kingdom (Anderson, 2000). Although most of the nematodes are free living, several species live as parasites on animals (Fig. 1.5.8). Nematodes are dioecious and heteroxenic (McClelland, 2005). They have a fluid-filled pseudocoel and a complete digestive system, with the mouth at the anterior extremity and the anus close to the posterior end (McClelland, 2005). The adults are endoparasites of the

gut and tissues, while the larval stages infect the body cavities or tissues of the intermediate or paratenic hosts.

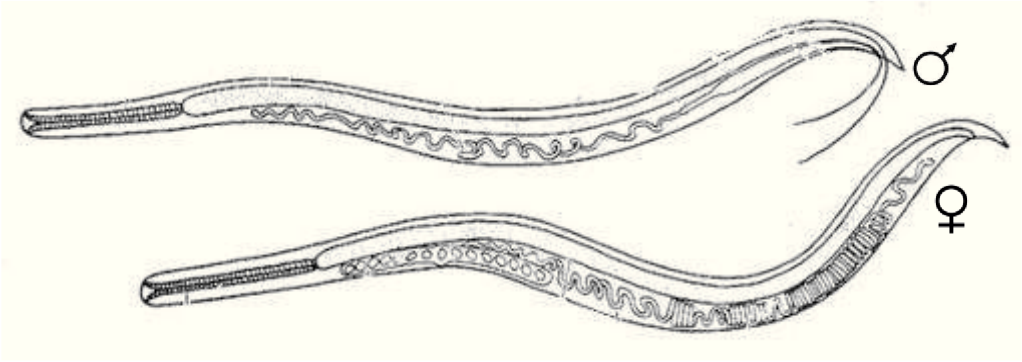

Figure 1.5.8. Male and female nematode parasites (after McClelland, 2005).

Adults of three families of nematodes have been reported in the gut (Camallanidae and Cystidicolidae) and the gonads (Philometridae) of tunas. Larval stages of several species of Ascaridoidea (particularly of the family Anisakidae) have been reported in the tissues of tuna worldwide (Munday *et a*l., 2003; Gibson *et al*., 2005). Adults of *Onchophora melanocephala* (Rudolphi, 1819) (Camallanidae) and anisakid larvae have been found in *A. rochei* and *T. thynnus* from the Mediterranean Sea (Gibson *et al.*, 2005; Mladineo *et al*., 2008).

Phylum Acanthocephala

Members of the phylum Acanthocephala, the so called spiny headed worms, are dioecious heteroxenic parasites. Their bodies lack intestine and have a characteristic thorny organ in the anterior part, which allows the adhesion of the adult parasites to the intestinal wall of the host (Fig. 1.5.9) (Taraschewski, 2005). The immature stages live encapsulated in the tissues of crustaceans and fish (intermediate or paratenic hosts). Several adult stages of species belonging to the families of Cavisomidae, Rhadinorhynchidae and Diplosentidae have been found in the intestine of tunas, whereas immature stages of the family of Polymorphidae have been found encysted in the tissues.

In the Mediterranean tunas two acantocephalan species have been reported: larval stages of *Bolbosoma vasculosum* (Rudolphi, 1819) (Polymorphidae) and adults of *Rhadinorhynchus pristis* (Rudolphi, 1802) (Rhadinorhynchidae) in *T. thynnus* (Gibson *et al*., 2005; Mladineo *et al*., 2008)

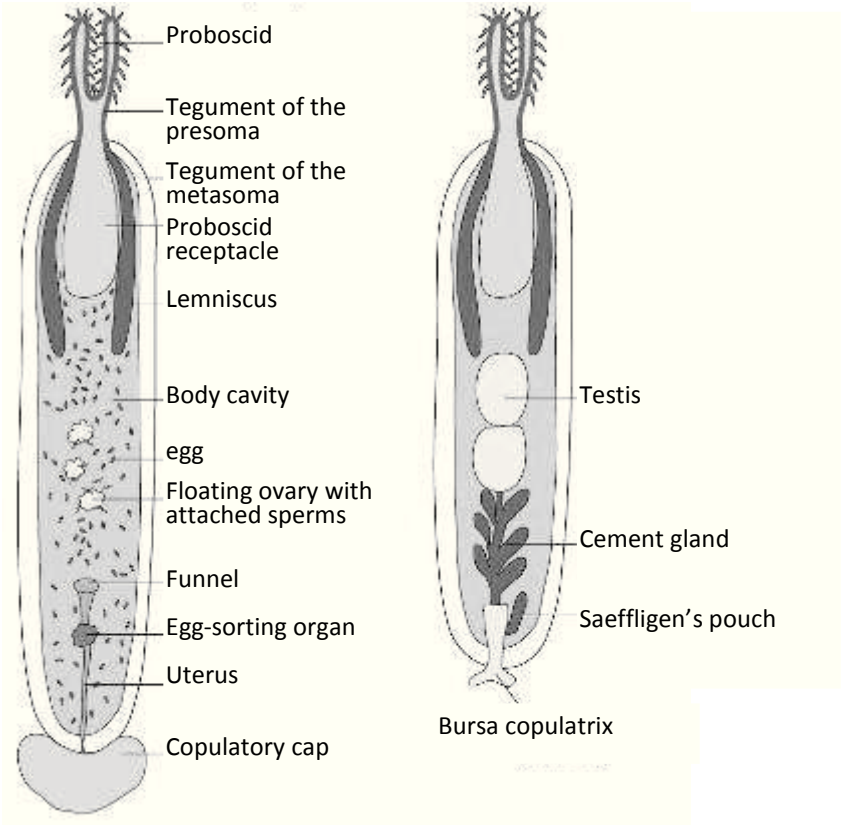

Figure 1.5.9. Male and female acanthocephalans (after Taraschewski, 2005).

Phylum Arthropoda subphylum Crustacea

Arthropods are dioecious invertebrate animals having an exoskeleton (external skeleton), a segmented body, and joined limbs (Kearn, 2004). They include insects, arachnids, and crustaceans. Among them, the crustaceans are the most diverse and ubiquitous subphylum of arthropods in the seas (Rohde, 2005). Most of the crustacean parasites are ectoparasites of a wide range of marine invertebrate and vertebrate organisms, but some species have adopted a mesoparasitic or endoparasitic life style (Rohde, 2005).

The crustacean parasites of tunas belong to two classes: Malacostraca (aegid and cymothoid isopods), and Maxillopoda (bomolochid, caligid, euryphorid, lernaeopodid, pennellid, pseudocycnid copepods) (Figs. 1.5.10-11). In the Mediterranean Sea four copepod species have been reported on *T. thynnus*: *Brachiella thynni* Cuvier, 1829 (Lernaeopodidae) reported by Aristotle (384-321 BC, see Brian, 1906), *Euryphorus brachypterus* (Gerstaecker, 1853) (Euryphoridae), *Pseudocycnus appendiculatus* Heller, 1865 (Pseudocycnidae), and *Pennella filosa* (Linnaeus, 1758) (Pennellidae) (Brian, 1906; Palese and Palese, 1992; Žilic *et al.*, 2007).

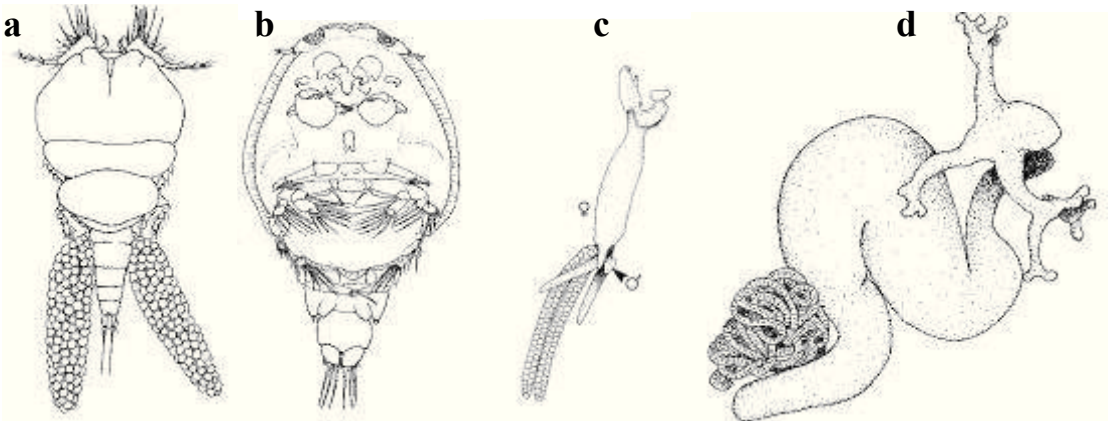

Figure 1.5.10. Copepod parasites (Boxshall, 2005): **a**, Bomolochidae; **b**, Caligidae; **c**, Lernaepodidae; **d**, Pennellidae.

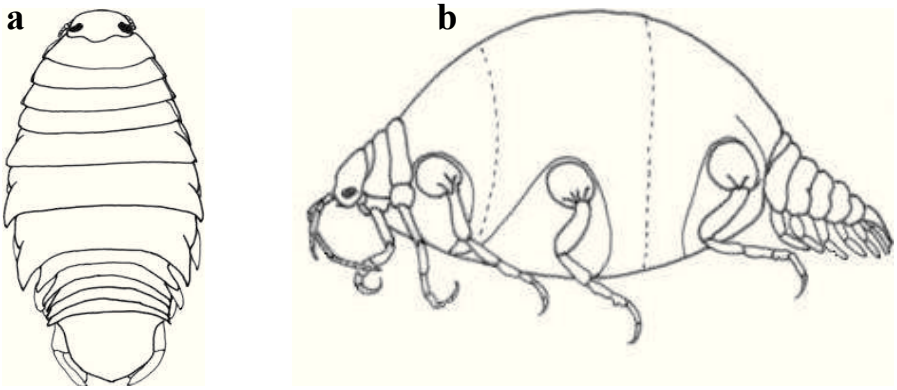

Figure 1.5.11. Isopod parasites (Lester, 2005): **a**, adult of cymothoid; **b**, larval stage of gnatiid.

1.6. PARASITES AS BIOLOGICAL TAGS IN MARINE FISH

Parasites have been successfully used as biological tags to investigate the biology, ecology, migration and population structure of marine organisms, particularly of commercially important fish species (MacKenzie, 2002). The basic principle underlying the use of parasites as tags in fish population studies is that fish can be infected with a parasite only when they enter in the geographic range of the parasite, and where and when the conditions are suitable for its transmission. If infected fish are found outside of this area, it can be inferred that these fish have been in that area at some time of their history (MacKenzie and Abaunza, 2005).

Natural parasitic tags offer several advantages when compared with artificial tags (Williams *et al*., 1992; Pawson and Jennins, 1996; Mosquera *et al*., 2003; MacKenzie and Abaunza, 2005):

- They are most appropriate in studies of delicate or deepwater species and crustaceans, which may shed their artificial tags during moult (Herrington *et al*., 1939).
- Parasites are cheaply sampled from routine samplings, as fish only need to be caught once, and can also provide preliminary information to aid the design of expensive and complex

artificial tagging experiments (Pawson and Jennins, 1996). Moreover, when fish have to be captured for tagging, it is possible to mark only a very small proportion of the population and even a smaller numbers of fish are recaptured.

- The use of parasites that have no pathological effects ensures that the biological approach is less biased by doubts concerning possible abnormal behaviour of tagged hosts.

However, there are some limitations in the use of parasites as tags, as indicated by Mosquera *et al.* (2003):

- What makes parasites useful in stock identification is the composition of the parasite population, which is itself a dynamical variable changing seasonally. Therefore, it is important to know whether or not the differences in the parasite distribution are consistent throughout the whole year.
- There are latitudinal changes in the intensity of infection, as well as in the number and kinds of parasites, which may simply reflect influences of the temperature or other physical factors on the parasite fauna.
- Parasite distribution may reflect variations in the distribution or abundance of intermediate hosts rather than the hosts being studied.
- There may exist long-term fluctuations in the parasite abundance that cannot be revealed by short-term scale studies.

The characteristics of a parasite to be used as indicator of aquatic host populations are shown in the following guidelines indicated by MacKenzie (1983, 1987), Williams *et al*. (1992), Mackenzie and Abaunza (1998, 2005), Mosquera *et al*. (2003). Parasites fulfilling all of these criteria are rarely found, so compromises usually have to be made:

- It should have significantly different levels of infection in the target host in different localities of the study area, *i.e.* differences in prevalence and/or mean intensity of infection. Infection data can be analysed according to prevalence, intensity and abundance of infection, as defined by Bush *et al*. (1997).
- It should have a lifespan, or remain in an identifiable form, in the target host long enough to cover the time scale of the investigation. The minimum acceptable lifespan will vary depending upon the type of study. For stock identification and recruitment studies, only parasites with lifespan of more than one year should be used, whereas for studies of seasonal migrations species with lower lifespan are suitable.
- Monoxenic parasites are the simplest to use. Heteroxenic parasites are more difficult to use because more information is required on the biotic and abiotic factors influencing their transmission between hosts. Given this information they can be used. Køie (1983) suggested

that digenetic trematodes have advantages as tags because they tend to be highly specific to the primary host, which is usually a mollusc. The endemic area of a digenean is therefore largely determined by the geographic distribution of its mollusc host.

- The level of infection should remain relatively stable from season to season and from year to year. Seasonal variations, however, can cause seasonal migrations of the subject host. Problems posed by annual fluctuations can be solved by following infections in single year classes of the target host and by avoiding comparisons of data from mixed cohorts.
- A good knowledge of the ranges of tolerance of the different parasite stages of a tag parasite, and of its hosts, to various environmental factors is important, because these factors act directly on the ectoparasites and on the free-living larval stages. They may also act indirectly by limiting the distributions of intermediate hosts.
- The parasite should be easily detected and identified. Examination of the host should involve the minimum of dissection.
- Parasites that are serious pathogens should be avoided.

One of the first attempts to use parasites as indicators in a study of fish biology was that of Dogiel and Bychovsky (1939) to distinguish between stocks of the sturgeon (*Acipenser* spp., Acipenseridae) in the Caspian Sea. Since then, a wide range of parasites from several different taxonomic groups have been found to be suitable biological indicators (Williams *et al*., 1992). Many different taxonomic groups of parasites have been used as tags to investigate several biological and ecological characteristics of numerous commercially important pelagic fish (Williams *et al*., 1992; MacKenzie and Abaunza, 2005). Almost all the studies on the pelagic fish concern the investigation of the population structure of the host species (population/stock separation, recruitment and seasonal migration), but also many contributions have dealt to investigate the diet, feeding behaviour and systematic of the host. A brief description of these applications is here reported (Williams *et al*., 1992):

- **Population separation**. These studies aim to identify, within a host species, populations which are distinguished by different patterns of behaviour at certain stages of their life history. Populations may differ in nurseries, feeding, or spawning grounds, or in some other behaviour.
- **Recruitment**. Parasites provide valuable information on fish recruitment, which is the incorporation of juveniles from nurseries to the stock. The choice of parasites as indicators of recruitment must fulfil two important requirements:
  - The parasite must infect recruits in the nursery and the adult host should not be susceptible to further infection;

- The parasite must have a life span long enough to be identifiable in the adults.

- **Seasonal migrations**. The adults of many species of fish undergo seasonal feeding and spawning migrations. A basic requirement in this type of study is that the parasite is acquired by the fish in certain area of the migratory range but not in others. Since the time scale of seasonal migration studies is less than 1 year, the longevity of the parasite is not always an important factor.
- **Diet and feeding behaviour**. Some species of parasite also provide information on host diet and feeding behaviour. To become infected with most of the heteroxenous helminth parasites, fish must eat the intermediate host. Since many helminths have some degree of specificity to their intermediate host, the presence of these parasites in fish indicates predation on specific organisms. Whereas examination of stomach contents shows only the very recent food items eaten by the fish, parasites can give an indication of the diet over a long period of time.
- **Phylogeny and systematics**. Parasites have been used successfully to clarify doubtful systematic relations between closely related hosts (Whittington, 2005; Oliva *et al.*, 2008). In cases of strict host specificity (oioxenic parasites) demonstrated by several monogeneans and trematodes, the parasite presence could be used as a diagnostic criterion for the host species, the parasite being sufficient to identify the host with precision (Lambert and El Gharbi, 1995).

A comprehensive revision on the use of parasites as tags of pelagic fish can be found in Williams *et al*. (1992) and MacKenzie (2002).

1.6.1. Methodological considerations for the application of parasites as biological tags to study the biology, ecology and migration of tunas in the western Mediterranean Sea

Several papers describe the general principles that should be applied to use parasites as tags for marine fish (*e.g.* Williams *et al*., 1992; MacKenzie and Abaunza, 1998, 2005). MacKenzie *et al*. (2008) focused the application of these guidelines on the studies of the small pelagic fish. Taking into account the experiences gained from the EU-funded project HOMSIR on stock identification research of the horse mackerel, *Trachurus trachurus* (Linnaeus, 1758), (Abaunza *et al*., 2004), Abaunza *et al*. (2008) discussed the aspects that should be considered in the sampling design for multidisciplinary approach including tag parasites, to stock identification research. These authors stated that the possible deficiencies and problems occurring in the sampling process and data analysis of fish population can be avoided, or at least limited, with an adequate sampling strategy. On the contrary, no any specific procedure to evaluate the usefulness of parasites as biological tags of the large pelagic fish as tunas exists. However, integrating the methodologies used in previous studies on tuna parasites (Lardeaux, 1982; Mackenzie, 1983;

Lester *et al.*, 1985; Jones, 1991; Rodríguez-Marín *et al.*, 2008) and those described in the studies of parasites as biological tags (MacKenzie and Abaunza, 1998, 2005; Mosquera *et al*., 2003) and the general guidelines for parasite sampling design (Abaunza *et al*., 2008) an *ad hoc* methodology could be developed.

In this chapter, several aspects related to sampling processes, parasitological procedures and statistical methods that should be considered to improve the design and the implementation of studies focused on the use of parasites as biological tags of tunas are evaluated according to MacKenzie and Abaunza (2005) and Abaunza *et al*. (2008). According to these authors, these are:

- Background
- Sampling target
- Sample size
- Replication of study in different times
- Replication of study in different areas
- Influence of host size
- Logistic and multidisciplinary approach
- Parasitological methods
- Outlines of the anatomy and physiology of the gills of Osteichthyes
- Statistical methods
- The selection of biological tags

Background

Previous studies on the use of parasites as tags for tunas concern mainly *Katsuwonus pelamis* and *Thunnus alalunga* from the Pacific Ocean (Lester *et al.*, 1985; Jones, 1991), and *T. alalunga*, *Thunnus albacares* and *Thunnus thynnus* from the Atlantic Ocean (Postel, 1963; Walters, 1980; Lardeaux, 1982; Mackenzie, 1983; Rodríguez-Marín *et al.*, 2008). According to MacKenzie and Abaunza (1998), they can be divided into two groups: studies that investigated the usefulness of selected parasites (Postel, 1963; Walters, 1980; MacKenzie, 1983) and studies on the parasite assemblages (Lardeaux, 1982; Lester *et al.*, 1985; Jones, 1991; Rodríguez-Marín *et al.*, 2008).

Specifically, Postel (1963) suggested *Hirudinella* cf. *oxystoma* Guiart 1938 as tag to study the populations of *T. alalunga* in the North Atlantic Ocean, and Walters (1980) proposed *Euryphorus brachypterus* and *Nasicola klawei* to distinguish the populations of *T. thynnus* from

the North Atlantic Ocean and Mediterranean Sea. MacKenzie (1983) described a systematic approach for the selection of tag parasites to trace the movement of *T. thynnus* in the North Atlantic Ocean.

Lardeaux (1982) analysed 219 heads and viscera of *T. albacares* from nine areas of the Atlantic Ocean between the years 1969 and 1972. He found 40 parasite species, although most of the didymozoids were identified at the genus level. This author used univariate descriptive analyses and multivariate analyses of abundance (canonical correspondence analysis of abundance and presence/absence data) to evaluate the differences between the parasite assemblages of the various areas.

Lester *et al*. (1985) analysed 875 *K. pelamis* caught by commercial and governmental vessels between 1979 and 1982 in 14 areas in the Pacific Ocean. The samples collected by the latter included gills and viscera while those collected by the commercial vessels included the heads and parts of the viscera. The parasitological analyses were carried out on the entire viscera and only the gills of one side were used to save time and effort. Similarity and dissimilarity of the parasite assemblages of the host schools were investigated using cluster analysis and multivariate canonical analysis.

Jones (1991) analysed 403 *T. alalunga* from 13 areas in the Pacific Ocean between 1985 and 1988. He examined heads and viscera of most of the specimens, although in order to save weight and space, several samples were limited to the head (cutted posteriorly to the opercula) or to the gills and viscera. He applied univariate analyses (Mann-Whitney U-test for the prevalence and box plot and ANOVA for the abundance) to study the correlation between the parasite infection and the host size and sex and to compare the prevalences between localities (Mann-Whitney U-test).

Rodríguez-Marín *et al.* (2008) analysed the skin and the heads of 228 *T. thynnus* from the Gulf of Biscay (North-eastern Atlantic Ocean) between 2001 and 2007. They estimated the significance of the differences of prevalence between the sampling years using the X^2 test and evaluated the usefulness of the parasites as tags.

Concerning the tunas studied in this thesis, *i.e.* the five tuna species of the Mediterranean Sea, there are several taxonomical data on the parasites of the gills of *Euthynnus alletteratus* and *T. thynnus* (Brian, 1906; Dollfus, 1926; Palombi, 1949; Arru and Garippa, 1995; Mariniello *et al*., 2000), while there is no information on the parasites of *Auxis rochei*, *K. pelamis* and *T. alalunga* from this area. There are no quantitative studies on their parasite assemblages neither on the parasites tags of tunas from this area, except for *T. thynnus* (Walters, 1980; Mladineo and Tudor, 2004; Mladineo *et al*., 2008, 2010).

Salvatore Mele. Gill metazoan parasites of tunas (Scombridae: Thunnini) from the western Mediterranean Sea: systematics, assemblages and use as biological tags. Tesi di dottorato in Riproduzione, Produzione, Benessere Animale e Sicurezza degli Alimenti di Origine Animale. Università degli studi di Sassari.

On the other hand, there are several studies on the parasite assemblages of *E. alletteratus* and *K. pelamis* from in the central-eastern and the south-western Atlantic Ocean (Bussiéras, 1972; Bussiéras and Baudin-Laurencin, 1973; Alves and Luque, 2006; Cissé *et al.*, 2007). Moreover, several taxonomical reports deal with parasites of *T. alalunga* and *T. thynnus* from the North Atlantic Ocean (Silas, 1962; Silas and Ummerkutty, 1967). Conversely there is no information on the parasites of *A. rochei* worldwide, even if there are ancient reports of gill parasites of *Auxis* sp. from the western Mediterranean Sea (Dollfus, 1926; Palombi, 1949) and the tropical areas of all Oceans (Silas, 1962; Silas and Ummerkutty, 1967).

Many studies on the parasites of tuna are aimed to surveys, to the use of parasites as biological tags (Lardeaux, 1982; Lester *et al.*, 1985; Jones, 1991; Rodríguez-Marín *et al.*, 2008). These studies have been carried out at the level of component community, assuming that the tuna studied was the only host available to the parasites in the area of study. However, this is not everywhere true, because several tuna species are sympatric in some areas of their distribution (*e.g. T. albacares*, *Thunnus obesus* and *K. pelamis* in the tropical areas, and *T. alalunga*, *T. thynnus* in the temperate areas of the Atlantic Ocean) and they share several parasite species (Silas, 1962; Silas and Ummerkutty, 1967). Thus, the distribution and migration of all potential definitive host species present in the area of study should be taken into account when studying the distribution of the parasites they share (Hayward *et al*., 1998; Huyse *et al*., 2005). For this reason, the application of an unique method (same sampling and parasitological protocol, same statistical tools and criteria to analyse the data) for all host species would allow a better understanding of the distribution of such parasites, since the application of different protocols, as occurred in previous studies, *e.g*., Lester *et al*. (1985) for *K. pelamis*, Jones (1991) for *T. alalunga*, makes it difficult to compare the results and hence to obtain general conclusions.

Summing up, the analysis of the literature shows that there are important differences among the levels of knowledge of the parasite fauna of the various tuna species inhabiting the western Mediterranean Sea. This fact implies that studies must start from different levels, in order to acquire the basic information to identify the parasite taxa and to describe the parasite assemblages of tunas from different localities of the western Mediterranean Sea.

Sampling target

The sampling units are considered discrete entities whose assemblage corresponds to the sampling universe (Kenkel *et al*., 1989). According to Poulin (2007) the different parasite assemblages at both the metapopulation and metacommunity levels are linked by exchanges of individual parasites via host migration or other dispersal routes. Then, in this study, the sampling

unit is the component community that infects a specific set of host specimens, *i.e.* of a tuna caught at definite locality and time, whereas the sampling universe is the metacommunity, which is the assemblage of all the component communities of all the tunas sampled in the western Mediterranean Sea at the same time.

Ideally the whole body of host should be analysed for parasites, as indicated in the guidelines proposed by MacKenzie and Abaunza (1998). If the whole body of the specimens is not possible to obtain, the organs and tissues that are the sites of infection of the potentially tag parasites should be identified (MacKenzie *et al*. 2005). In fact, because of the high economical value and size of tunas, in none of the previous studies dealing with the use of tuna parasites as tags the whole fishes were examined, but only the anatomical parts with a limited (or null) commercial interest (*e.g*. gills, head and viscera) (Lardeaux, 1982; Lester *et al*., 1985; Jones, 1991; Rodríguez-Marín *et al.*, 2008). It is important to stress that only the gills are always available, because they are easier to store (compared with the voluminous head and viscera (Jones 1991), and in addition they really represent a waste of the slaughter process, whereas several parts of the head (*i.e.* muscles of head, pharyngeal region and heart) and viscera (*i.e*. stomach, intestine and gonads) are used by fishermen of the Mediterranean region to prepare traditional dishes (Gallart Jornet *et al.*, 2005; Addis *et al*., 2012).

Several studies indicated that the parasites of the gills of tunas have narrow microhabitats, the didymozoids in particular (Yamaguti, 1970; Mladineo *et al*., 2010; Rodríguez-Marín *et al*., 2008), but also the polyopisthocotylean monogeneans (Dawes, 1947; Rohde, 1979; Kearn, 2004). The significance of the site specificity of tuna parasites is not yet known, but it is useful to know if these parasites have (or not) specific distribution patterns (Anderson and May, 1978), since this information allows to investigate the correlation between the distribution of the parasites within the body of the host and the mechanisms of orientation and migration in the host body, as well as some aspects of parasitocoenology, physiology, diseases and population biology of parasites (Kurochkin, 1985; Mladineo and Tudor, 2004; Mladineo *et al*., 2010). Moreover, site specificity can be helpful by limiting sample dimensions and thus saving time for parasitological examination. This is an important characteristic to be evaluated when estimating the usefulness of a parasite as tag (MacKenzie and Abaunza, 2005).

Therefore, for all these reasons, and because of the richness of the gill parasitofauna (Silas, 1962; Silas and Ummerkutty, 1967; Gibson *et al*., 2005), the gill parasite assemblages were analysed to investigate the usefulness of tuna parasites as tags. However, when possible, the whole head were sampled and analysed, in order to increase the information on parasite assemblages.

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Outlines of the anatomy and physiology of the gills of Osteichthyes

The gills of Osteichthyes are the primary organ of respiration and they also play a major role in other functions, such as osmoregulation and acid-base balance (Kearn, 2004). The gills of tunas (as those of all Osteichthyes) are a paired organ composed by four holobranchs on each side of the head. Each holobranch has a bony arch from which two rows of tapering primary gill lamellae (= gill filaments) are projected (Kearn, 2004). Thus, the whole holobranch is composed by an arch and an anterior (post-trematic) and a posterior (pre-trematic) hemibranch, each hemibranch consisting of one of the two rows of primary lamellae. The arches have a "J" shape, and are in continuity with the basihyoid bone anteriorly and with the pharyngeal tissue posteriorly. The holobranchs are composed by five bone pieces: basibranchial, hyobranchial, cerathobranchial, epibranchial, pharyngobranchial (Stiassny, 2000). The inner side of the arch can hold numerous gill teeth, *i.e.*, skin protuberances useful to retain the food in the mouth cavity (Ankenbrandt, 1984; Stiassny 2000), that vary in number and shape among species (Fig. 2.1.2).

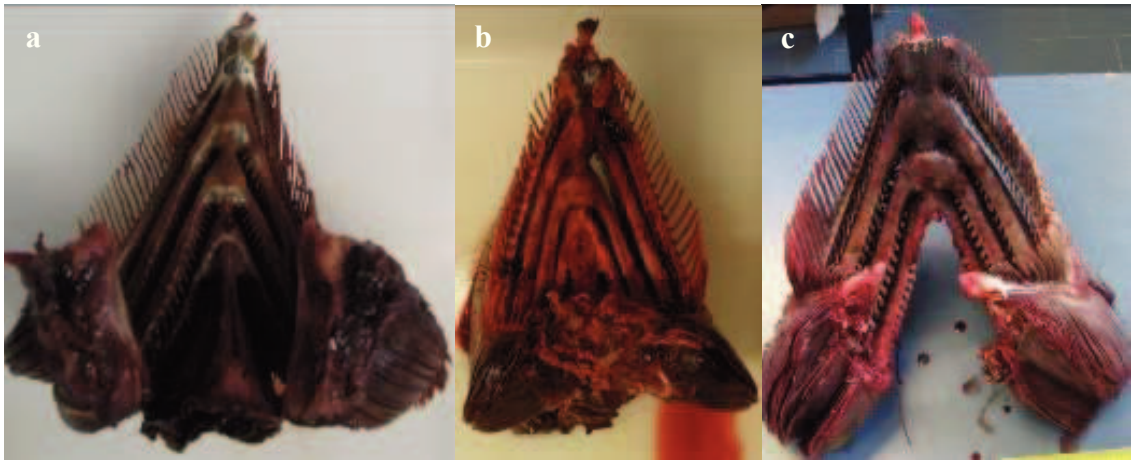

Figure 1.6.1.1. Gills of tunas from the western Mediterranean Sea: **a**, *Katsuwonus pelamis*; **b**, *Thunnus alalunga*; **c**, *Thunnus thynnus*.

The number of primary lamellae varies between holobranchs, the first and second usually have the highest number, and the anterior hemibranch has a few more than the posterior one. The length of a primary lamella depends on its position on the gill arch. The number of primary lamellae increases as fish grow. Primary lamellae are flattened like a knife blade. A cartilaginous rod, extending along each primary lamella from the gill arch to the tip, provides support. The primary lamella is the functional unit of the gill and carries two rows of closely spaced, flap-like secondary lamellae, which project in opposite directions from the two surfaces of the primary lamella (Kearn, 2004). The number, dimension and direction of the secondary lamellae change

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between species and position in the gills, having the greater surface in the second and third holobranchs. The holobranchs receive venous blood from the afferent branchial arteries, branches of the ventral aorta. These branches penetrate in the holobranchs from the basybranchial region and travel along the gill arches, and then bifurcate in two arteries. These supplies secondary vessels, called afferent arteries of the gill filaments, one of them penetrates on each gill filament from the middle of the inner margin of the gill filament. It immediately splits into two branches, one going through the basal region of the filament and the other through the apical one. Several capillaries departing from these arterioles penetrate in the secondary lamellae, producing a fenestrate *retia mirabilia*, separated from the lamellar epithelium of the secondary lamellae by a thin connective layer (Olson, 2000; Stiassny, 2000). This is the main site of gas exchange (Kearn, 2004). The blood flowing inside of the secondary lamellae moves in the opposite direction to that of the water, flowing between the secondary lamellae. Particularly, blood flows from the afferent (inner) border of each primary lamella to the efferent (outer) border of the primary lamella. This counter-current arrangement is an important adaptation for maximizing gas exchange (Kearn, 2004). Almost the total of the oxygenated blood drains from the secondary lamellae entering in the efferent gill filament artery of the outer margin of the gill filaments to become arterial systemic blood, whereas a small part re-enters the filaments to supply nutrients and return to the heart via the venous system (Stiassny, 2000). The efferent artery of the gill filaments is in continuity with the efferent gill artery, root of the dorsal aorta that moves the blood to the rest of body. Gills have muscles innervated by the ninth and tenth nerves (Hughes, 1984).

Sample size

Campbell *et al.*, (2007) stated that the time and effort required to carry out a parasite tag study depends on the sample size, the dimensions of the host specimens and the abundance of parasites. Effectively, one of the major problems to be solved when designing a sampling is to establish how many specimens (replicates) are needed to obtain a representative sampling unit of the sampling universe (Greenwood and Robinson, 2006). Thus, an estimation of these technical limits during the first analysis of each species might be helpful to save time and effort along the entire sampling period. For example Lester *et al*. (1985) analysed only one side of the head and gills and the whole viscera of the 875 specimens of *K. pelamis* available.

Previous studies on tag parasites of tuna sampled a number of hosts varying according to locality and year (between 4 and 163) (Lardeaux, 1982; Lester *et al*., 1985; Jones, 1991; Rodríguez-Marín *et al.*, 2008). According to Lardeaux (1982), the low homogeneity of sample

size of the various host populations affects the power of comparisons, particularly using parametric tools. A small sample (less than five specimens) is not representative of a population (Zar, 1996; Greenwood Robinson, 2006; Zuur *et al*., 2007), particularly if the latter is constituted by a greater than 10000 replicates. Conversely, a high number of specimens allows decreasing the uncertainty of the results. For instance, a sample size of more than 1000 will assure a good estimation of the 95% confidence intervals of the rare parasites (prevalence < 0.01%) (Simon and Schill, 1984; Jovani and Tella, 2005). Studies with this number of specimens are usually carried out in the plans of surveillance and eradication of pathogens harmful to human and livestock. Nevertheless, it is an excessive sampling effort for studies of parasite component communities, because it is effort and time consuming, redundant and sometimes without ethic justification (Simon and Schill, 1984; Dell *et al*., 2002). Jovani and Tella (2005) examined the relationships between prevalence and sample size, indicating that size samples between 10 and 30 specimens are acceptable. Statistical books indicate that a sample size equal and greater than 30 is useful for the common statistical analysis of communities (Zuur *et al.*, 2007; Kufs, 2011).

In conclusion, a compromise between a feasible sampling effort and the representativeness of results should be achieved (Legendre and Legendre, 2003; Abaunza *et al*., 2008).

Replication at different times

All the previous studies on tag parasites of tunas have been carried out during several years (3-5) (Lardeaux, 1982; Lester *et al*., 1985; Jones, 1991; Rodríguez-Marín *et al.*, 2008). A similar range of time allowed to detect temporal changes (annual and seasonal) in the distribution of parasites of pelagic fish and hence could be useful to understand the spatio/temporal distribution of parasites (Rodríguez-Marín *et al*., 2008; Campbell *et al.*, 2007).

According to Ferrer Castelló *et al*. (2007), the study of the temporal variability should be conducted in well defined areas, in order to consider constant the factors related to the substrate. In this way, the studies from traditional tuna traps *tonnara* in Italy and *almadraba* in Spain assure the sampling in the same locality (from several centuries). The samples from game fishing tournaments could also be useful, because the sampling area is well defined and they are yearly replicated. The study of seasonal change of the parasite assemblages is strongly limited by the availability of fish only during the fishing season, the collaboration of fishermen, the weather conditions and the logistic costs. In fact, all the Mediterranean tuna species are usually available only during the summer spawning season, when the different Mediterranean tuna species are intensively fished (Di Natale, 2009).

In the present study, considering the constrain imposed by the duration of the PhD study,

for some species, it was possible to replicate the sampling within a maximum of three years. Although it is a short period, it is enough to reveal differences in epidemiological data between years, as demonstrated for other pelagic fish (MacKenzie *et al*., 2008).

Replication in different areas

According to Abaunza *et al*. (2008) samples representative of the different oceanographic and ecological areas where the fish inhabit should be analysed. Nevertheless, fish sampling is often limited to particular localities of the range of species distribution to *e.g*. those where fish congregate and there are commercial fleets working (Abaunza *et al.*, 2008; Campbell *et al.*, 2007). In fact, all previous studies on tag parasites of tunas have been carried out on samples caught where the fish harvesting is made for commercial purposes (Lardeaux, 1982; Lester *et al*., 1985; Jones, 1991; Rodríguez-Marín *et al.*, 2008). Similarly, the study of the parasite assemblages of tunas within the western Mediterranean Sea is strongly limited to these localities where commercial and scientific efforts take place.

Therefore, to develop this study it was suitable the collaboration with the Spanish and Italian research institutes working in the three sub areas of the Mediterranean Sea: Alboran Sea, Balearic Sea and Tyrrhenian Sea.

Influence of host size

As indicated by several studies on parasites of pelagic fish, the levels of infection of several parasites can be cumulative with age (Williams *et al*. 1992; Gibson and Jones, 1993; MacKenzie *et al*., 2008). Generally this is due to the accumulation of parasites in relation to their large life span, but sometimes to the increase of the microhabitat with the increasing of the host size, or also to an effective change of the behaviour of the host. To evaluate the possible correlation between the levels of infection and the host age, the parasite fauna of tuna at different age classes should be investigated (MacKenzie and Abaunza, 1998; Campbell *et al*., 2007; MacKenzie *et al*., 2008). For this purpose it would be better to analyse the correlation between the levels of infection and the age of the host rather than the size, because the analysis of the age is more accurate than that of length of fish, which is influenced by the effects of different environmental conditions on fish growth (Mackenzie and Abaunza, 1998). Nevertheless, the analysis of otoliths, scales or spines for the age determination are not always available. In fact numerous studies on parasite assemblages of pelagic fish, and particularly those concerning tunas (Lardeaux, 1982; Lester *et al*., 1985; Jones, 1991; Rodríguez-Marín *et al.*, 2008) have been carried out using the host length rather than the host size.

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Therefore in this study, only host length was taken into account to analyse the pattern of parasite distribution.

Logistic and multidisciplinary approach

The collection of biological data of host (*i.e.* species identification, sex, age, size and weight) and the transport and storing of samples seem to be one of the major logistic problems for the studies of parasites of tunas (Lester *et al*., 1985; Jones, 1991). One way to overcome this drawback is to ask for collaboration and support to research institutes or institutions that routinely collect this kind of information. Moreover, collaborations with research institutes allows sampling fish specimens belonging to size groups other than those available from commercial fisheries. Gills and heads of the specimens sampled for routine biological sampling taken by officers are easily available for parasitological analyses. This collaboration allows performing several analyses on the same samples and it is useful to create multidisciplinary research groups (Cadrin *et al*., 2005).

Parasitological methods

MacKenzie and Abaunza (1998) indicated that the host samples should preferably be examined fresh. However, if this is not possible, they should be deep-frozen or preserved in 10% buffered formaldehyde-saline solution as soon as possible after capture. Freezing is a practical technique to store, in fact, the parasitological studies on the gills (and head) of tuna have been carried out on deep frozen samples (Lardeaux, 1982; Lester *et al*., 1985; Jones, 1991; Rodríguez-Marín *et al.*, 2008). Nevertheless, identification of parasites is easier with fresh samples, because freezing makes identification difficult (Berland, 1984).

The parasites collected should be prepared for identification and stored using specific fixatives and staining methods according to taxa (Kennedy, 1979; Berland, 1984; Cribb and Bray, 2010). In some studies on tunas, parasites have not been identified to the species level, but grouped in morphotypes (Lardeaux, 1982; Jones, 1991). This should be avoided whenever possible, because it generates confusion and minimises the possibilities of providing useful information about the ecological features of both parasites and hosts (Cribb and Bray, 2010). Anyway, if for any reason species identification is not possible, the morphotypes should be described in detail to identify them unequivocally.

Statistical methods

Most of the studies on the parasite assemblages of tuna analyse the data according to the

standard methods proposed by MacKenzie *et al.* (1982) and Bush *et al*. (1997). Providing measures of the prevalence, mean abundance and mean intensity is recommended to describe the levels of infection of parasites (MacKenzie and Abaunza, 2005). Moreover, the confidence intervals or the range of the values should be presented in order to give a description of the data dispersion (Rózsa *et al*., 2000). Almost all previous work on tag parasites of tunas included at least data on the prevalence and the abundance or intensity of infection (Jones, 1991; Rodríguez-Marín *et al.*, 2008).

In the past, although it is known that the parasite are distributed in hosts according to a negative binomial distribution (Anderson and May, 1978; Anderson and Gordon, 1982; Poulin, 2007), the parametric tools were widely used because they were broadly used in ecological studies and they were included in the most popular statistical softwares (Magurran, 2004; Zuur *et al*., 2007). For this reason, almost all studies adopted univariate and multivariate parametric tools to analyse parasite populations (e.g. Chavez *et al*., 2007; Ferrer-Castelló *et al*., 2007). Concerning the studies on the parasites of tunas, univariate methods have been used to investigate the differences of single parasite species between pairs of localities of *T. albacares* (see Lardeaux, 1982) and *T. alalunga* (see Jones, 1991). Among them, only Jones (1991) applied a statistical test (Mann-Whitney U-test) to evaluate the significance of results.

Multivariate analyses are increasingly being used (MacKenzie and Abaunza, 2005), because they summarise the differences between parasite assemblages considering several variables. Concerning studies on the parasites of tunas, Lardeaux (1982) and Lester *et al*. (1985) used cluster analysis and multivariate canonical analysis to study the similarity and dissimilarity of the parasite assemblages of *T. albacares* and *K. pelamis* from the Pacific Ocean, respectively. Lester *et al*. (2001) and Timi (2007) demonstrated that the application of both univariate and multivariate analyses is useful to detect ecological differences between host populations, but to date only Lardeaux (1982) used both analyses to study the parasite assemblages of *T. albacares* from the central Atlantic Ocean. Recently, the mathematical theory of non parametric tools has been improved (Rózsa *et al*., 2000; Zuur *et al*., 2007; Badin and Daraio, 2011) and they are increasingly used in fish parasitology (Praichel and Muzall, 2009; Pérez-del-Olmo *et al*., 2009; Carballo *et al*., 2012).

Therefore, non parametric tools are a useful tool to investigate the similarity and dissimilarity of the tuna parasite assemblages from the western Mediterranean Sea.

The selection of biological tags

The ideal characteristics of a tag parasite are described by Williams *et al*. (1982),

MacKenzie and Abaunza (2005). In accordance with MacKenzie and Abaunza (1998, 2005) they should show significant differences in prevalence, mean intensity and mean abundance among the areas of study; the levels of infection should be constant among years and the lifespan of the parasites should be larger than the phenomenon of the study; parasites should be easily detected, and finally, they should not be harmful or cause changes in the behaviour of the host. Among the studies on parasites as biological tags of tunas Jones (1991) evaluated the difference between localities, Rodríguez- Marín *et al*. (2008) compared the levels of infection between years, and Lester *et al*. (1985) estimated the possible lifespan of each parasite species of *K. pelamis*. These authors also rejected some parasites from the analyses because their search was time consuming. Nevertheless, the application of these rules is subjected to the arbitrary choice of the researcher.

In this study the general rules suggested by MacKenzie and Abaunza (1998) are followed, but in the case that the protocol cannot be strictly followed an alternative method will be adopted.

1.7. AIM AND OBJECTIVES

Aim

The aim of this study is to provide new scientific information on the gill parasites of the tunas of the western Mediterranean Sea, in particular on their taxonomy, ecology and structure of assemblages, with the practical goal of evaluating the use of these parasites as tags for studies on host biology, ecology and migrations.

Objectives

In order to achieve this aim, the following objectives have been established:

- to improve the knowledge of the parasite assemblages of the gills/head of tunas from the western Mediterranean Sea;
- to evaluate the geographical and temporal changes in the parasite assemblages.

In relation to these objectives the following tasks have been carried out:

- Identification of the parasites on the basis of a morphological study.
- Description of the levels of infection of the parasite species according to host size, sex, sampling area and time.
- Description of the parasite diversity at the infracommunity and component community levels.
- Comparison and testing of the differences between the parasite assemblages using univariate and multivariate non parametric analyses.
- Compilation of a checklist of the parasites of the gills of each tuna species throughout its range.
- Development of a method to assess the usefulness of parasites as tags to study the biology, ecology and migration of tunas of the western Mediterranean Sea.

CHAPTER 2. MATERIALS AND METHODS

2.1. FISH SAMPLING

The five species of Mediterranean tunas: *Auxis rochei*, *Euthynnus alletteratus*, *Katsuwonus pelamis*, *Thunnus alalunga* and *Thunnus thynnus*, were selected for the study (Fig 1.3.2). A total of 334 specimens were sampled from seven localities of the western Mediterranean Sea and the Levantine Sea (eastern Mediterranean Sea) (Fig. 2.1.1).

Sampling was carried out from 2006 to 2011 during the tuna fishing seasons (May to October), by the traditional tuna trap fishery (*almadraba* in Spain and *tonnara* in Italy) and by trolling in collaboration with big-game competitions (Table 2.1.1). The specimens belonging to the species *A. rochei*, *E. alletteratus*, *K. pelamis* and *T. alalunga* were collected within the framework of two national scientific projects (*Grandes Pelágicos del Mediterráneo y Atlántico suroriental* GPM-3 and GPM-4) developed by the Málaga Oceanographic Center of the *Instituto Español de Oceanografía*. The specimens of *T. thynnus* were collected during the sampling of the ICCAT project of the *Dipartimento di Scienze della Vita e dell'Ambiente* of the University of Cagliari.

Immediately after landing, tunas were measured (LF, fork length), weighed (TW, total weight) and sexed; then the gills were extracted; wherever possible the entire head, including gills and hearth, was sampled. Samples were labelled, stored individually in plastic bags and frozen at -20° C (Figs. 2.1.2-5). When it was possible, a fresh sub-sample of gills was examined to obtain alive parasites for precise species identification (Pérez-del-Olmo, 2008). Pathological alterations were recorded and samples were fixed in 10% buffered formalin for histopathological analysis.

In addition, to prevent the possible lost of biometrical data, the length of the lower limb of the first left gill arch (LLL) (Fig. 2.1.6) of each specimen was recorded, in order to evaluate the correlation between the fork length and LLL. This correlation was used to estimate some missing

data of *T. alalunga* and *T. thynnus* (Annex 1).

The specific materials used in each study are described in the Materials and Methods sections of the corresponding chapters (Sections 3.2, 4.2, 5.2, 6.2).

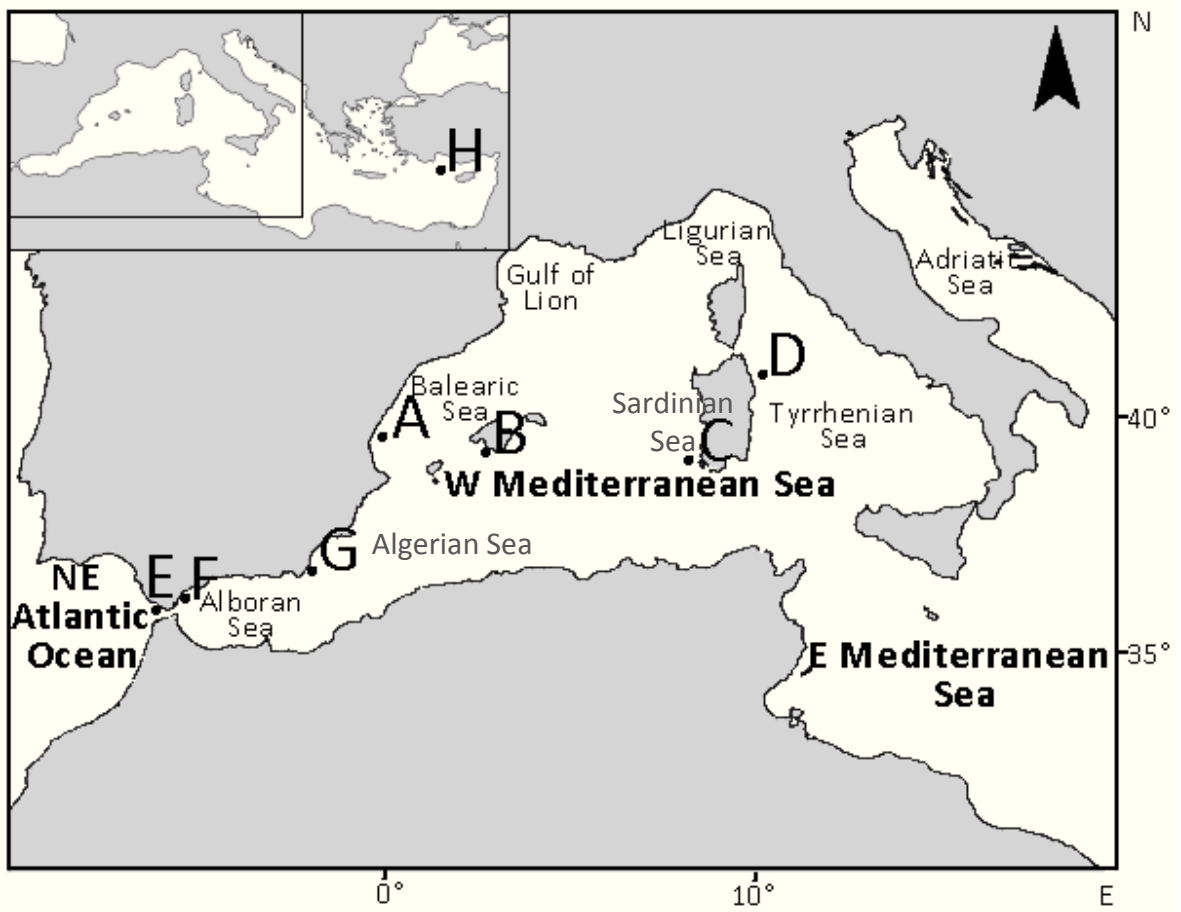

Figure 2.1.1. Map of the sampling localities in the Western Mediterranean Sea: **A**, Gulf of Valencia, Balearic Sea; **B**, S'Estanyol du Mitjorn, Balearic Sea; **C**, Isle of San Pietro, Sardinian Sea; **D**, Tavolara, Tyrrhenian Sea; **E**, Tarifa, Strait of Gibraltar; **F**, Puerto Banús, Alboran Sea; **G**, La Azohía, Algerian Sea; **H**, Levantine Sea, Eastern Mediterranean Sea.

Table 2.1.1. Data of the tuna sampling according to species, year, locality, gear and project funding. N, number of specimens collected. Sampling areas: A, Gulf of Valencia, Balearic Sea; B, S'Estanyol du Mitjorn, Balearic Sea; C, Isle of San Pietro, Sardinia Sea; D, Tavolara, Tyrrhenian Sea; E, Tarifa, Strait of Gibraltar; F, Puerto Banús, Alboran Sea; G, La Azohía, Algerian Sea; H, Levantine Sea, eastern Mediterranean Sea.

Species	N	Year	Sampling area	Locality	Gear	Project
Auxis rochei	33	2008	G	La Azohía	TunaTrap	GPM-3
" "	9	2008	E	Tarifa	TunaTrap	GPM-3
" "	30	2011	G	La Azohía	TunaTrap	GPM-4
Euthynnus alletteratus	63	2008	G	La Azohía	TunaTrap	GPM-3
" "	22	2008	E	Tarifa	TunaTrap	GPM-3
" "	22	2009	G	La Azohía	TunaTrap	GPM-3
" "	6	2010	A	Gulf of Valencia	Purse seine	GPM-4
" "	20	2011	G	La Azohía	TunaTrap	GPM-4
" "	23	2011	A	Gulf of Valencia	Trolling	GPM-4
Katsuwonus pelamis	4	2008	B	Balearic Sea	Trolling	GPM-3
" "	31	2008	F	Puerto Banus	Trolling	GPM-3
Thunnus alalunga	30	2008	B	Balearic Sea	Trolling	GPM-3
Thunnus thynnus	30	2006	C	San Pietro	TunaTrap	ICCAT
" "	4	2006	D	Tavolara	Trolling	ICCAT
" "	19	2007	C	San Pietro	TunaTrap	ICCAT
" "	10	2007	H	Levantine Sea	Trolling	ICCAT

GPM-3 and GPM-4, project *Grandes Pelágicos del Mediterráneo y Atlántico suroriental* of the *Instituto Español de Oceanografía*; ICCAT project of the *Dipartimento di Scienze della Vita e dell'Ambiente* of the University of Cagliari.

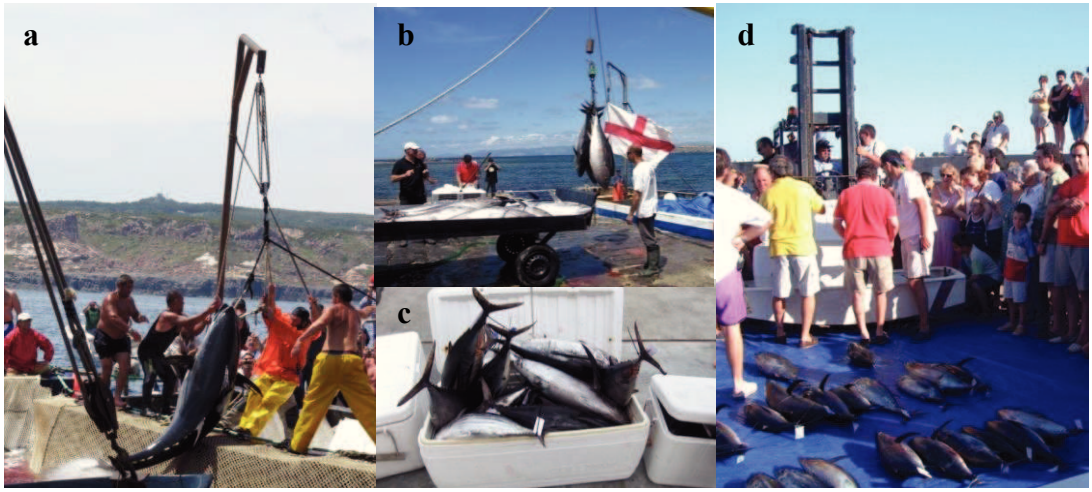

Figure 2.1.2. Fish sampling: **a-b**, harvest and landing of *Thunnus thynnus* at the tuna trap (*tonnara*) of the Isle of San Pietro. **c-d**, landing of *Katsuwonus pelamis* and *Thunnus alalunga* at S'Estanyol du Mitjorn (big game competition).

Figure 2.1.3. a-b, measuring tunas; **c**, weighting tunas.

Figure 2.1.4. a, biological sampling and data recording. **b**, sex and gonad maturation assessment.

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Figure 2.1.5. Gill sampling.

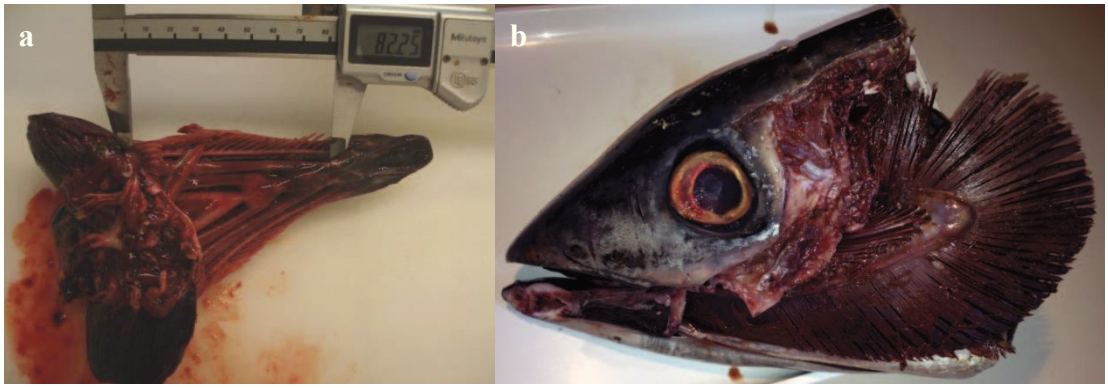

Figure 2.1.6. a, measuring the LLL. **b**, head of tuna *thunnus thynnus* after cutting off the operculum.

2.2. PARASITE COLLECTION

Fresh and thawed samples were placed on a laboratory tray, and the bag washed with filtered sea water or saline solution (and 0.85% NaCl), respectively; the rinse was sieved with a 180 μm mesh, poured into a conical container and left to sediment for 30 minutes, the supernatant discarded and the sediment observed under a stereomicroscope for parasites. If the sample was the whole head, the left operculum was cutted to expose the first holobranch (Fig. 2.1.6). Then the LLL of the first holobranch was recorded. Subsequently, the holobranchs were excised and analysed separately by naked eye and under a stereomicroscope for parasites (Fig. 2.2.1). After gill analysis each holobranch was washed with filtered sea water (or saline solution) and the rinse examined as described for the plastic bags.

Figure 2.2.1. Examination of gill holobranchs: with the naked eye and under stereo-microscope.

The holobranchs were numbered (1-4), from the anterior-external to the posterior-internal, and divided into three main parts (the gill arch and two hemibranchs) (Fig. 2.2.2-3). The holobranch surface was divided into five regions (A1 hypobranchial area; A2 and A3, cerathobranchial area; A4, epi-cerathobranchial joint area; A5, epibranchial area), each divided into four sub regions (inner, upper, outer and vessel regions). Each hemibranch was divided into 15 regions, comprising the basal (B1 to B5), central (C1 to C5) and distal regions (D1 to D5) of the gill filaments. Each filament has an inner margin (with afferent gill artery) and an outer margin (with efferent artery) (Fig. 2.2.4).

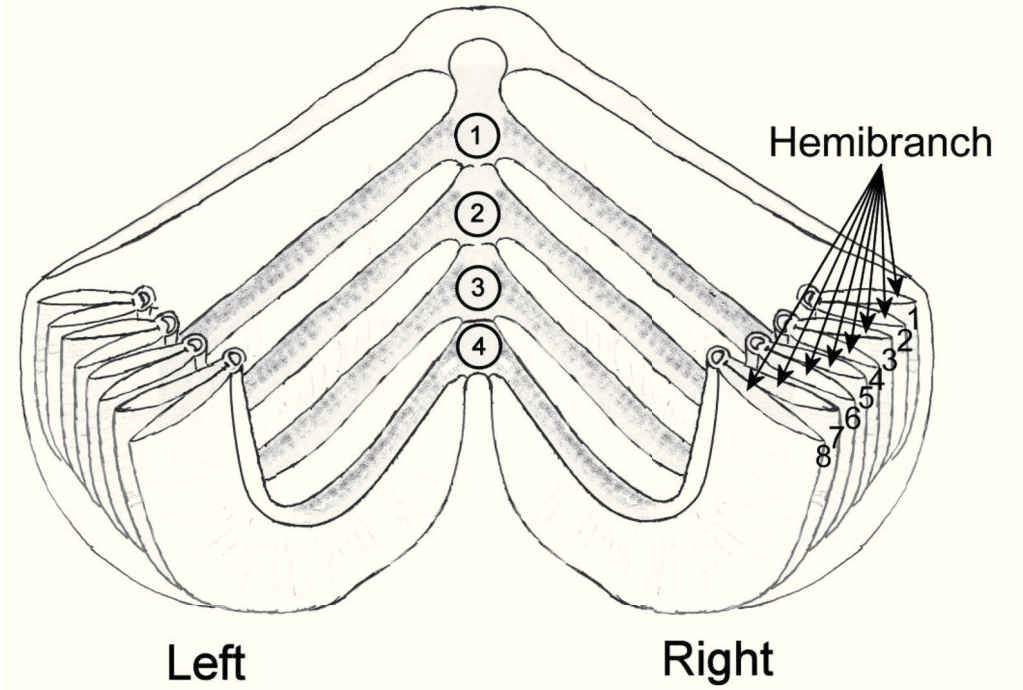

Figure 2.2.2. Schematic drawing of the gill holobranchs.

Salvatore Mele. Gill metazoan parasites of tunas (Scombridae: Thunnini) from the western Mediterranean Sea: systematics, assemblages and use as biological tags. Tesi di dottorato in Riproduzione, Produzione, Benessere Animale e Sicurezza degli Alimenti di Origine Animale. Università degli studi di Sassari.

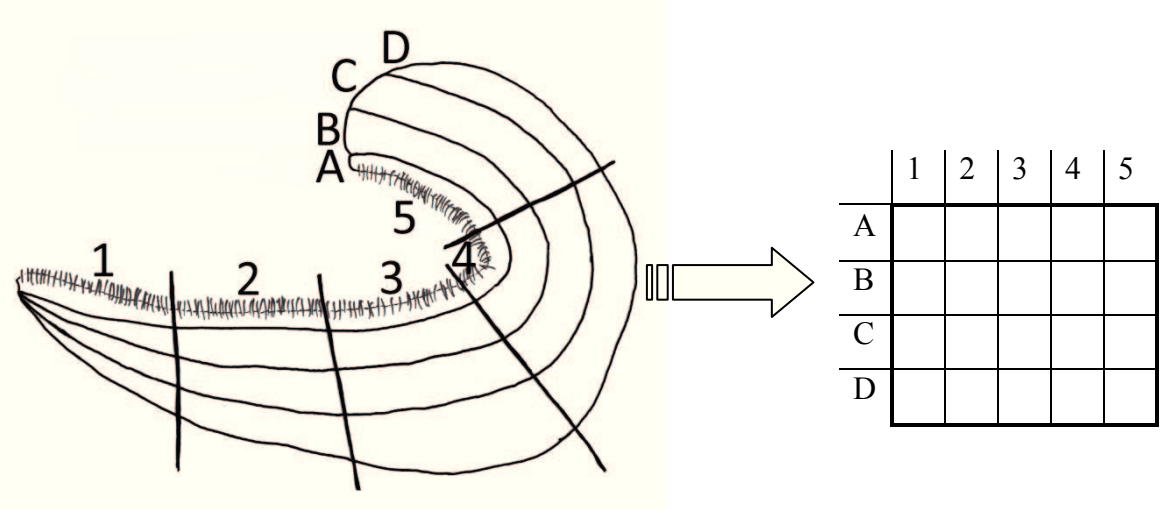

Figure 2.2.3. Schematic drawing showing the subdivision of the gill arch (A1-A5), and the basal (B1-B5), central (C1-C5) and distal parts (D1-D5) of the hemibranchs.

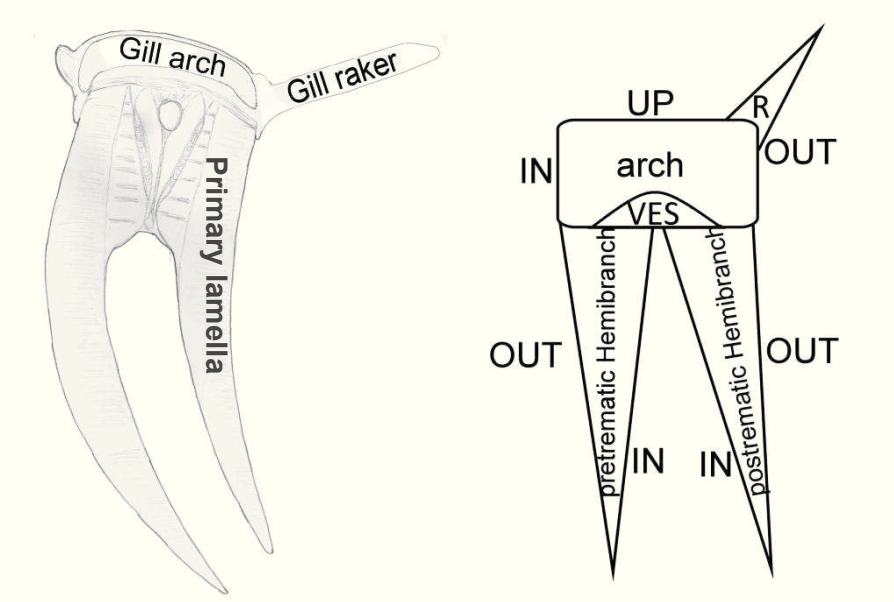

Figure 2.2.4. Schematic drawing of the gill arch and gill filaments. Abbreviations: IN, inner side of gill arch and gill filaments; OUT, outer side of gill arch and gill filaments; R, gill raker; UP, upper side of gill arch; VES, vessel region of the arch.

On the basis of this scheme, the exact location of parasites and lesions were recorded using a specific form (Annex 2).

The information of host (species, ICCAT code, sampling code, size, weight, sex, LLL, date and site of sampling, date of analysis) and each parasite specimen found (database code, location, sex) were recorded in a specifically designed database (Fig. 2.2.6).

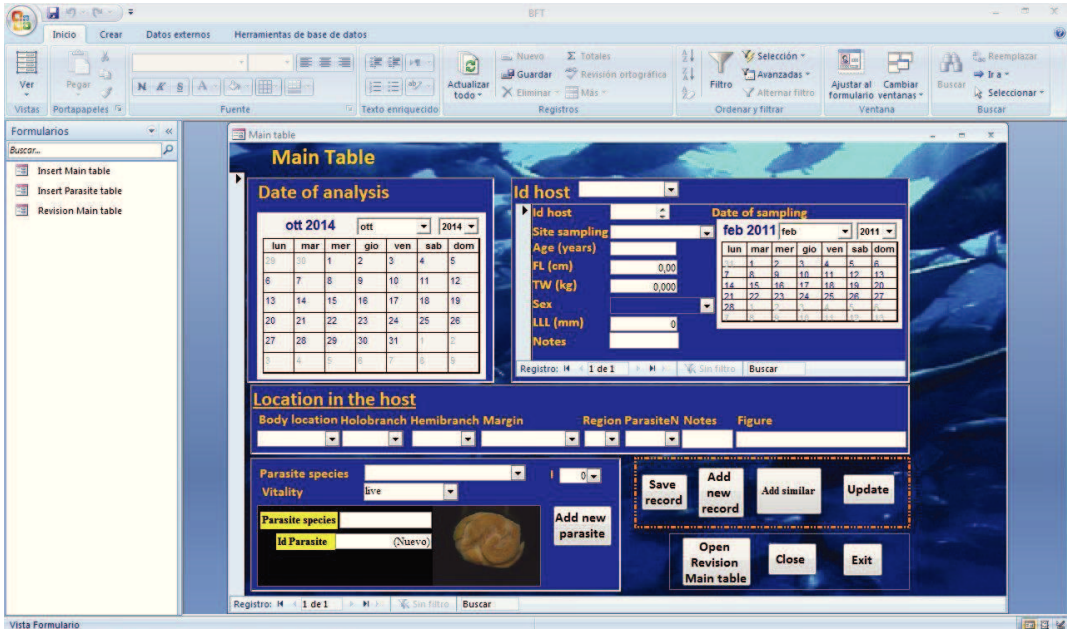

Figure 2.2.5. Screenshot of the first page of the digital database for *Tunnus thynnus*.

All parasites were counted and stored in 70% ethanol, except some selected specimens stored in 96% ethanol for genetic studies. The tubes were labelled with the code of host, date of sampling, location and taxon of parasite, name of the fixative solution.

2.3. PREPARATION TECHNIQUES OF PARASITES

For microscopical examination and species identification parasites were prepared according to standard protocols (Kennedy, 1979; Berland, 1984; MacKenzie and Abaunza, 2005; Cribb and Bray, 2010). Didymozoids were excysted, washed in saline solution, flattened under a coverslip, fixed in 70% ethanol, stained with modified Malzacher's technique and mounted in Canada balsam (Annex 3). Monogeneans and copepods were washed in saline solution, cleared with glycerin-alcohol or lactophenol and observed as temporary mounts. Fresh and mounted parasites were microphotographed with a digital system, and drawings of specimens were made using a drawing tube attached to a stereo- and /or light microscope.

2.4. SPECIES IDENTIFICATION

Parasites were identified to the lowest taxonomic level possible. The following textbooks and specialised papers were used for species identification; for monogeneans: Price (1939), Dawes (1947), Palombi (1949), Lamothe-Argumedo (1997), Chisholm and Whittington (2007); for trematodes: Ishii (1935), Yamaguti (1958, 1970), Podznyakov (1993), Murugesh and Madhavi (1995) and Podznyakov and Gibson (2008); for copepods: Brian (1906), Vervoort (1962), Cressey and Cressey (1980), Kabata (1979, 1992) and Boxshall and Halsey (2004); for

isopods: Haswell (1882), Richardson (1905), Bruce (1983) and Brusca (1985). Apart from this literature, several specialised papers and specific identification keys were used, and they are cited in the Materials and Methods sections of the corresponding chapters (sections 3.2, 4.2, 5.2, 6.2, 7.2).

2.5. STATISTICAL ANALYSES

Parasite assemblages were analysed at the infra- and component community levels. The correlation between the levels of infection and the host size, as well as the differences of the parasite assemblage between dates of samplings and areas were estimated. The definitions of the parasitological terms used are shown below.

Parasite population and assemblage

Definition of the parasite assemblages

Population. It includes all of the individuals of one species, whatever their developmental stage, living in one locality and forming a potentially interbreeding group (Magurran, 2004).

Parasite population. It comprises all of the individuals of a single parasite species in a definite place and time (Bush *et al*., 1997). However, it is used to consider only the individuals at one stage of their life-cycle in one host population (*e.g*. adult worms inside their definitive host) (Poulin, 2007).

Infrapopulation. All the individuals of a parasite species in a host (Bush *et al*., 1997)

Component population. It includes all of the individuals of a specified life history phase of a parasite species occurring in one/several hosts living at a definite place and time. It is a subset of the suprapopulation (Bush *et al*., 1997).

Suprapopulation. It includes all the life stages of a parasite species in a definite place and time Bush *et al*. (1997).

Community. It is a group of populations of different species that live in the same place and interact with each other in some way (Magurran, 2004).

Infracommunity. It includes all the infrapopulations of all parasite species infecting a single individual host.

Component community. It includes all the infrapopulations of parasites associated with some sub set of a host species (Bush *et al.*, 1997).

Assemblage. It is a non random unstructured ensamble of parasite populations (Rohde *et al*. 1998).

Statistical descriptors of the parasite assemblages

Infracommunity level. Two descriptors were used to evaluate the diversity of the parasite assemblages: the mean species richness per host (R) and the mean total abundance (np) (Bush *et al*., 1997).

Species richness per host. It is the number of parasite species in each individual host, *i.e*. number of species in each infracommunity.

Mean species richness per host (R). It is the mean number of parasite species in each infracommunity.

Total abundance. It is the number of parasite specimens in each individual host, *i.e*. the number of specimens in each infracommunity.

Mean total abundance (np). It is the mean number of parasite specimens in each infracommunity.

Component community. The parasite species richness (S) was used to estimate the number of parasites species included in the parasite assemblage; the Berger-Parker index (d) was calculated to evaluate the dominance according to Magurran (2005).

Species Richness. It is the number of (parasite) species present (Frosini, 2003).

Berger-Parker index (d). It is a dominance measure, and it expresses the proportional abundance of the most abundant species:

$$d = \frac{N\,max}{N\,tot}$$

where N max is the number of individuals of the most abundant species and N tot the total abundance, *i.e*. all the parasite specimens of the component community (Magurran, 2004).

Parasitological indices of prevalence and abundance

Prevalence (P%), mean abundance (MA) and mean intensity (MI) of each parasite species were calculated according to Bush *et al.* (1997). The confidence interval of the prevalence was computed using the Sterne's exact method with the free software Quantitative Parasitology 3.0 (Reiczigel and Rózsa, 2005). The confidence intervals of the mean abundance and mean intensity were calculated using the bias-corrected and accelerated bootstrap of Efron and

Tibshirani (1993) implemented in Quantitative Parasitology 3.0 (Reiczigel and Rózsa, 2005).

Prevalence (P%). It is the number of infected hosts (N inf.) with a parasite species divided by the number of examined host (N):

$$P\% = \frac{N\ inf.}{N}$$

Commonly it is expressed as a percentage (Bush *et al*., 1997).

Abundance. It is the number of parasites living in a host (abundance ≥ 0) (Bush *et al*., 1997).

Mean abundance (MA). It is the arithmetic mean of the number of individuals of a parasite species per examined host (Bush *et al*., 1997):

$$MA = \frac{number\ of\ parasite\ specimens}{N}$$

Intensity. It is the number of parasites living in an infected host (intensity >0), *i.e*. the number of individuals of an infrapopulation (Bush *et al*., 1997).

Mean Intensity (MI). It is the arithmetic mean of the number of individuals of a particular parasite species divided by the number of host infected whit that parasite species (Bush *et al*., 1997):

$$MI = \frac{number\ of\ parasite\ specimens}{N\ inf.}$$

Correlation between the levels of infection and host size

Spearman's rank correlation coefficient (ρ) was used to evaluate possible correlations between the host size and the abundance of infection (Zar, 1996); its significance was tested ($p \leq 0.05$) using the statistical package *stats* of the R software (R Development Core Team, 2011).

Comparisons between the parasite assemblages

Qualitative analyses

Component community dissimilarity was evaluated with the metric Marczewski-Steinhaus distance, and the non metric Bray-Curtis distance (Magurran, 2004) using the statistical package *vegan* of the R software (R Development Core Team, 2011).

Marczewski-Steinhaus dissimilarity measure (MS). It is a complementary measure of the Jaccard similarity (1-Jaccard), that is a metric measure of similarity between two groups of species (Chao *et al*., 2005):

$$MS = 1 - \frac{A}{A + B + C}$$

where A is the number of shared species and B and C the number of unshared species of each group.

Bray-Curtis dissimilarity measure (BC). It is a complementary measure of the Sorensen similarity (1-Sorensen), that is a semi metric measure of similarity between two groups of species (Chao *et al*., 2005):

$$BC = 1 - \frac{2A}{2A + B + C}$$

Jaccard and Sorensen coefficients are almost equivalent, except that the Sorensen index gives double weight to positive co-occurrences (A) (Dalirsefat *et al*., 2009). Their complementary measures (MS and BC) allow describing the dissimilarity between several pairwise of group using multivariate statistical tools.

Quantitative analyses

Univariate analyses

The differences of prevalences were evaluated with the Fisher's exact test using the free software Quantitative Parasitology 3.0 (Reiczigel and Rózsa, 2005).

The differences in mean abundance and mean intensity were evaluated with the bootstrap rank Welch test (Efron and Tibshirani, 1993) using the free software Quantitative Parasitology 3.0 (10,000 replications) (Reiczigel and Rózsa, 2005).

Multivariate analyses

To identify possible differences between parasite assemblages from the different localities, non-Metric Multidimensional Scaling (NMDS) and Cluster Analysis (CA) were performed on a Bray-Curtis similarity matrix with the average method, considering prevalence, mean abundance and mean intensity of the parasite species that showed almost a significant difference between two host groups (Field *et al.*, 1982). The vectors of each variable were included in NMDS graphs in order to find out the principal factor driving the separation according to the sampling areas. This factor was used to compute the NMDS and the CA of the parasite assemblages grouped as ectoparasite-, endoparasite- assemblages of the gills (or of the head if available). The coefficient of cophenetic correlation (Rc) was computed to evaluate how the hierarchical structure of the CA represented the effective distance between the parasite assemblages. Multivariate analyses were carried out using the *vegan* package of the R software (R Development Core Team, 2011).

CHAPTER 3. METAZOAN PARASITES OF THE HEAD OF THE BULLET TUNA *AUXIS ROCHEI* (OSTEICHTHYES: SCOMBRIDAE) FROM THE WESTERN MEDITERRANEAN SEA

3.1. INTRODUCTION

The bullet tuna *Auxis rochei* is an epipelagic neritic fish distributed worldwide in tropical and subtropical oceans and in the Mediterranean Sea (Uchida, 1981). The limits of its distribution are not well known, mainly because this species may be confused with its congener, the frigate tuna *Auxis thazard*, another cosmopolitan fish (Di Natale *et al*., 2009). In fact, the systematics of the genus *Auxis* is still controversial; some decades ago various authors considered the existence of only one species (Collignon, 1961; Nair *et al.*, 1970) while others proposed the existence of two distinct species (Shomura *et al.*, 1994; Yesaki and Arce, 1994; Collette and Aadland, 1996). Currently, the latter hypothesis is accepted (Catanese *et al.*, 2008). However, recent genetic and morphometric studies carried out in the Mediterranean Sea agree with the existence of only one *Auxis* species in the Mediterranean Sea and adjacent areas of the Atlantic Ocean (Orsi Relini *et al.*, 2009), indicating that the two species are still confused and that probably misidentifications occur even in scientific papers.

The bullet tuna is a multiple spawner with asynchronous oocyte development (Macías *et al.*, 2006). In the eastern Atlantic Ocean its spawning takes place from April to June in the Gulf of Guinea, and from October to March in the Congo-Angola area (Rudomiotkina, 1984). In the western Atlantic Ocean and Gulf of Mexico two different spawning peaks have been observed, one in April and one in August (Collette and Nauen, 1983). In the Mediterranean Sea this species spawns in several areas from May to September (Alemany *et al*., 2010; Kahraman *et al*., 2010). Little is known about its migrations between the Atlantic Ocean and the Mediterranean Sea; it has been suggested that it performs a trophic migration along the north-western coast of Africa during the autumnal upwelling season (Grudtsev, 1992) and a spawning migration from the

Atlantic Ocean to the western Mediterranean Sea in the summer (Sabatés and Recasens, 2001).

The bullet tuna is an opportunistic predator, feeding on a variety of planktonic invertebrates, small sized cephalopods and fish larvae, and its diet becomes more piscivorous in larger specimens (Mostarda *et al*., 2007). It is prey of large pelagic predators (tunas, billfishes and sharks) (Valeiras and Abad, 2006).

This fish is the most abundant tuna in the Mediterranean Sea, where it represents an important element of the food web (Mostarda *et al*., 2007). It is exploited by artisanal fisheries, representing the 39% of the total tuna catches in the Mediterranean area (*i.e*. 9829 t out of 25318 t in 2010; FAO, 2011).

Despite its economic and ecological importance, no information is available on the parasites of *A. rochei* in the Mediterranean Sea. Moreover, from the bibliographic data it is difficult to compile a specific parasite list of this species, because of the aforementioned misidentifications with *A. thazard*, and also because in some scientific works the fish have been identified only at the genus level. In the Atlantic Ocean, the parasites have been mostly attributed to *A. thazard* (Vervoort, 1962; Mogrovejo *et al.* 2004), but it is likely that in many cases the host was *A. rochei*.

Considering the genus *Auxis*, a great number of parasites have been reported in the head of this host from various localities: several axinid, gastrocotylid and hexostomid monogeneans, a number of didymozoid trematodes, and some bomolochid and caligid copepods (Silas, 1967; Cressey and Cressey, 1980; Gibson *et al*. 2005). However, in the Mediterranean Sea only two parasites of the head have been described in *Auxis* sp.: the didymozoid *Didymozoon auxis* and the poliopysthocotylean *Hexostoma auxisi* (Dollfus, 1926; Palombi, 1949).

As widely explained in the chapter 1.6, parasitological studies are useful to investigate the biology, ecology, migration and population structure of marine organisms (MacKenzie, 2002), and they have been successfully used as tags to clarify taxonomic relationships between closely related hosts (Whittington, 2005). In cases of strict host specificity, as for several monogeneans and trematodes, the parasites could also be used as diagnostic criteria for host species identification (Lambert and El Gharbi, 1995). For instance: Oliva *et al.* (2008) found different monogeneans and didymozoids in *Scomber japonicus* Houttuyn, 1782 (Osteichthyes: Scombridae) from the Atlantic Ocean (now *Scomber colias* Gmelin, 1789) and *S. japonicus* from the Pacific Ocean, suggesting the need of a taxonomical revision of the hosts, as afterward confirmed by molecular studies (Catanese *et al*., 2010; Cheng *et al*., 2011); Châari *et al*. (2010) proposed that three of the four subspecies of *Tylosurus acus* (Lacepède, 1803) (Osteichthyes**:**

Belonidae) could be discriminated by specific axinid monogeneans.

The aim of this study is to investigate the metazoan parasites of the head of *A. rochei* from the western Mediterranean Sea, and to evaluate their possible use as biological tags to improve the knowledge of the bio-ecological aspects of this fish species.

3.2. MATERIALS AND METHODS

Sixty-three bullet tuna, caught in the traditional trap fishery of La Azohía (Algerian Sea, western Mediterranean Sea, area G in Fig. 2.2.1 (37°32'59"N, 1°10'44"W) in May 2008 (N = 33) and May 2011 (N = 30), were examined for parasites (Table 3.2.1). Immediately after landing fish were measured (FL range 33–44 cm), weighed (total weight range 0.6–1.5 kg) and sexed (32 males and 31 females). In addition, nine specimens of bullet tuna (six males and three females) caught in the traditional trap fishery of Tarifa, Gibraltar Strait, western Mediterranean Sea, area E in Fig. 2.2.1 (36° 0'59"N, 5°37'44"W), with FL range 38–44 cm and total weight range 1.0–1.6 kg, were examined for comparative purposes (Table 3.2.1). After each sampling, the heads of fish were excised, stored individually in plastic bags and frozen at -20° C. The samples were dissected and processed as described in Chapter 2.2.

Table 3.2.1. Sampling data of *Auxis rochei* according to locality and year.

Locality	Year	N	mean FL (cm)	Range FL (cm)
La Azohía (Algerian Sea)	2008	33	37	33-44
La Azohía (Algerian Sea)	2011	30	38	33-44
Tarifa (Gibraltar Strait)	2008	9	41	38-44

In addition to the general references cited in the Chapter 2, the following specific literature was used for species identification: for monogeneans, Mogrovejo and Santos (2002) and Mogrovejo *et al*. (2004); for copepods, Lin and Ho (2006).

The parasitological terms used are as defined in the Chapter 2.5; prevalence (P%), mean abundance (MA) and mean intensity (MI) of each parasite species and their confidence intervals were calculated according to the methods described in the Chapter 2.5.

Possible correlations between parasite abundance and host size were assessed using the Spearman rank correlation coefficient.

The levels of infection of each parasite species were calculated according to host size, locality and year, dividing hosts into five groups (Table 3.2.2): small fish (FL 33-37 cm) of La Azohía in 2008 (AS08) and 2011 (AS11), and large fish (FL = 38-44) of La Azohía in 2008 (AL08) and 2011 (AL11), and of Tarifa in 2008 (TL08).

Table 3.2.2. Sampling data of *Auxis rochei* according to locality, year and size.

Host group	Locality	Year	N	mean FL (cm)	Range FL (cm)	Size category
AS08	La Azohía (Algerian Sea)	2008	11	35	33-37	Small
AL08	La Azohía (Algerian Sea)	2008	22	39	38-44	Large
AS11	La Azohía (Algerian Sea)	2011	10	36	33-37	Small
AL11	La Azohía (Algerian Sea)	2011	20	40	38-44	Large
TL08	Tarifa (Gibraltar Strait)	2008	9	41	38-44	Large

The differences between the parasite assemblages these five host groups and between host sexes were evaluated using the Fisher's exact test for prevalence and the bootstrap t-test for mean abundance and mean intensity (Ròzsa *et al*. 2000).

Non-Metric Multidimensional Scaling (NMDS) and Cluster Analysis (CA) were performed as described in the Chapter 2.5, considering prevalence, mean abundance and mean intensity of each parasite species to identify possible differences between the five host groups.

Component community parameters (species richness; Berger-Parker index, d) were calculated according to Magurran (2004).

The dissimilarity between the parasite assemblages of the five groups of *A. rochei* were evaluated according to the methods described in the Chapter 2.5.

A datasheet of presence/absence was created on the basis of the published records of parasites of the head of *Auxis* sp. according to the geographical region (Table 3.2.3). These data allowed to evaluate the dissimilarity between the parasite fauna of the head of *A. rochei* from the western Mediterranean Sea (ArM) and those of *A. thazard* from the Atlantic Ocean (AtA), Indian Ocean (AtI), and Pacific Ocean (AtP), according to the methods described in the Chapter 2.2.

Table 3.2.3. Published data of the parasites of the head of *Auxis thazard* (no data for *A. rochei*) according to locality: AtA, Atlantic Ocean; AtI, Indian Ocean; AtP, Pacific Ocean (no data for the Mediterranean Sea apart from *Didymozoon auxis* and *Hexostoma auxisi* in *Auxis* sp. (Dollfus, 1926; Palombi, 1949). Numbers represent literature sources: 1, Silas (1962); 2, Vervoort (1965); 3, Yamaguti (1970); 4, Murugesh and Madhavi (1995); 5, Fuentes Zambrano (1997); 6, Mogrovejo and Santos (2002); 7, Mogrovejo *et al*. (2004); 8, Chisholm and Whittington (2007). **[t]**, parasite reported in the tropical area of the host distribution; **[w]**, parasite reported in the temperate area of the host distribution.

Parasite / Locality	AtA	AtI	AtP
Monogenea			
Allopseudaxine macrova (Unnithan, 1957) **[t]**	7	7	7
Capsala gouri (Chauhan, 1951) **[t]**		8	
Capsala magronum (Ishii, 1936) **[t]**	8		
Capsala manteri (Price, 1951) **[t]**	8		6
Capsala manteriaffinis (Mamaev, 1968) **[t]**		8	8
Capsala notosinense (Mamaev, 1968) **[t]**		8	
Churavera triangula (Mamaev, 1967) **[t]**	7		8
Hexostoma auxisi Palombi, 1943 **[t, w]**	7		
Hexostoma keokeo Yamaguti, 1968 **[t]**	7	7	7
Hexostoma thynni (Delaroche, 1811) **[t, w]**	1		
Homostoma chura Unnithan, 1965 **[t]**		1	
Metapseudaxine ventrosicula Mamaev, 1967 **[t]**			1
Neohexostoma euthynni (Meserve, 1938) **[t, w]**			1
Neohexostoma mochimae Fuentes Zambrano, 1997 **[t]**	5		
Didymozoidae			
Didymosulcus wedli (Ariola, 1902) **[t]**		4	
Didymozoon auxis Taschenberg, 1879 **[t, w]**	7		3
Copepoda			
Caligus bonito Wilson, 1905 **[t, w]**	7		
Unicolax mycterobius (Vervoort, 1965) **[t]**	2		

3.3. RESULTS

A total of seven parasite species were found in the head of the bullet tuna from the western Mediterranean Sea (Table 3.3.1, Figs. 3.3.1-2). Most of the parasites found in the samples from La Azohía were adult didymozoids (72% of all specimens), with two species, *Didymozoon auxis* and Nematobothriinae gen. sp. 1; followed by monogeneans (21% of all specimens), with three species: *Allopseudaxine macrova*, *Churavera triangula*, *Hexostoma auxisi*; and copepods (7% of all specimens), with two species: *Caligus bonito* and *Unicolax mycterobius*. *Didymozoon auxis* was the dominant species (d = 0.63), showing the highest infection parameters (P% = 59%; MA = 1.9; MI = 3.3, Table 3.3.2). Unidentified juveniles (post-larval stages) of didymozoids were also found (P% = 52%, MA = 3.2, MI = 6.0). The parasites of the bullet tuna from Tarifa are shown in the Table 3.3.3; a total of three species were found: *A. macrova*, *D. auxis* and *U.*

micterobyus. Similarly to La Azohía, *D. auxis* was the dominant species (d = 0.87).

Table 3.3.1. Parasites of the head of *Auxis rochei* from the western Mediterranean Sea with indication of location. *, new geographical record; #, new host record.

Parasite / Year	Location
Monogenea	
A. macrova *#	Gill filaments
C. triangula *#	Gill filaments
H. auxisi#	Gill filaments
Didymozoidae	
D. auxis#	Outer side of gill filaments
Didymozoid sp. (juveniles) *#	Gill arch and pharynx
Nematobothriinae gen. sp. 1 *#	Periorbital tissues
Copepoda	
C. bonito *#	Gill chamber
U. mycterobius *#	Nasal cavity

No significant differences in P%, MA and MI of each parasite species were found between host sexes and sampling years in the samples from La Azohía, thus the data were pooled for subsequent analyses. Regarding the possible correlations between the abundance and the host size in the samples from La Azohía, apart from a negative correlation between the abundance of *U. mycterobius* and the FL (-0.27, p = 0.003), no other significant relationship was found.

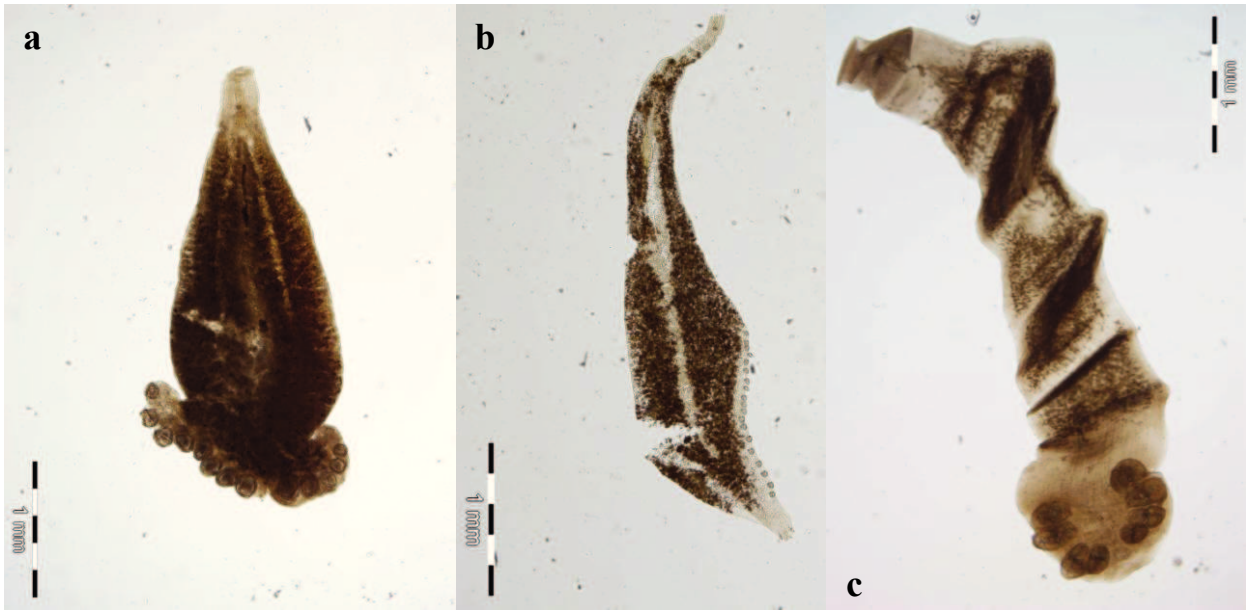

Figure 3.3.1. Monogeneans *ex* the gills of *Auxis rochei*: **a**, *Allopseudaxine macrova*; **b**, *Churavera triangula*; **c**, *Hexostoma auxisi*.

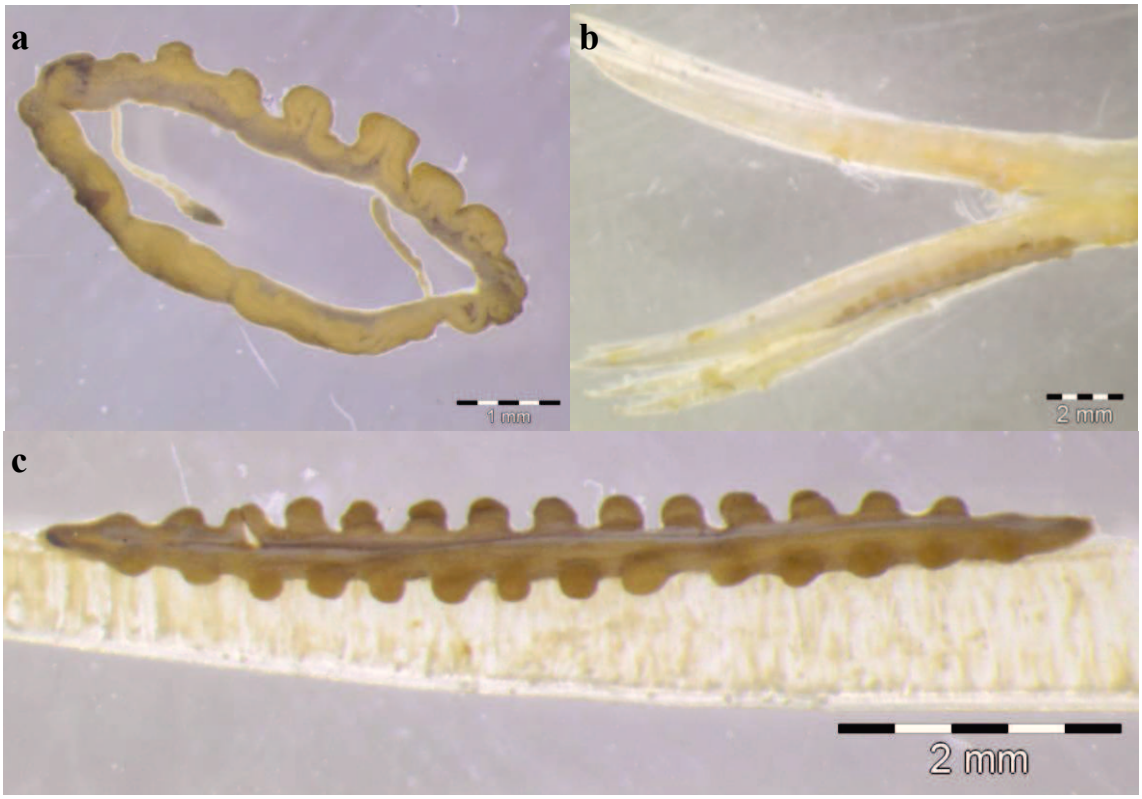

Figure 3.3.2. a, *Didymozoon auxis ex* the gills of *Auxis rochei*; **b-c**, *D. auxis in situ*.

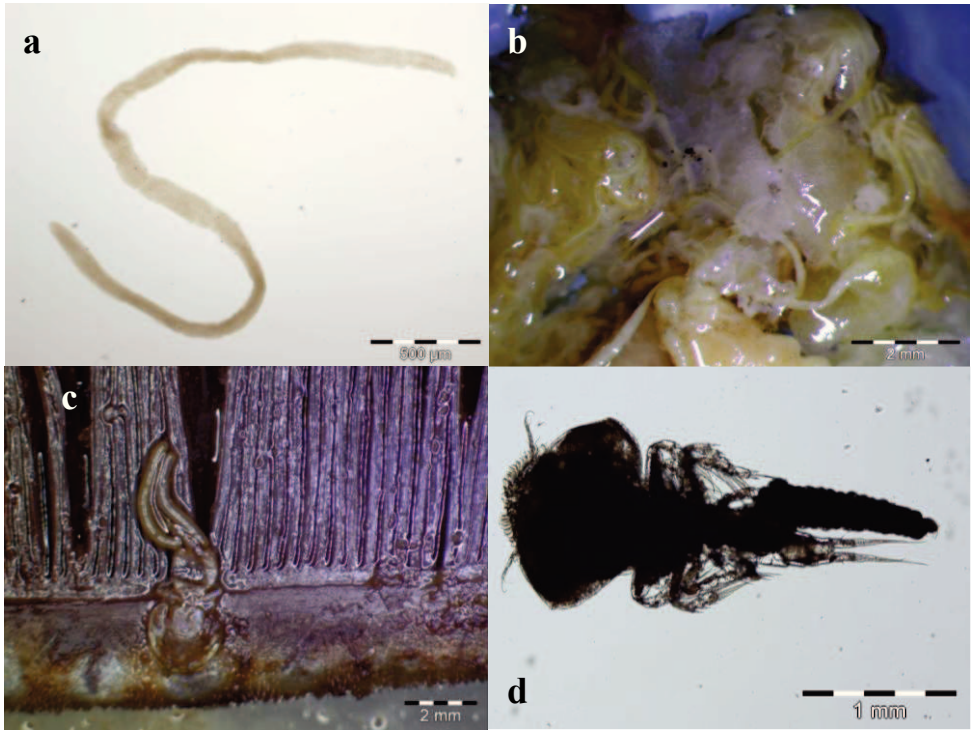

Figure 3.3.3. Parasites of *Auxis rochei*: **a**, post-larval stage of a didymozoid *ex* the gill arch; **b**, Nemathobothriinae gen. sp. 1 *ex* the fatty tissues of the eye; **c**, female of *Caligus bonito ex* the gill arch; **d**, female of *Unicolax mycterobius ex* the olfactory sinus.

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Table 3.3.2. Prevalence (P%), mean abundance (MA), mean intensity (MI) of the parasites of the head of *Auxis rochei* from La Azohía according to sampling year, (95% confidence intervals in parentheses).

Parasite / Year	2008			2011			Total		
Parameter	P%	MA	MI	P%	MA	MI	P%	MA	MI
Monogenea									
A. macrova	15 (6-32)	0.2 (0.0-0.3)	1.0 (1.0-1.0)	20 (9-38)	0.2 (0.1-0.4)	1.2 (1.0-1.3)	18 (7-29)	0.2 (0.1-0.3)	1.1 (1-1.3)
C. triangula	6 (1-19)	0.2 (0.0-0.4)	2.5 (2.0-2.5)	7 (1-21)	0.1 (0.0-0.3)	1.5 (1.0-1.5)	6 (2-16)	0.1 (0.0-0.4)	2.0 (1.0-2.5)
H. auxisi	6 (1-19)	0.6 (0.0-2.1)	9.0 (2.0-9.0)	7 (1-21)	0.1 (0.0-0.3)	1.5 (1.0-1.5)	6 (2-16)	0.3 (0.1-1.4)	5.3 (1.3-12.5)
Didymozoidae									
D. auxis	58 (40-73)	2.1 (1.1-3.7)	3.6 (2.1-6.1)	60 (42-76)	1.8 (1.1-3.0)	3.0 (1.9-4.6)	59 (46-71)	1.9 (1.3-2.9)	3.3 (2.3-4.7)
Didymozoid sp. (juveniles)	60 (45-79)	5.5 (3.3-9.4)	8.6 (5.7-14.1)	40 (24-58)	0.6 (0.3-0.9)	1.5 (1.2-1.8)	52 (40-64)	3.2 (2.0-5.3)	6.0 (4.0-9.8)
Nematobothriinae gen. sp. 1	6 (1-19)	0.1 (0.0-0.2)	1.5 (1.0-1.5)	43 (26-62)	0.5 (0.3-0.8)	1.2 (1.0-1.5)	24 (15-36)	0.3 (0.2-0.5)	1.3 (1.1-1.5)
Copepoda									
C. bonito	18 (8-35)	0.2 (0.1-0.3)	1.0 (1.0-1.0)	7 (1-21)	0.1 (0.0-0.2)	1.0 (1.0-1.0)	13 (6-24)	0.1 (0.1-0.2)	1 (-)
U. mycterobius	9 (3-24)	0.1 (0.0-0.2)	1.0 (1.0-1.0)	7 (1-21)	0.1 (0.0-0.2)	1.0 (1.0-1.0)	8 (3-17)	0.1 (0.0-0.1)	1 (-)

Table 3.3.3. Prevalence P (%), mean abundance (MA), mean intensity (MI) of the parasites of the head of *Auxis rochei* from Tarifa from 2008 (95% confidence intervals in parentheses).

Parasite / Parameter	P (%)	MA	MI
Monogenea			
A. macrova	11 (1-44)	0.1 (0.0-0.3)	1.0 (1.0-1.0)
Didymozoidae			
D. auxis	78 (44-96)	1.4 (0.7-2.3)	1.9 (1.1-2.7)
Copepoda			
U. micterobius	11 (1-44)	0.1 (0.0-0.4)	1.0 (1.0-1.0)

Table 3.3.4 shows the levels of infection in the hosts grouped according locality and size (pooling years) and the results of the statistical analyses. *Didymozoon auxis* showed higher values of P% in the large fish of La Azohía (AL08+AL11) and Tarifa (TL08) than in the small fish of La Azohía (AS08+AS11). Three parasites had differences of MA: *A. macrova* had higher MA in the large specimens of La Azohía (AL08+AL11) than in the small ones (AS08+AS11); Nematobothriinae gen. sp. 1 had higher MA in the small and large fish of La Azohía (AS08+AS11 and AL08+AL11) than in Tarifa (TL08); *C. bonito* had higher MA in the large fish of La Azohía than in the large fish of Tarifa (AL08+AL11 *vs* TL08).

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Table 3.3.4. Prevalence P (%), mean abundance (MA) and mean intensity (MI) of the parasites of the head of *Auxis rochei* according to locality and size (95% confidence intervals in parentheses). AS08+AS11, small fish from La Azohía; AL08+AL11, large fish from La Azohía; TL08, large fish from Tarifa.* and #, significant differences ($p \leq 0.05$).

Parasite / Group	AS08+AS11			AL08+AL11			TL08		
Parameter	P%	MA	MI	P%	MA	MI	P%	MA	MI
Monogenea									
A. macrova	5(0-23)	0.1 (0-0.1)*	1.0 (-)	24 (13-39)	0.3 (0.1-0.4)*	1.1 (1.0-1.3)	11 (1-44)	0.1 (0.0-0.33)	1.0 (-)
C. triangula	5(0-23)	0.1 (0-0.1)	1.0 (-)	7 (2-20)	0.1 (0.0-0.3)	1.7 (1.0-2.0)	0 (0-32)	0.0 (-)	0.0 (-)
H. auxisi	14 (4-35)	0.9 (0.1-3.9)	6.3 (1.0-11.3)	2 (0-13)	0.1 (0.0-0.1)	2.0 (N/A)	0 (0-32)	0.0 (-)	0.0 (-)
Didymozoidae									
D. auxis	33 (16-55)*#	1.4 (0.4-4.1)	4.3 (1.7-10.3)	71 (56-84)*	2.2 (1.4-3.3)	3.1 (2.1-4.4)	78 (44-96)#	1.4 (0.7-2.3)	1.9 (1.1-2.7)
Nematobothriinae gen. sp.	24 (10-46)	0.3 (0.1-0.4)*	1.0 (-)	24 (13-39)	0.3 (0.2-0.6)#	1.4 (1.1-1.6)	0 (0-32)	0.0 (-)*#	0.0 (-)
Copepoda									
C. bonito	5(0-23)	0.1 (0-0.1)	1.0 (-)	17 (8-31)	0.2 (0.1-0.3)*	1.0 (-)	0 (0-32)	0.0 (-)*	0.0 (-)
U. mycterobius	20 (7-47)	0.2 (0.0-0.4)	1.0 (-)	5 (0-16)	0.1 (0.0-0.1)	1.0 (-)	11 (1-44)	0.1 (0.0-0.33)	1.0 (-)

The non-metric Multidimensional scaling (NMDS) plots and the cluster analysis (CA) diagrams of the species with significant differences of prevalence and mean abundance between the three host groups of the Table 3.3.3 are shown in Fig. 3.3.3. The cophenetic index of the dendrograms indicated on each graph is representative of the real distance between host groups (Rc =0.95-1.00). The CA and NMDS based on the prevalence of *D. auxis* separated the small fish of La Azohía (AS08+AS11) from the large fish of La Azohía (AL08+AL11) and Tarifa (TL08). The CA based on the mean abundance of Nematobothrinae gen. sp. 1 separated the parasite assemblage of the fish of Tarifa from those of La Azohía (AL08+AL11 and AS08+AS11), while this separation is less evident in the NMDS plot. The CA and NMDS of the mean abundance of ectoparasites (*A. macrova* and *C. bonito*) separated the parasite assemblages of the large specimens of La Azohía (AL08+AL11) and those of the other groups (AS08+AS11 and TL08).

The cluster analyses of the parasite assemblages according to the indices of dissimilarity based on the presence/absence data of parasite species in the three groups of *A. rochei* from the western Mediterranean Sea (AL08+AL11, AS08+AS11, TL08) are shown in Fig. 3.3.4. Two clusters can be identified: one with the samples of Tarifa (T08), and one with all the groups of La Azohía (AL08+AL11, AS08+AS11).

The cluster analyses of the parasite assemblages according to the indices of dissimilarity based on the presence/absence data of parasite species in the parasites assemblages of *A. rochei* from the Mediterranean Sea (present study) and *A. thazard* from other areas of the world (Table 3.2.2) are shown in the Fig 3.3.5. The parasite assemblage of *A. rochei* from the Mediterranean Sea (ArM) is grouped with that of *A. thazard* (AtA) from the Atlantic Ocean and well separated from those of the Indian and Pacific *A. thazard* (AtI and AtP).

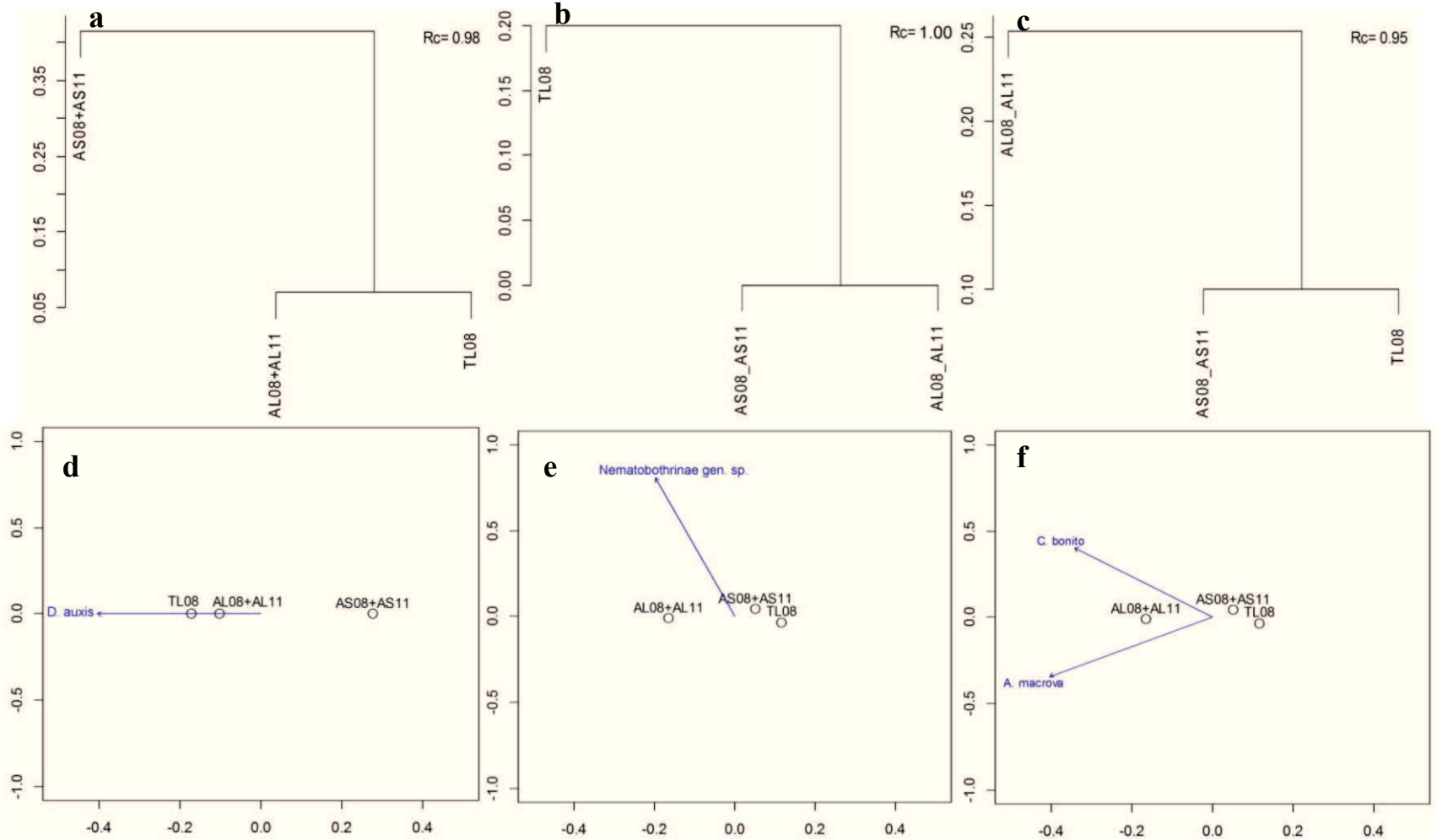

Figure 3.3.4. Cluster dendrograms (CA) (**a**, **b**, **c**) and Non-metric multidimensional scaling (NMDS) plots (**d**, **e**, **f**) based on the Bray-Curtis distance of the parasite species of the head of *Auxis rochei* from the Mediterranean Sea that showed differences between at least one pairwise of host groups according to size and locality. **a**, **d,** CA and NMDS of the prevalence of *Didymozoon auxis*, **b**, **e,** mean abundance of Nematobothriinae gen. sp. 1; **c**, **f,** mean abundance of ectoparasites (*Allopseudaxine macrova* and *Caligus bonito*). AS08+AS11, small fish from La Azohía; AL08+AL11, large fish from La Azohía; TL08, large fish from Tarifa.

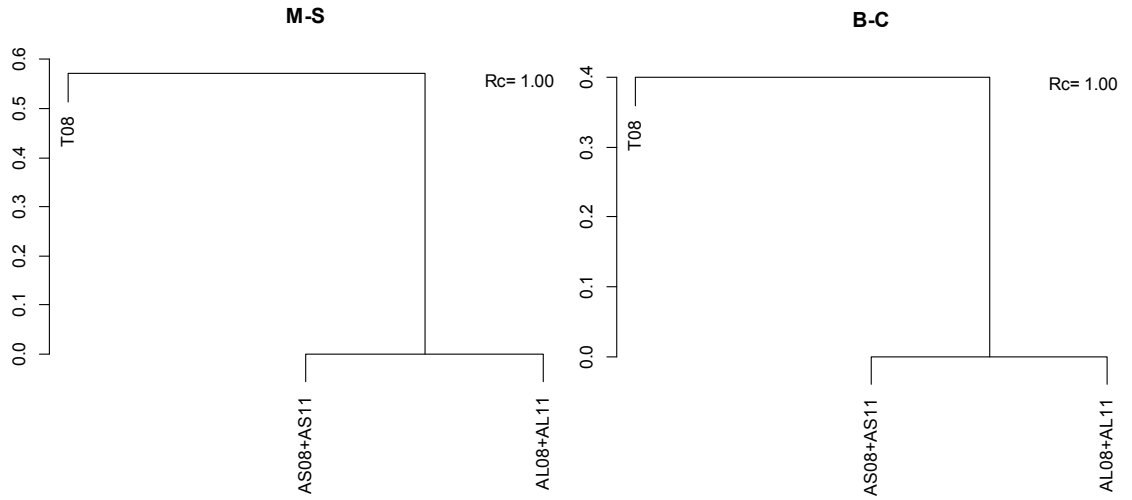

Figure 3.3.5. Cluster dendrograms (group-average linkage) of the parasites of the head of *A. rochei* from the Mediterranean Sea according to locality and size, using Marczewski-Steinhaus (M-S) and Bray-Curtis (B-C) dissimilarity measures based on the presence/absence of parasites. AS08+AS11, small fish from La Azohía; AL08+AL11, large fish from La Azohía; TL08, large fish from Tarifa.

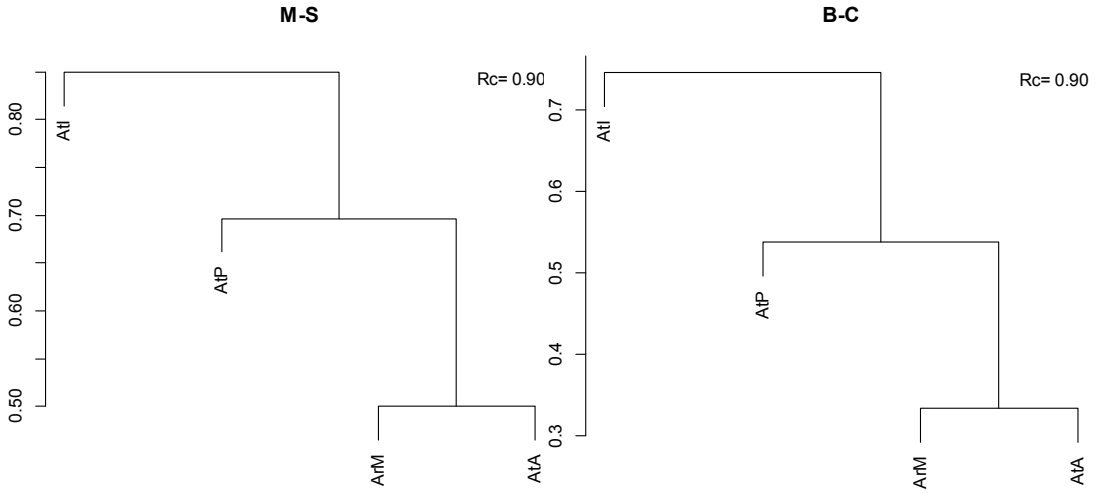

Figure 3.3.6. Cluster dendograms (group-average linkage) of the parasite of the head of *Auxis rochei* from the western Mediterranean Sea (ArM), and of *A. thazard* from the Atlantic Ocean (AtA), Indian Ocean (AtI) and Pacific Ocean (AtP), using Marczewski-Steinhaus (M-S) and Bray-Curtis (B-C) dissimilarity measures based on the presence/absence of parasites.

3.4. DISCUSSION

The present work is the first study on the parasite assemblages of the head of *Auxis rochei* from the Mediterranean Sea. The parasites of the head of the genus *Auxis* have been studied in several areas of the world, but the confusion in the systematic of the species of this genus and the misidentification of the specimens belonging to the two congeneric species (*Auxis thazard* and *A. rochei*) makes the specific information on the parasitofauna of the single species scarce and not

clear, and no information is available on the parasites of the head of *A. rochei* from the Mediterranean Sea. For instance, several parasites (*Caligus asymmetricus* Pillai in Kabata, 1965; *Caligus biseriodentatus* Shen, 1957; *Caligus pelamydis* Krøyer, 1863; *Caligus productus* Dana, 1849; *Didymozoon auxis*; *Hexostoma auxisi*; and *Unicolax collateralis* Cressey *et* Cressey, 1980) have been recorded in host specimens belonging to *Auxis* sp. (Dollfus, 1926; Palombi 1949; Cressey and Cressey, 1980).

Some of the parasites found in this study, *i.e*. *Allopseudaxine macrova*, *Caligus bonito*, *Churavera triangula,* Nematobothriinae gen. sp. and *Unicolax mycterobius*, are reported for the first time from the Mediterranean Sea, while *D. auxis* and *H. auxisi* have been previously recorded in *Auxis* sp. from this area (Dollfus, 1926; Palombi, 1949). Due to the absence of parasitological data of *A. rochei*, all the parasites found are new for this host species.

The parasite fauna of the head of *A. rochei* has a lower species richness than that of the Atlantic *A. thazard*, and similar to those of *A. thazard* from the Indian and Pacific Oceans, but it shares more species with the Atlantic *A. thazard*. In fact, all the parasites found in the present study, except Nematobothriinae gen. sp., have been found in the Atlantic *A. thazard*. It should be stressed that the location of this parasite in the fatty tissues of the eye can make difficult its detection, and this may justify the absence of record in the Atlantic *A. thazard*. Considering the genetic homogeneity of *Auxis* sp. from the central-eastern Atlantic Ocean and the Mediterranean Sea (Orsi Relini *et al.*, 2009), the high similarity of the parasite fauna of the Mediterranean *A. rochei* with that of the Atlantic *A. thazard*, and the fact that it includes many species with high host specificity, these data would be compatible with the existence of an unique host species in the Atlantic Ocean and Mediterranean Sea. The available information is still not enough to confirm this hypothesis, but it suggests that misidentifications of *Auxis* species may have occurred (as mentioned above), and also point up the need of further studies to confirm the coexistence of two *Auxis* species in the Atlantic Ocean. On the other hand, among the parasites of *A. rochei* from the Mediterranean Sea, just *A. macrova* is shared with the Indian *A. thazard*, and three species (*A. macrova*, *C. triangula* and *D. auxis*) with the Pacific one. The differences between the parasite fauna of the Mediterranean *A. rochei* and those of the Indian and Pacific *A. thazard* are likely due to geographical barriers, as shown for other scombrids (Rohde, 1989; Oliva *et al*., 2008).

The parasites of the head of *Auxis* spp. can be divided into three categories: those specific to the genus *Auxis* (*C. triangula*, *D. auxis, H. auxisi, Hexostoma keokeo, Neohexostoma mochimae, U. micterobyus*); those that infect tunas (*A. macrova, Capsala gouri*, *Capsala magronum*, *Capsala manteri*, *Capsala manteriaffinis*, *Capsala notosinense*, *Hexostoma thynni*,

Homostoma chura, *Metapseudaxine ventrosicula*, *Neohexostoma euthynni*, *U. collateralis*); and those that infect pelagic fish in general (*C. asymmetricus*, *C. biseriodentatus*, *C. bonito*, *C. pelamydis*, *C. productus*, *Didymosulcus wedli*). Among the parasites specific to *Auxis* sp., *D. auxis* and *U. micterobyus* occur worldwide (Silas and Ummerkutty, 1967; Mogrovejo *et al*., 2004) and in *A. rochei* from the western Mediterranean Sea (present results), whereas the monogeneans seem to have a local distribution. For instance, *N. mochinae* was described only in *A. thazard* from the tropical western Atlantic Ocean (Fuentes Zambrano, 1997); *H. keokeo* in *A. thazard* from tropical areas of the western Atlantic, Indian and Pacific Oceans; *C. triangula* in *A. thazard* from tropical areas of the western Atlantic and Pacific Oceans (Mogrovejo *et al*., 2004) and in *A. rochei* from the Mediterranean Sea (present results); *H. auxisi* in *A. thazard* from the tropical western Atlantic Ocean (Mogrovejo *et al*., 2004) and in *A. rochei* from the Mediterranean Sea (present results). The presence of *D. auxis* and *U. micterobyus* worldwide can be correlated with a common ancestor of *Auxis* spp., and the absence of *H. keokeo* and *N. mochimae* in the western Mediterranean *A. rochei* suggests that it is separated from the *Auxis* spp. populations from other areas, such as those of the south-western Atlantic, Indian and Pacific Oceans. In fact, the studies of highly host specific parasites, such as polyopisthocotylids, can contribute to the understanding of the spatial dynamics of both host and parasites (Oliva *et al.*, 2008; Châari *et al.*, 2010).

Didymozoon auxis and *A. macrova* showed differences of prevalence between small and large Mediterranean fish. Didymozoids are generally considered tropical parasites, because their immature stages are widely present in tropical areas (Cribb *et al*., 2000), whereas they seem less frequent in the Mediterranean Sea (Culurgioni *et al*., 2012). *Allopseudaxine macrova*, has been previously reported in other tunas from the tropical areas (*e.g*. in *A. thazard*, *Euthynnus alletteratus* and *Katsuwonus pelamis* from the south western Atlantic Ocean; Mogrovejo *et al*., 2004; Alves and Luque, 2006), but it has never been reported in the same species from the Mediterranean Sea, and the levels of infection of *A. macrova* in the large Mediterranean fish are similar to those reported in *E. alletteratus* and *K. pelamis* from tropical waters (Alves and Luque, 2006). Therefore, these differences could be a consequence of the origin of the two host groups (large and small Mediterranean fish) from different areas, suggesting that the large fish could get infected in the tropical area of the Atlantic Ocean, where the presence of didymozoid intermediate hosts is higher and the levels of infection of *A. macrova* are greater. This hypothesis agrees with Grutsev (1992), who described an autumnal trophic concentration of *A. rochei* in this area, and with Sabatés and Recasens (2001), that suggested a genetic migration of this species from the Western Sahara coasts to the north-western Mediterranean Sea.

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Moreover, considering that didymozoids infect the definitive host through the food web, the difference of infection of *D. auxis* in large and small Mediterranean fish can also indicate a change of diet with size (Williams *et al*. 1992), as already described by Mostarda *et al*. (2007) for *A. rochei* in the Tyrrhenian Sea. This author indicated that the large fish (>35 cm) are able to predate on fast-swimming preys (*e.g.* juveniles and adults of fish and cephalopods), which are among the most common intermediate hosts for didymozoids (Anderson, 1999; Vidal and Haimovici, 1999; Felizardo *et al*. 2011).

The analyses of the total parasite assemblage of *A. rochei* from the western Mediterranean Sea showed that the prevalence of *D. auxis* is the most important factor to discriminate the groups of the large and small fish, and these results are confirmed by the cluster analysis and the NMDS of didymozoids, while the cluster analysis of ectoparasites did not show a clear difference between large and small fish. Didymozoids are considered useful tags because they live encysted in the host tissues and cannot be lost during migration, and this fact strengthens the usefulness of *D. auxis*. On the other hand, ectoparasites can be lost during the host migration (Lester *et al*., 1985), and they are rarely considered useful tags; nevertheless, *A. macrova* could help to follow the seasonal migration of the bullet tuna from the tropical Atlantic area to the Mediterranean Sea.

CHAPTER 4. METAZOAN PARASITES OF THE HEAD OF THE ATLANTIC LITTLE TUNNY *EUTHYNNUS ALLETTERATUS* (OSTEICHTHYES: SCOMBRIDAE) FROM THE WESTERN MEDITERRANEAN SEA

4.1. INTRODUCTION

The Atlantic little tunny *Euthynnus alletteratus* is a pelagic fish that inhabits the inshore tropical and subtropical waters of both sides of the Atlantic Ocean, including the Mediterranean Sea, Caribbean Sea and Gulf of Mexico (Collette and Nauen, 1983). The population structure of this species is complex: in the eastern Atlantic Ocean there are two populations, one in the central-eastern and one in the southern eastern Atlantic Ocean. In the Mediterranean Sea there is another population, which is considered independent from the Atlantic ones (Gaykov and Bokhanov, 2008). Finally, in the western Atlantic Ocean there is one single population (Yoshida, 1979).

The Atlantic little tunny is considered less migratory than other tuna species, and its migration patterns are not well known. In the eastern Atlantic Ocean, tagging results confirmed the existence of two populations and indicated the possibility of migrations between the Atlantic Ocean and the Mediterranean Sea (Rey and Cort, 1981). The status of the Mediterranean population is quite unknown, although the 27% of the total volume of catches of this species (5573 t in 2010) is landed in this area (Catarci, 2004; FAO, 2011).

As described in the chapter 1.6, parasitological studies can be useful to investigate the biology, ecology, migration and population structure of marine organisms (Mackenzie, 2002). The parasites of *E. alletteratus* have been studied in the western Atlantic Ocean by Price (1939), Hendrix (1994), Fernandez (2002, 2009), Alves and Luque (2006) and Justo and Kohn (2005); in the eastern Atlantic Ocean by Vertoor (1962); and in the Mediterranean Sea by Palombi 1949, Akmirza (2006) and Lin Ho (2006).

The aim of this study is to investigate the metazoan parasites of the head of *E. alletteratus*

from the western Mediterranean Sea, and to evaluate their possible use as biological tags to improve the knowledge of the bio-ecological aspects of this fish.

4.2. MATERIALS AND METHODS

One hundred forty-six heads of Atlantic little tunny, caught in the western Mediterranean Sea between 2008 and 2011, were examined for parasites (Table 4.2.1). Among them, 105 specimens were caught in the traditional trap fishery of La Azohía (Algerian Sea, western Mediterranean Sea), area G of the Fig. 2.2.1 (37°32'59"N, 1°10'44"W) in June 2008 (N = 63), 2009 (N = 22) and 2011 (N = 30), whereas 22 specimens were caught in the traditional trap fishery of Tarifa in June 2008 (Gibraltar Strait, western Mediterranean Sea), area E of the Fig. 2.2.1 (36° 0'59"N, 5°37'44"W). The rest of samples, 29 fish, were caught by purse seine (N = 6) and trolling (N = 23) in the Gulf of Valencia, western Mediterranean Sea (area A of the Fig. 2.2.1) in October 2010 and 2011, respectively. Immediately after landing fish were measured (total fork length range 19–73 cm) and sexed (55 males and 70 females). After each sampling, the heads were excised, stored individually in plastic bags and frozen at -20° C.

The heads of all specimens were processed as described in the chapter 2.2.

Table 4.2.1. Sampling data of *Euthynnus alletteratus* according to locality and year.

Host group	Locality	Year	N	mean FL (cm)	Range FL (cm)	Size category
A08	La Azohía (Algerian Sea)	2008	63	61	54-69	Adults
A09	La Azohía (Algerian Sea)	2009	22	65	58-73	Adults
A11	La Azohía (Algerian Sea)	2011	20	53	38-63	Adults
T08	Tarifa (Gibraltar Strait)	2008	22	61	58-65	Adults
V10	Gulf of Valencia (Balearic Sea)	2010	6	20	19-22	Juveniles
V11	Gulf of Valencia (Balearic Sea)	2011	23	31	31-37	Juveniles

In addition to the general references cited in the chapter 2, the following specific literature was used for species identification of copepods: Lin and Ho (2006).

The parasitological terms used are as defined in the Chapter 2; prevalence (P%), mean abundance (MA) and mean intensity (MI) of each parasite species and their confidence intervals were calculated according to the methods described in the Chapter 2.2.

Possible correlations between parasite abundance and host size were assessed using the Spearman rank correlation coefficient.

The levels of infection of each parasite species were calculated according to locality and year, dividing hosts into six groups (Table 4.2.1): adult fish from La Azohía of 2008 (A08), 2009 (A09) and 2011 (A11); from Tarifa of 2008 (T08); and juvenile fish from the Gulf of Valencia of 2010 (V10) and 2011 (V11).

The differences between the parasite assemblages of these six host groups and between host sexes were evaluated using the Fisher's exact test for prevalence and the bootstrap t-test for mean abundance and mean intensity (Ròzsa *et al*., 2000).

The results were compared with previously published quantitative data of *E. alletteratus* parasites from the Brazilian waters of the south-western Atlantic Ocean (coded B) (Alves and Luque, 2006).

Non-Metric Multidimensional Scaling (NMDS) and Cluster Analysis (CA) were performed as described in the Chapter 2.2, considering prevalence, mean abundance and mean intensity of each parasite species to identify possible differences between the considered host groups (A08, A09, A11, B, T08, V10, V11).

Component community parameters (species richness; Berger-Parker index, d) were calculated according to Magurran (2005).

The dissimilarity between the parasite assemblages of the six groups of *E. alletteratus* from the western Mediterranean Sea (Table 4.2.1), were evaluated according to the methods described in the Chapter 2.2.

A datasheet of presence/absence was created on the basis of the published data of the parasites of the head of *E. alletteratus* according to locality (Table 4.2.2). These data served to evaluate the dissimilarity between the parasite fauna of the head of *E. alletteratus* from the western Mediterranean Sea and the Atlantic Ocean, according to the methods described in Chapter 2.2.

Table 4.2.2. Published data of the parasites of the head of *Euthynnus alletteratus* from the Atlantic Ocean according to locality. Numbers represent literature sources: 1, Palombi (1949); 2, Bussiéras (1972); 3, Cressey and Cressey (1980); 4, Hendrix (1994); 5, Alves and Luque (2006); 6, Lin and Ho (2006); 7, Chisholm and Wittington (2007). **[t]**, parasite reported in the tropical area of the host distribution. **[w]**, parasite reported in the temperate area of the host distribution.

	Eastern Atlantic Ocean	Western Atlantic Ocean
Monogenea		
Allopseudaxine macrova (Unnithan, 1957) **[t]**		5
Capsala gouri (Chauhan, 1951) **[t]**		7
Capsala magronum (Ishii, 1936) **[t]**		7
Capsala manteri (Price, 1951) **[t]**	2	7
Capsala onchidiocotyle (Setti, 1899) **[w]**		7
Hexostoma euthynni Meserve, 1938 **[t, w]**		2, 5
Hexostoma keokeo Yamaguti, 1968 **[t]**		5
Hexostoma lintoni Price, 1961		2
Hexostoma thunninae Parona *et* Perugia, 1889 **[w]**		1
Udonella caligorum Johnston, 1835 **[t]**		4
Metapseudaxine ventrosicula Mamaev, 1967 **[t]**		
Digenea		
Lobatozoum multisacculatum Ishii, 1935 **[t]**		5
Crustacea		
Caligus coryphaenae Steenstrup *et* Lütken, 1861 **[t, w]**		
Caligus bonito Wilson, 1905 **[t, w]**		5
Caligus pelamydis Krøyer, 1863 **[t, w]**		5
Caligus productus Dana, 1849 **[t]**		
Ceratocolax euthynni Vervoort, 1965 **[t]**	3	3
Isopoda gen. sp. **[t]**		5
Pseudocycnus appendiculatus Heller, 1865 **[t, w]**	3	5
Unicolax anonymous (Vervoort, 1965) **[t]**	6	3
Unicolax collateralis Cressey *et* Cressey, 1980 **[t, w]**		6

4.3. RESULTS

A total of 13 parasite species/taxa were found in the head of the Atlantic little tunny from the western Mediterranean Sea (Tables 4.3.1-3, Figs. 4.3.1-11), all of them found in the samples of La Azohía. Most of the parasites from this locality were adult didymozoids (75% of all specimens), with seven species: *Didymocystis* sp. 1, *Didymocystis* sp. 2, Didymozoinae gen. sp., *Didymozoon* sp., *Melanocystis* sp., Nematobothriinae gen. sp. 2, and Nematobothriinae gen. sp. 3; followed by monogeneans (17% of all specimens), with two species: *Capsala manteri*, *Hexostoma thunninae*; and copepods (8% of all specimens), with four species: *Caligus bonito*, *Ceratocolax euthynni*, *Pseudocycnus appendiculatus* and *Unicolax collateralis*.

Table 4.3.1. Prevalence (%) of the parasites of the head of *Euthynnus alletteratus* from the western Mediterranean Sea according to host groups and location (95% confidence intervals in parentheses). A08, A09, A11, fish from La Azohía of 2008, 2009 and 2011; T08, fish from Tarifa of 2008; V10 and V11, fish from the Gulf of Valencia of 2010 and 2011. *, new geographical record; #, new host record. Greek letters, significant differences ($p \leq 0.05$).

Parasite species / Host group	A08	A09	A11	V10	V11	T08	Location
Monogenea							
C. manteri∗	3(1-10)	9 (2-29)	0 (0-17)	0 (0-41)	13 (4-32)α	0(0-15)	Gill arch
H. thunninae	64 (51-75)αβ	64 (42-81)γδ	70 (47-86)εζ	0 (0-41)αγε	17 (6-39)βδζη	50(29-71)η	Gill filaments
Didymozoidae							
Didymocystis sp. 1 ∗#	0 (0-6) α	0 (0-15) β	25 (10-47) αβγ	0 (0-41)	9 (2-29)	0(0-15)γ	Nasal sinus
Didymocystis sp. 2∗#	8 (3-17)	9 (2-29)	20 (7-42)	33 (6-73)	13 (4-32)	9(2-29)	Gill rakers
Didymozoinae gen. sp. ∗#	0 (0-6)	5 (0-22)	0 (0-17)	0 (0-41)	0 (0-15)	0(0-15)	Outer margin of gill filaments
Didymozoon sp. ∗#	27(17-40)αβ	59 (38-78)αγ	75 (53-90)βδεζ	17 (1-59)δ	39 (21-61)εη	9(2-29)γζη	Inner margin of gill filaments
Melanocystis sp. ∗#	8 (3-17)αβγ	46 (26-66)αδ	100 (83-100)βδεζη	17 (1-59)ε	65 (43-82)γζθ	18(6-39)ηθ	Pharyngeal mucosa
Nematobothriinae gen sp. 2∗#	0 (0-6)αβ	14 (4-34)αγ	45 (24-68)βγδ	0 (0-41)	4 (0-21)δ	0(0-15)	Operculum
Nematobothriinae gen sp. 3∗#	19 (11-31)αβ	32(15-55)αγ	35 (17-58)δε	0 (0-41)	0 (0-15)βδ	0(0-15)γε	Pharyngeal tissues
Crustacea							
C. bonito∗	19 (11-31)α	32 (15-55)β	25 (10-47)γ	0 (0-41)	35 (18-57)δ	0(0-15)αβγδ	Gill chamber
C. euthynni ∗	2 (0-8)	0 (0-15)	9 (2-29)	0 (0-41)	0 (0-15)	0(0-15)	Nasal sinus (rosette)
P. appendiculatus	60 (48-72)α	50 (29-71)	50 (29-71)β	0 (0-41)αβ	41 (22-62)	41(22-62)	Gill filaments
U. collateralis	0 (0-6)αβ	0 (0-15)γδ	25 (10-47)αγ	0 (0-41)	39 (21-61)βδε	5(0-22)ε	Nasal sinus

Table 4.3.2. Mean abundance of the parasites of the head of *Euthynnus alletteratus* from the western Mediterranean Sea according to host groups (95% confidence intervals in parentheses). A08, A09, A11, fish from La Azohía of 2008, 2009 and 2011; T08, fish from Tarifa of 2008; V10 and V11, fish from the Gulf of Valencia of 2010 and 2011. Greek, indicate significant differences ($p \leq 0.05$).

Parasite species / Host group	A08	A09	A11	V10	V11	T08
Monogenea						
C. manteri	0.4(0.0-2.1)	4.2(0.0-16.7)	0.0(-)	0.0(-)	0.2(0.0-0.5)	0.0 (N/A)
H. thunnina	1.8(1.3-2.6)α, β	3.0(1.6-6.2)γ	2.3(1.2-4.2)εζ	0.0(-)αγε	0.2(0-0.3)βζ	1.0(0.5-2.2)
Didymozoidae						
Didymocystis sp. 1	0.0 (-)α	0.0(-)β	0.3(0.1-0.4) αβγδ	0.0(-)δ	0.1(0.0-0.2)	0.0 (-)γ
Didymocystis sp. 2	0.1(0.0-0.2)	0.1(0.0-0.4)	0.6(0.1-1.6)	0.5(0.0-1.2)	0.3(0.0-0.7)	0.1(0.0-0.4)
Didymozoinae gen. sp.	0.0 (-)	0.2(0.0-0.6)	0.0(-)	0.0(-)	0.0(-)	0.0 (N/A)
Didymozoon sp.	2.1(1.0-3.9)αβ	3.5(1.8-5.8)γδ	8.8(4.2-18.0)	0.3(0.0-0.7)αγ	2.4(0.9-5.2)	0.2(0.0-0.5)βδ
Melanocystis sp.	0.2(0.1-0.5)αβγ	46.2(20.6-86.4)αδεζη	1.3(1.1-1.4)βδθικ	0.2(0.0-0.3)εθλ	0.9(0.5-1.2)γζλμ	0.2(0.1-0.3)ηκμ
Nematobothriinae gen sp. 2	0.0 (-)α	0.1(0.0-0.3)β	0.7(0.3-1.0)αβγδ	0.0(-)γ	0.0(0.0-0.1)δ	0.0 (-)
Nematobothriinae gen sp. 3	0.3 (0.2-0.6)αβγ	0.6(0.2-1.1)δεζ	0.4(0.2-0.7)ζηθ	0.0(-)αδζ	0.0(-)βεη	0.0 (-)γεθ
Copepoda						
C. bonito	0.2 (0.1-0.3)αβ	0.4(0.1-0.6)γδ	0.5(0.2-0.9)	0.0(-)αγε	0.5(0.2-0.8)εζ	0.0 (-)βδζ
C. euthynni	0.0 (0.0-0.1)	0.0(-)	0.1(0.0-0.4)	0.0(-)	0.0(N/A)	0.0 (-)
P. appendiculatus	1.5 (1.1-2.0)αβγδε	0.6(0.4-1.0)αζ	0.7(0.4-1.1)βη	0.0(-)γζηθι	0.6(0.3-0.8)δθ	0.6(0.2-0.8)ει
U. collateralis	0.0 (-)α	0.0(-)βγ	0.4(0.2-0.9)β	0.0(-)δ	0.6(0.3-0.9)αγδε	0.1(0.0-0.1)ε

Table 4.3.3. Mean intensity of the parasites of the head of *Euthynnus alletteratus* from the western Mediterranean Sea according to host groups (95% confidence intervals in parentheses). A08, A09, A11, fish from La Azohía of 2008, 2009 and 2011; T08, fish from Tarifa of 2008; V10 and V11, fish from the Gulf of Valencia of 2010 and 2011. Greek letters, significant differences ($p \leq 0.05$).

Parasite species / Host group	A08	A09	A11	V10	V11	T08
Monogenea						
C. manteri	13.5(1.0-13.5)	46.0(-)	0.0 (-)	0.0 (-)	1.7(1.0-1.7)	0.0 (-)
H. thunnina	2.8(2.1-4.1)α	4.6(2.9-9.6)	3.2(1.9-5.6)	0.0 (-)	1.0(N/A)α	2.0(1.2-3.9)
Didymozoidae						
Didymocystis sp. 1	0.0 (-)	4.0(-)	1(-)	0.0 (-)	1.0 (-)	0.0 (-)
Didymocystis sp. 2	1.2(1.0-1.4)	1.5(-)	3.0(1.0-4.8)	1.5(-)	2.0(1.0-2.7)	1.5(-)
Didymozoinae gen. sp.	0.0 (-)	4.0(-)	0.0 (-)	0.0(-)	0.0 (-)	0.0 (-)
Didymozoon sp.	7.7(4.3-12.4)α	5.9(3.5-8.7)β	11.7(6.1-23.7)	2.0(-)	6.0(2.7-11)	2.0(-)αβ
Melanocystis sp.	2.2(1.0-4.2)α	101.7(52.0-155.1)αβγ	1.3(1.1-1.4)ε	1.0(-)	1.3(1.1-1.7)βι	1.0(-)γε
Nematobothriinae gen sp. 2	0.0 (-)	1.0(-)α	1.4(1.0-1.7)α	0.0 (-)	1.0(-)	0.0 (-)
Nematobothriinae gen sp. 3	1.7(1.3-2.5)	1.9(1.1-2.4)	1.1(1.0-1.3)	0.0 (-)	0.0(-)	0.0 (-)
Copepoda						
C. bonito	1.0 (-)	1.1(1.0-1.3)	1.8(1.0-2.4)	0.0 (-)	1.4(1.0-1.6)	0.0 (-)
C. euthynni	0.0 (-)	0.0 (-)	1.5(-)	0.0 (-)	0.0(-)	0.0 (-)
P. appendiculatus	2.5(2.1-3.0)αβγδ	1.3(1.0-1.7)α	1.4(1.0-1.8)β	0.0 (-)	1.3(1.0-1.6)γ	1.3(1.0-1.6)δ
U. collateralis	0.0 (-)	0.0 (-)α	1.8(-)α	0.0 (-)	1.4(1.0-1.9)	1.0(-)

The dominant species of the parasite assemblages of La Azohía were *Didymozoon* sp. in 2008 and 2011 (d = 0.33 and 0.56, respectively), and *Melanocystis* sp. in 2009 (d = 0.79). Considering the other localities, *Didymocystis* sp. 2, *Didymozoon* sp., *H. thunninae*, *Melanocystis* sp., *P. appendiculatus* and *U. collateralis* were found in Tarifa, where *H. thunninae* was the dominant species (d = 0.46) (Table 4.3.4), and Didymozoinae gen. sp., *C. euthynni* and Nematobothrinae gen. sp. 3 were not found in the Gulf of Valencia, where *Didymocystis* sp. 2 and *Didymozoon* sp. were the dominant species in 2010 and 2011, respectively.

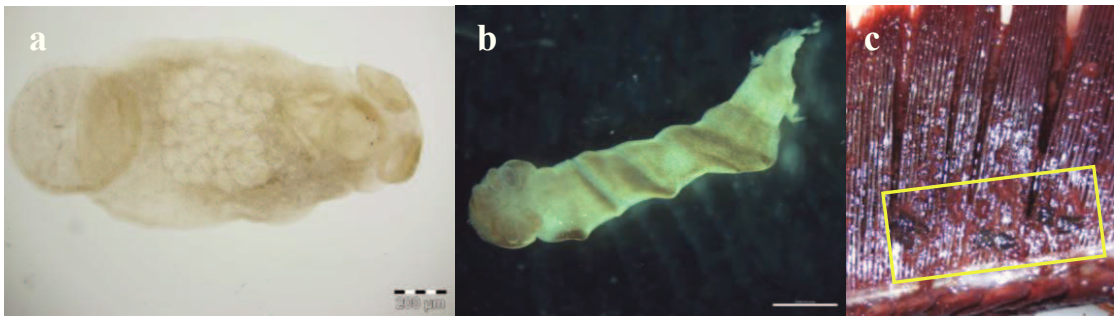

Figure 4.3.1. Monogeneans *ex* the gills of *Euthynnus alletteratus*: **a**, *Capsala manteri*; **b**, *Hexostoma thunninae*; **c**, *H. thunninae in situ.*

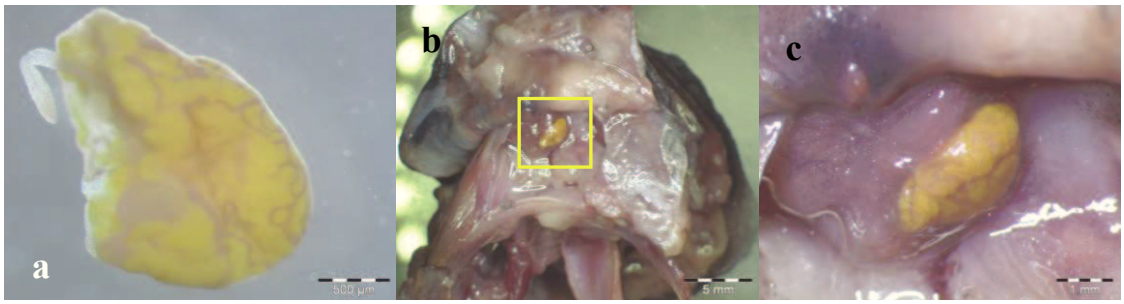

Figure 4.3.2. a, *Didymocystis* sp. 1 *ex* the olfactory rosette of *Euthynnus alletteratus*. **b-c**, *Didymocystis* sp. 1 *in situ.*

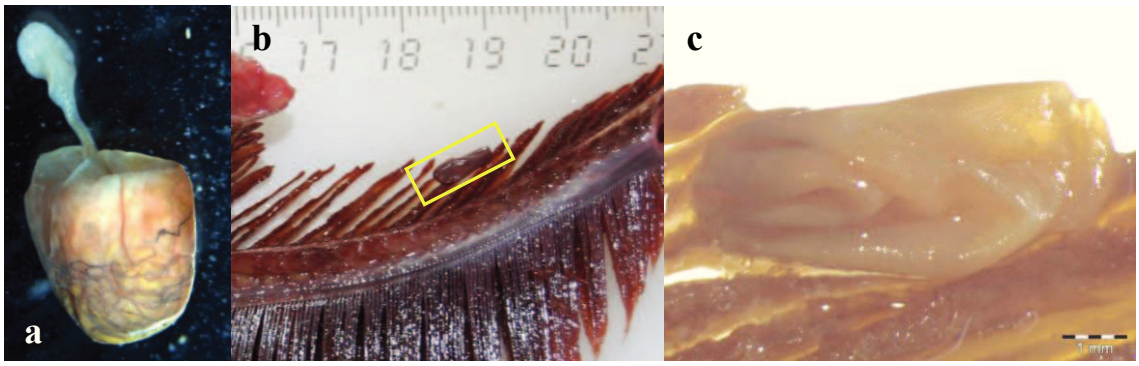

Figure 4.3.3. a, *Didymocystis* sp. 2 *ex* the gill rakers of *Euthynnus alletteratus*. **b-c**, *Didymocystis* sp. 2 *in situ.*

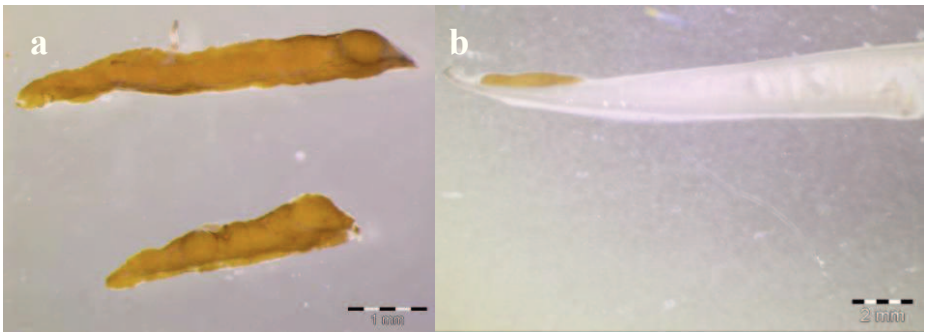

Figure 4.3.4. **a**, Didymozoinae gen. sp. *ex* outer margin of the gill filaments of *Euthynnus alletteratus*. **b**, Didymozoinae gen. sp. *in situ.*

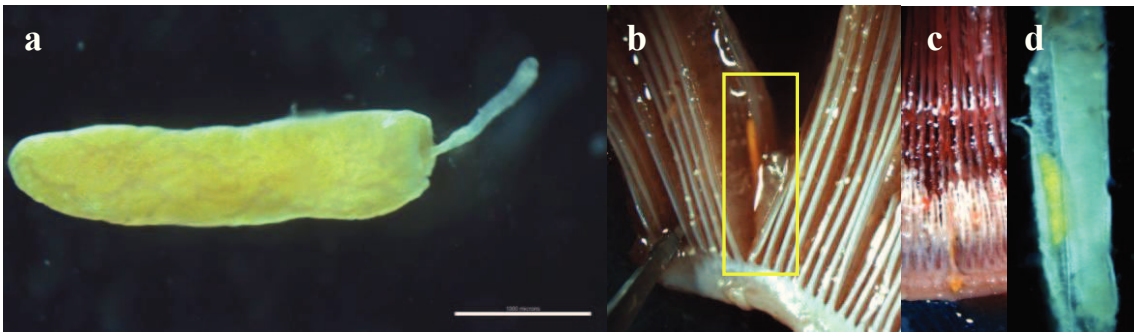

Figure 4.3.5. a, *Didymozoon* sp. *ex* the gills of *Euthynnus alletteratus*. **b-d**, *Didymozoon* sp. *in situ.*

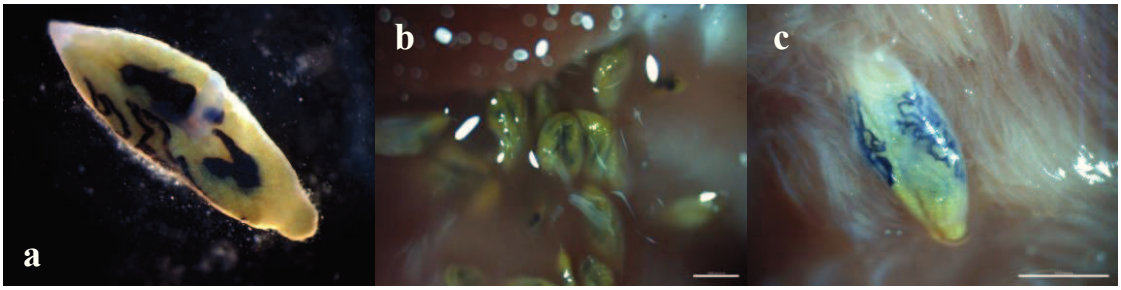

Figure 4.3.6. a, *Melanocystis* sp *ex* the pharyngeal region of *Euthynnus alletteratus*. **b-c**, *Melanocystis* sp. *in situ*.

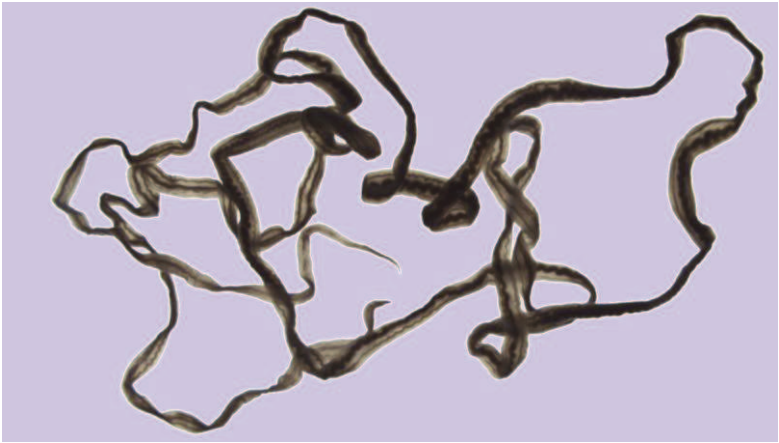

Figure 4.3.7. Nematobothriinae gen. sp. 2 *ex* the operculum of *Euthynnus alletteratus*.

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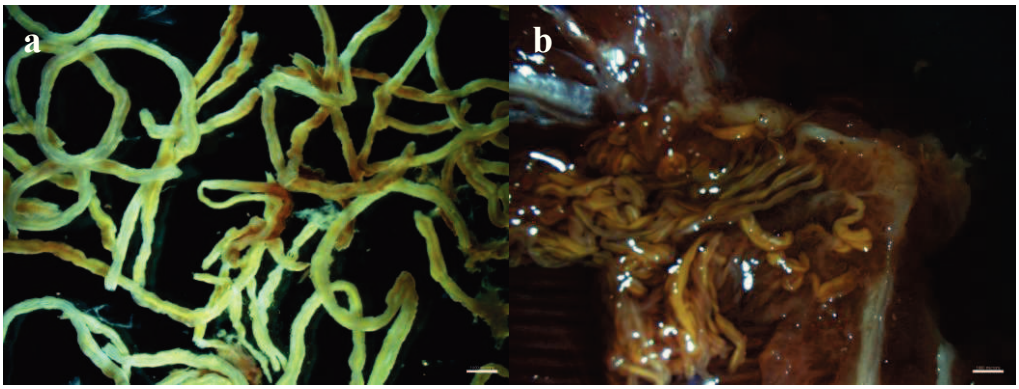

Figure 4.3.8. a, Nematobothriinae gen. sp. 3 *ex* the pharyngeal region of *Euthynnus alletteratus*. **b**, Nematobothriinae gen. sp. 3 *in situ.*

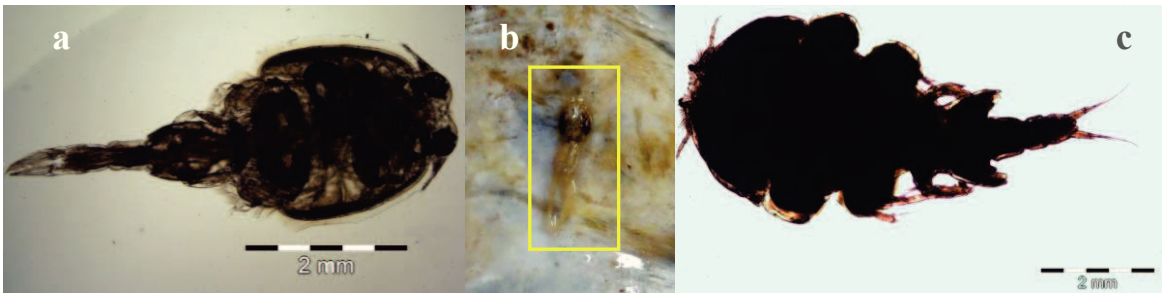

Figure 4.3.9. Copepods of *Euthynnus alletteratus*: **a**, male of *Caligus bonito ex* the gills; **b**, female of *C. bonito ex* the operculum; **c**, female of *Ceratocolax euthynni ex* the olfactory rosette.

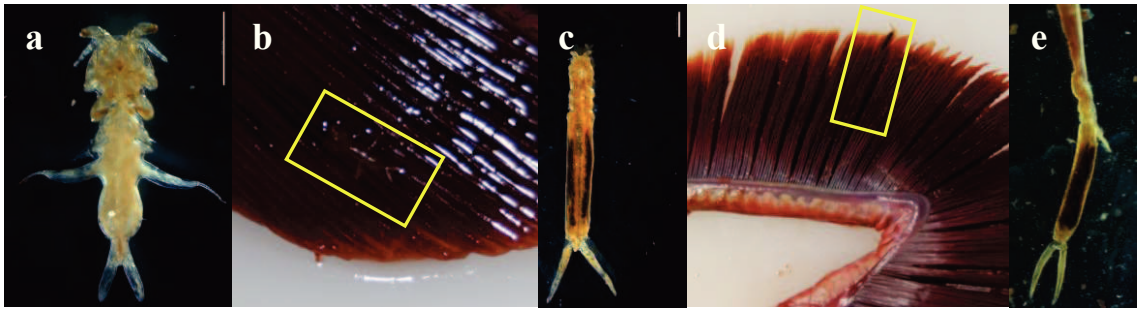

Figure 4.3.10. *Pseudocycnus appendiculatus ex* the gills of *Euthynnus alletteratus*: **a**, male; **b**, male *in situ*; **c**, female; **d-e**, female *in situ*.

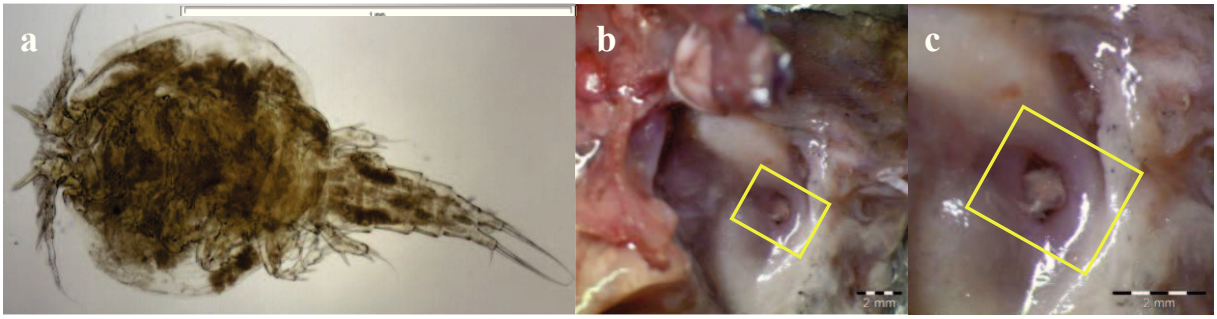

Figure 4.3.11. a, female *of Unicolax collateralis ex* the nasal sinus of *Euthynnus alletteratus*. **b-c**, female of *U. collateralis in situ.*

Salvatore Mele. Gill metazoan parasites of tunas (Scombridae: Thunnini) from the western Mediterranean Sea: systematics, assemblages and use as biological tags. Tesi di dottorato in Riproduzione, Produzione, Benessere Animale e Sicurezza degli Alimenti di Origine Animale. Università degli studi di Sassari.

No significant differences in P%, MA and MI of each parasite species were found between host sexes, and no significant relationship was found between the abundance and the host size.

The Table 4.3.1 shows the P% of the parasites of six host groups from the Mediterranean Sea (locality and year), and the results of the statistical analysis. Several species of La Azohía showed significant differences between sampling years. The didymozoids had higher values of P% in the samples of 2011 than those of 2009 and 2008, and in that of 2009 than that of 2008. Particularly, the P% of *Didymozoon* sp., and *Melanocystis* sp. and Nematobothriinae gen. sp. 2 was higher in 2009 and 2011 (A09 and A11) than in 2008 (A08); the P% of Nematobothriinae gen. sp. 2 was higher in 2011 (A11) than in 2009 (A09); the P% of Nematobothriinae gen. sp. 3 was higher in 2009 (A09) than in 2008; (A08); the P% of *Didymocystis* sp. 1 and *U. collateralis* was higher in 2011 (A11) than in 2008 (A08) and 2009 (A09). Comparing the parasite assemblages of the same year from different localities of the western Mediterranean Sea, in 2011 the P% of *Didymozoon* sp., *H. thunninae* and *Melanocystis* sp. was higher in La Azohía (A11) than in the Gulf of Valencia (V11), and in 2008 the P% of Nematobothriinae gen. sp. 2 was higher in La Azohía (A08) than in Tarifa (T08).

The Table 4.3.2 shows the MA of the parasites of the six host groups from the Mediterranean Sea (locality and year). Several parasites from La Azohía showed significant differences of MA between sampling years: the MA of *P. appendiculatus* was higher in 2008 (A08) than in 2009 (A09) and 2011 (A11); the MA of *Melanocystis* sp. was higher in 2009 (A09) than in 2011 (A11), and lower in 2008 (A08) than in 2009 (A09); the MA of three species was lower in 2008 (A08) than in 2011 (A11), *i.e. Didymocystis* sp. 1, *Melanocystis* sp., Nematobothriinae gen. sp. 2; the MA of three species was lower in 2009 (A09) than in 2011, *i.e. Didymocystis* sp. 1, Nematobothriinae gen. sp. 2. and *U. collateralis.* Furthermore, the mean abundance of some species was higher in the samples from the Gulf of Valencia of 2011 than of 2010, *i.e*. *C. bonito*, *Melanocystis* sp., *P. appendiculatus* and *U. collateralis.* Comparing the samples of the same year from different localities, in 2008 the MA of *C. bonito*, *Didymozoon* sp., Nematobothriinae gen. sp. 3, and *P. appendiculatus* was higher in La Azohía (A08) than in Tarifa, (T08); the MA of *H. thunninae*, Nematobothriinae gen. sp. 2 was higher in La Azohía (A11) than in the Gulf of Valencia (V11).

The Table 4.3.3 shows the MI of the parasites of the six host groups from the Mediterranean Sea (grouped for locality and year). Two parasites from La Azohía had significant differences of MI between years: the MI of *P. appendiculatus* was higher in 2008 (A08) than in 2009 (A09) and 2011 (A11); and that of *Melanocystis* sp. was higher in 2009 (A09) than in 2008

(A08). Comparing the parasite assemblages of the same year from different localities, in 2008 the MI of *Didymozoon* sp. and *P. appendiculatus* was higher in La Azohía (A08) than in Tarifa (T08).

The Table 4.3.4 shows the P% of the parasites of the Mediterranean hosts pooled, that recorded in the Atlantic little tunny (Alves and Luque, 2006), and the results of the statistical analysis.

Table 4.3.4. Prevalence (%) of the parasites of the head of *Euthynnus alletteratus* from the western Mediterranean Sea (WM, groups pooled) and from the south-western Atlantic Ocean (B, Alves and Luque 2006; 95% confidence intervals in parentheses). α, significant differences ($p \leq 0.05$).

Parasite species / Host group	WM	B
Monogenea		
A. macrova	0 (0-6)α	15α
C. magronum	0 (0-6)α	9α
C. manteri	5(2-9)	0
H. euthynni	0 (0-6)α	32α
H. keokeo	0 (0-6)α	15α
H. lintoni	0 (0-6)α	13α
H. thunninae	53 (45-61)α	0 α
M. ventrosicula	0 (0-6)α	11α
Didymozoidae		
Didymocystis sp. 1	5 (2-9)	0
Didymocystis sp. 2	12 (7-18) α	0α
Didymozoinae gen. sp.	0 (0-4)	0
Didymozoon sp.	37 (29-45)α	0α
L. multisacculatum	0 (0-6)α	15α
Melanocystis sp.	35 (28-43)α	0α
Nematobothriinae gen sp. 2	8 (5-14)α	0 α
Nematobothriinae gen sp. 3	17 (11-23)α	0 α
Crustacea		
C. bonito	21 (15-28)α	7 α
C. pelamydis	0 (0-6)α	11α
C. euthynni	2 (1-7)	0
Isopoda gen. sp.	0 (0-6)	4
P. appendiculatus	47 (39-55)α	24α
U. collateralis	11 (7-17)α	0α

Most of the species showed significant differences between the two localities (17/22), and among these, 7 have higher P% in the Atlantic Ocean (B) (*Allopseudaxine macrova*, *Capsala magronum*, *Caligus pelamydis*, *Hexostoma keokeo*, *Lobatozoum multisacculatum*, *Metapseudaxine ventrosicula*) and 10 in the western Mediterranean Sea (*C. bonito*, *Didymocystis*

sp. 1, *Didymocystis* sp. 2, *Didymozoon* sp., *H. thunninae*, *Melanocystis* sp., Nematobothriinae gen. sp. 2, Nematobothriinae gen. sp. 3, *P. appendiculatus* and U. *collateralis*).

The non-metric Multidimensional scaling (NMDS) plots and the cluster analysis (CA) diagrams of the species that had significant differences of prevalence between the Mediterranean groups are shown in Fig. 4.3.12. The cophenetic index of the dendrograms indicated that the graph of ectoparasites is more representative than that of didymozoids (Rc = 1.00 and 0.85, respectively). The CA and NMDS of didymozoids showed that the P% of *Didymocystis* sp. 2, *Didymozoon* sp., *L. multisacculatum*, *Melanocystis* sp. and Nematobothriinae sp. 2 separate the samples of the Atlantic Ocean (B) to those of the western Mediterranean Sea, in particular of La Azohía (A08, A09, A11) and Tarifa (T08). The P% of *Didymocystis* sp. 1, *Didymocystis* sp. 2, *Melanocystis* sp. and Nematobothriinae gen. sp. 1 separated the groups of La Azohía and Gulf of Valencia of 2011 (A11 and V11) from the other groups of the western Mediterranean Sea (A08, A09, V10) and Atlantic Ocean (B). The CA and NMDS of the prevalence of the ectoparasites showed that several species distinguished the group of the Atlantic Ocean (B) from the western Mediterranean samples, *i.e. A. macrova*, *C. bonito*, *C. magronum*, *C. pelamydis*, *H. keokeo*, *H. lintoni*, *H. thunninae M. ventrosicula*. The P% of *C. manteri*, *C. bonito*, *P. appendiculatus* and *U. collateralis,* separated the group of the Atlantic Ocean from that of La Azohía of 2009 (A09) and (B) the other Mediterranean groups.

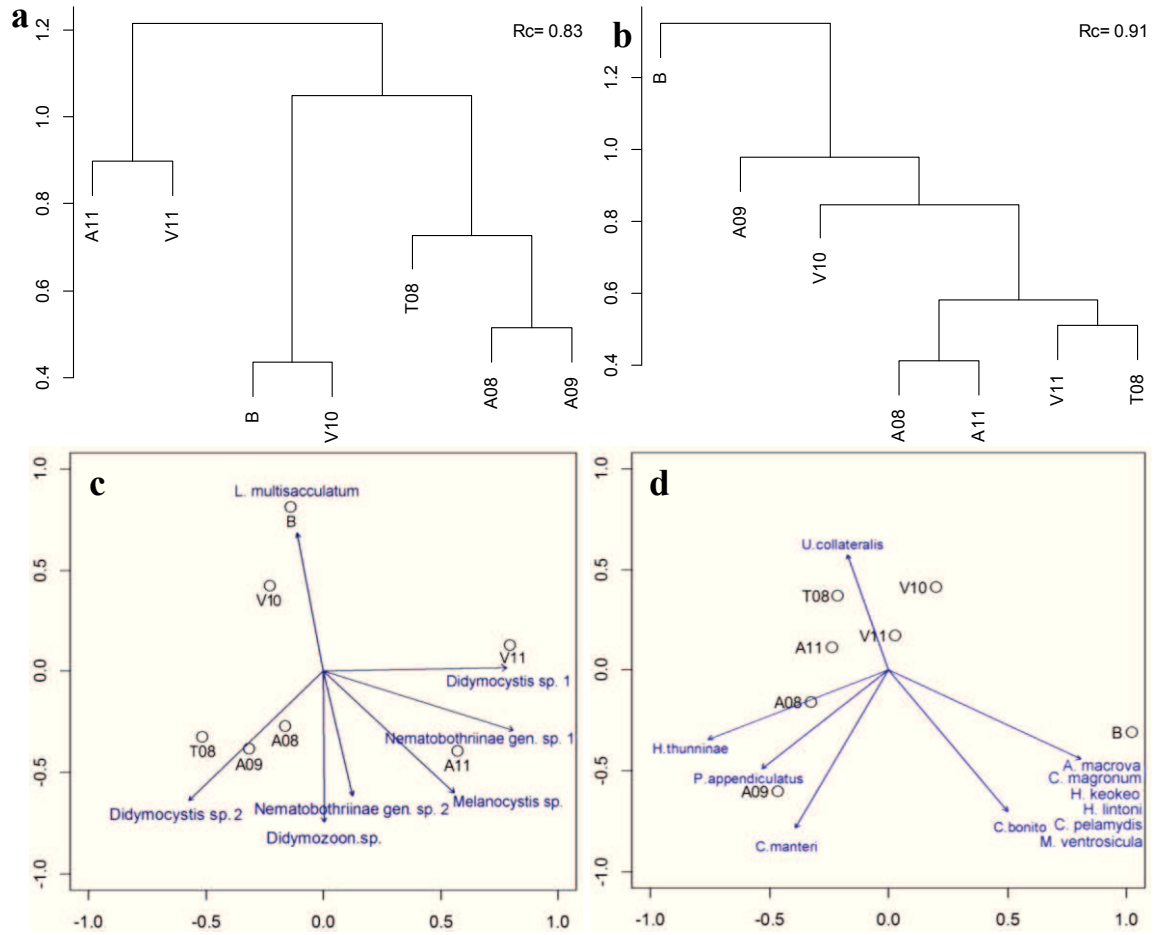

Figure 4.3.12. Cluster dendrograms (**a**, **b**) and NMDS plots (**c**, **d**) based on the Bray-Curtis distance of the data of the species that showed differences between at least one pairwise of host groups according to locality and year. **a**, **c,** CA and NMDS of the prevalence of didymozoids, **b**, **d,** CA and NMDS of the prevalence of ectoparasites. A08, A09 and A11, samples from La Azohía of 2008, 2009 and 2011; T08, samples from Tarifa; V10 and V11, samples from the Gulf of Valencia of 2010 and 2011; B, data from the Atlantic Ocean (Alves and Luque, 2006).

The NMDS plot and the CA diagrams of the species that had significant differences of mean abundance among the Mediterranean groups are shown in Fig. 4.3.13. The cophenetic index of the dendrograms indicated that the graph of didymozoids is more representative than that of ectoparasites (Rc =0.99 and 0.76). The CA and NMDS based on the MA of didymozoids showed that *Melanocystis* sp. separated the samples of La Azohía of 2009 (A09) from all the others of the western Mediterranean Sea. The MA of *Didymocystis* sp. 1, *Didymozoon* sp., Nematobothriinae gen. sp. 2 and Nematobothriinae gen. sp. 3 distinguished the samples of La Azohía of 2011 from the others of the western Mediterranean Sea. The CA and NMDS based on the MA of ectoparasites showed that the MA of *P. appendiculatus* separated the group of La

Azohía of 2008 from the others of the western Mediterranean Sea; the MA of *H. thunninae* and *U. collateralis* separated the groups of La Azohía (A08, A09, A11) from those of Tarifa (T08) and of the Gulf of Valencia (V10 and V11).

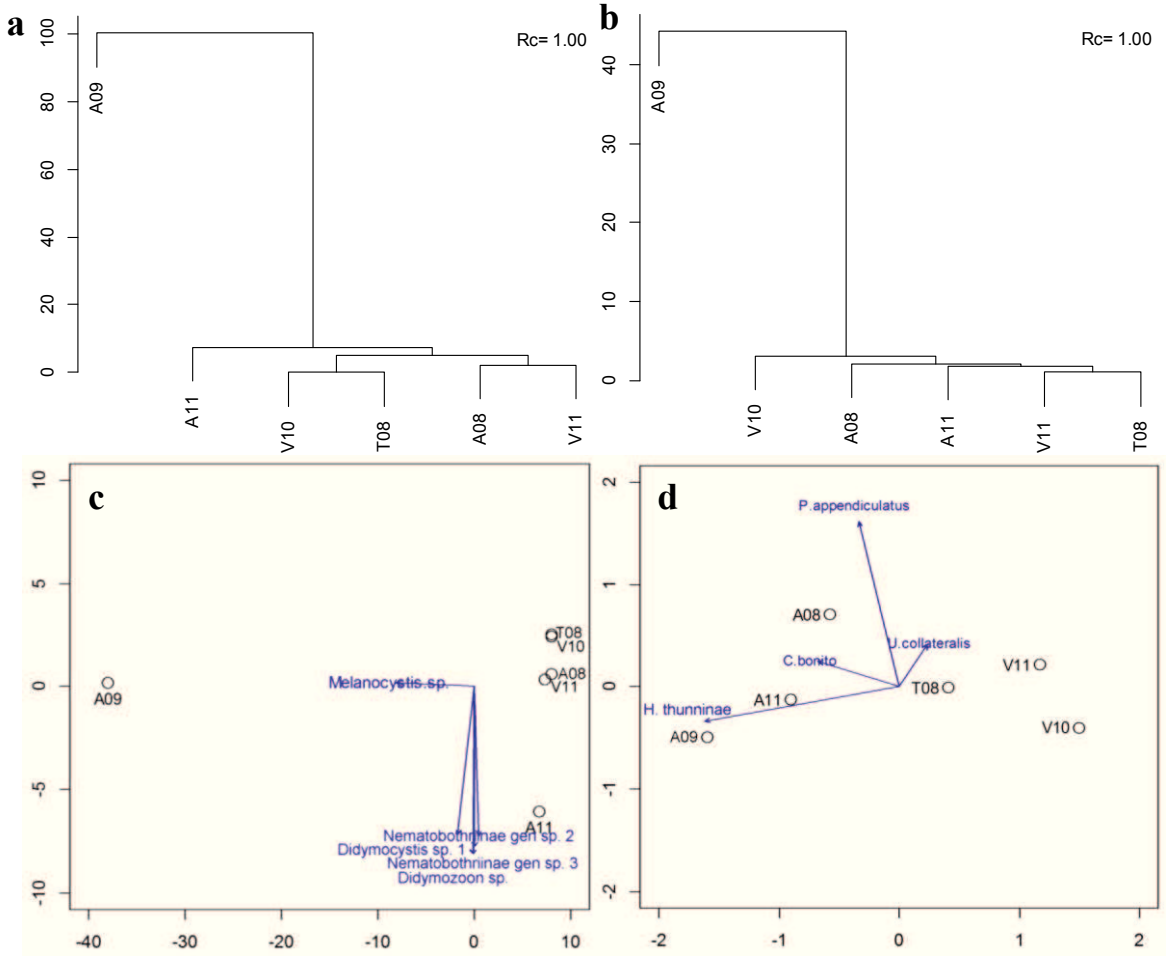

Figure 4.3.13. Cluster dendrograms (**a**, **b**) and NMDS plots (**c**, **d**) based on the Bray-Curtis distance of the data of the species that showed differences between at least one pairwise of host groups according to locality and year. **a**, **c**, CA and NMDS of the mean abundance of didymozoids. **b**, **d**, CA and NMDS of the mean abundance of ectoparasites. A08, A09 and A11, samples from La Azohía of 2008, 2009 and 2011; T08, samples from Tarifa; V10 and V11, samples from the Gulf of Valencia of 2010 and 2011.

The NMDS plot and the CA diagrams of the species that had significant differences of mean intensity between the Mediterranean groups are shown in Fig. 4.3.14. The cophenetic index of the dendrograms indicated that the graph of didymozoids and ectoparasites are both highly representative (Rc =1.00). The CA and NMDS based on the MI of didymozoids showed that the MI of *Melanocystis* sp. distinguished the samples of La Azohía of 2009 (A09) from the others of the western Mediterranean Sea. A similar result is shown in the CA and NMDS of ectoparasites, where the MI of *H. thunninae* also distinguished the samples of La Azohía of 2009

(A09) from the other groups.

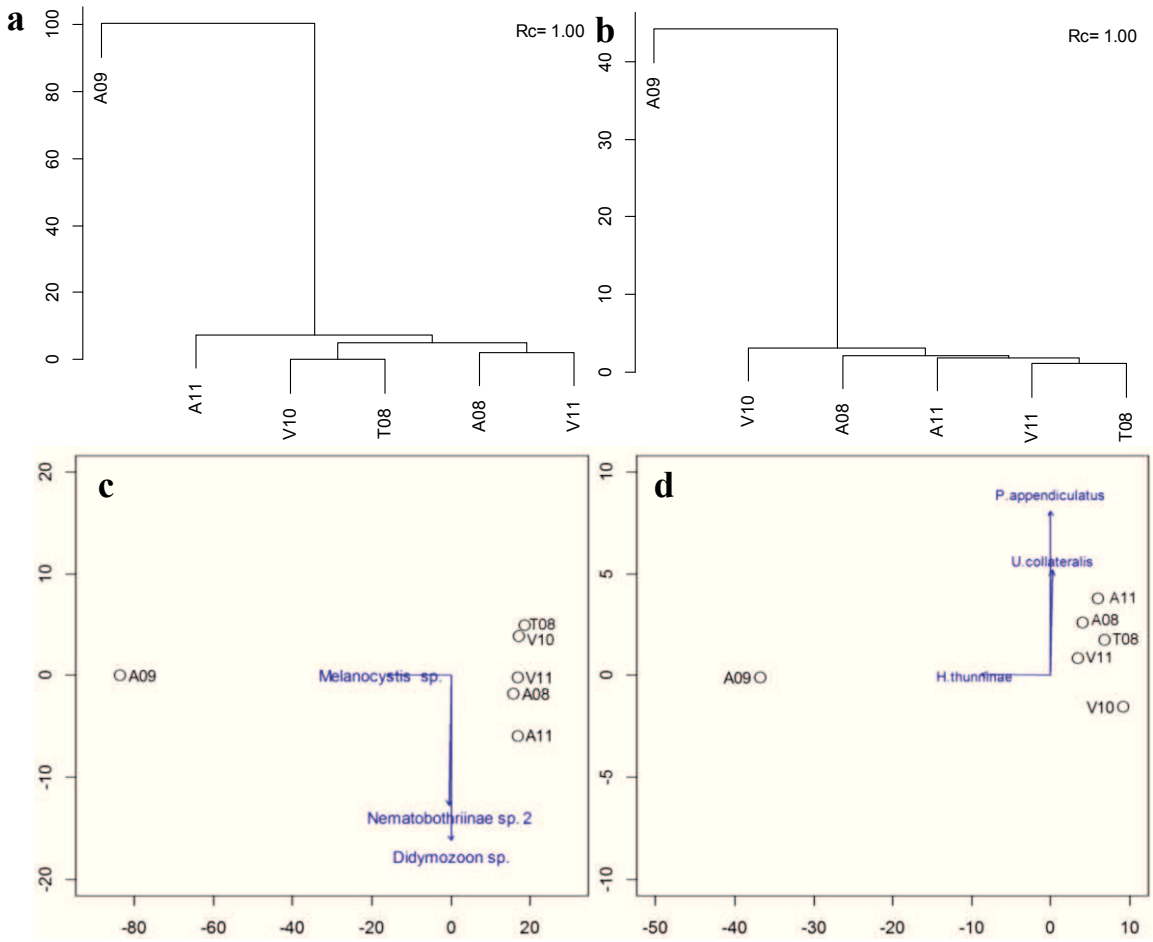

Figure 4.3.14. Cluster dendrograms (**a**, **b**) and NMDS (**c**, **d**) plots based on the Bray-Curtis distance of the data of those species that showed differences between at least one pairwise of host groups according to locality and year. **a**, **c,** CA and NMDS of the mean intensity of didymozoids, **b**, **d,** CA and NMDS of the mean intensity of ectoparasites. A08, A09 and A11, samples from La Azohía of 2008, 2009 and 2011; T08, samples from Tarifa; V10 and V11, samples from the Gulf of Valencia of 2010 and 2011.

The cluster analysis of the indices of dissimilarity based on the presence/absence of parasite species in the assemblages of the six groups of *Euthynnus alletteratus* from the western Mediterranean Sea (A08, A09, A11, T08, V10, V11) and on the published data from the eastern and western Atlantic Ocean (Table 4.2.2) are shown in Fig. 4.3.15. In both dendrograms three main clusters can be identified: the fish from the eastern Atlantic Ocean, the fish from the western Atlantic Ocean, and all the Mediterranean groups.

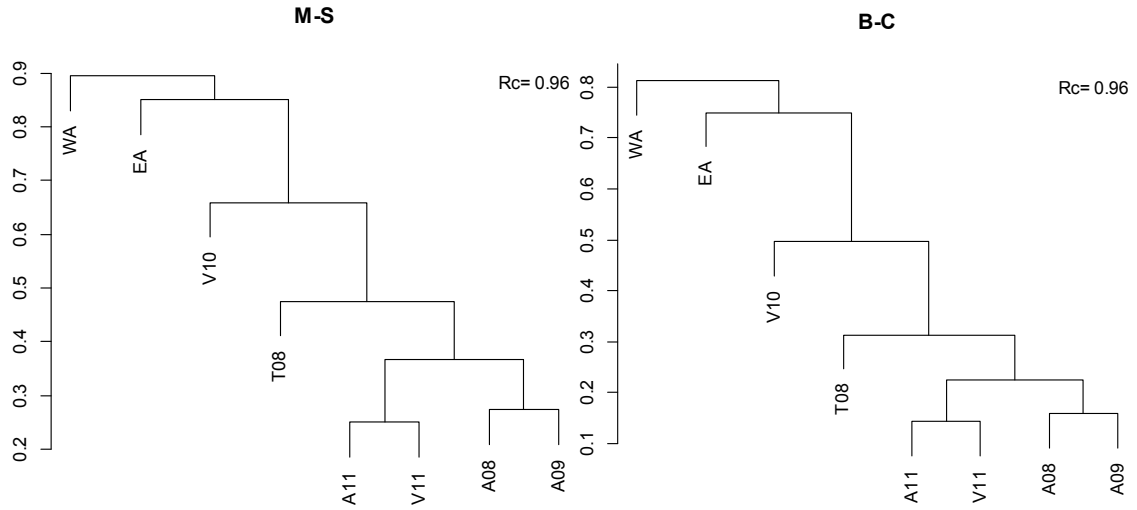

Figure 4.3.15. Cluster dendrograms (group-average linkage) of the parasites of the head of *Euthynnus alletteratus* from the Mediterranean Sea and Atlantic Ocean, according to locality and year, using Marczewski-Steinhaus (M-S) and Bray-Curtis (B-C) dissimilarity measures based on the presence/absence of parasites. A08, A09, AS11, fish from La Azohía of 2008, 2009, 2011, respectively; T08 fish from Tarifa of 2008, V10 and V11, fish from the Gulf of Valencia of 2010 and 2011, respectively; EA, eastern Atlantic Ocean and WA, western Atlantic Ocean (see Table 4.2.2).

4.4. DISCUSSION

The metazoan parasite fauna on the head of the Atlantic little tunny from the Atlantic Ocean and Mediterranean Sea includes a total of 29 species/taxa. Among them, four species have been previously reported from the Mediterranean Sea: *Hexostoma thunninae* and *Unicolax collateralis* from the same host, and *Capsala onchidiocotyle* and *Pseudocycnus appendiculatus* from *Thunnus thynnus* (Palombi, 1949; Arru and Garippa, 1994; Lin and Ho, 2006). Some of the parasites found in this study (*Capsala manteri*, *Caligus bonito, Didymocystis* sp. 1, *Didymocystis* sp. 2, Didymozoinae gen. sp., *Didymozoon* sp., *Ceratocolax euthynni*, *Hexostoma thunninae*, *Melanocystis* sp., Nematobothriinae gen. sp. 2 and Nematobothriinae gen. sp. 3) are reported for the first time in the Mediterranean Sea. *Euthynnus alletteratus* is a new host record for *Didymocystis* sp. 1, *Didymocystis* sp. 2, Didymozoinae gen. sp., *Didymozoon* sp., Nematobothriinae gen. sp. 2 and Nematobothriinae gen. sp. 3. The identification of didymozoids has not been done at the species level because of the lack of specific literature and of the difficulties for the preparation of the microscope slides for the morphological analysis. In fact, the identification of the species of this family is mainly based on the general aspect of the body and on the relative position and morphology of the reproductive organs, but these characters were not fully recognizable in the examined specimens, because they were mostly concealed by the abundant presence of yellowish eggs in the uterus or because the body was damaged

(particularly Nematobothriinae gen. sp. 2 and Nematobothriinae gen. sp. 3).

The parasite fauna of *E. alletteratus* from the western Mediterranean Sea has a lower richness than that from the Atlantic Ocean, because of the lack of several tropical parasites recorded in the Atlantic host (see Table 4.2.2).

The parasites of the head of *E. alletteratus* can be divided into three categories: Mediterranean (*Didymocystis* sp. 1, *Didymocystis* sp. 2, Didymozoinae gen. sp., *Didymozoon* sp., *H. thunninae*, *Melanocystis* sp., Nematobothriinae gen. sp. 2 and Nematobothriinae gen. sp. 3); Atlantic tropical (*Capsala magronum*, *Caligus productus*, *H. euthynni*, *Hexostoma keokeo*, *Lobatozoum multisacculatum* and *Metapseudaxine ventrosicula*); and those of both areas (*Allopseudaxine macrova, C. manteri*, *Caligus asymmetricus*, *C. bonito*, *C. pelamydis, P. appendiculatus* and *U. collateralis*). The first group of parasites seems to be highly specific to *E. alletteratus*, because the parasites have been found only in this host species. The parasites of the second group, the tropical ones, are shared between several tuna species; *e.g.*, *C. magronum* and *H. keokeo* are shared with *Auxis thazard* from the south-western Atlantic Ocean (Mogrovejo *et al*., 2004). The third group is less homogeneous than the others, because it includes species shared between tunas species (*i.e.*, *A. macrova*, *C. manteri*, *P. appendiculatus* and *U. collateralis*) and species more generalist shared between several pelagic fish (*Caligus asymmetricus*, *C. bonito* and *C. pelamydis,*). Among the parasites of the third group, in this same study *A. macrova* was also found in *Auxis rochei* from La Azohía and Tarifa, particularly in the large hosts (see chapter 3.3), whereas it is absent in *E. alletteratus* from the same localities. This fact suggests that *A. rochei* could have acquired the parasite outside of the Mediterranean Sea, likely in a tropical Atlantic area where *A. macrova* infects several tuna species (*A. thazard*, *E. alletteratus*, *Katsuwonus pelamis*, *Thunnus albacares*), and where the Mediterranean specimens of *E. alletteratus* do not migrate.

Most of the parasites of the western Mediterranean *E. alletteratus* are didymozoids, while only one didymozoid species (*L. multisacculatum*) have been reported in the south-western Atlantic Ocean (Alves and Luque, 2006). This species has also been observed in *K. pelamis* from the tropical Atlantic area (Bussiéras and Baudin-Laurencin, 1973; Alves and Luque, 2006), whereas it was not found in the Mediterranean Sea. These differences suggest that the fish population of the Mediterranean Sea is well separated from the south-western Atlantic one. Also the low similarity of the parasite fauna of the head of the adults of *E. alletteratus* from la Azohía with that of the adults from the south-western Atlantic Ocean suggests that the distance between these areas represent a barrier to the dispersion of the host and to its helminth parasites. This is in

accordance with other studies on scombrid fish, *e.g. Scomber colias*, that showed differences in the helminth fauna between Madeira (central-eastern Atlantic Ocean) and the Brazilian waters (south-eastern Atlantic Ocean) (Oliva *et al*., 2008).

The levels of infection of several species (*Didymocystis* sp. 1, *Didymozoon* sp. 2, *H. thunninae*, *Melanocystis* sp., Nematobothriinii gen. sp. 2, Nematobothriinii gen. sp. 3 and *U. collateralis*) changed between years. These annual differences can be due to several factors, such as environmental conditions, migration of fish from different localities, host size, etc. For instance, taking into account the samples from La Azohía, it is likely that the differences between the parasite assemblages of the different sampling years could be due to the migration of fish from different localities. On the other hand, the seasonal changes can not be considered to explain the difference of level of infection between years, because the samplings were replicated during the same period of the year. In addition, the lack of significant differences between hosts (*e.g.* size, sex ratio) indicated that these factors should not cause the differences. For example, the higher levels of infection of didymozoids observed in the 2011 group from La Azohía in relation to those sampled in 2008 and 2009 suggest that the host group of 2011 migrated from an area within the Mediterranean Sea with a higher abundance of their intermediate stages than those of the fish from the other years.

Concerning the differences between the parasite assemblages of the groups of the adult and juvenile fish, it must be considered that the occurrence of didymozoids can be influenced by the diet of the host. In fact, the diet of *E. alletteratus* changes as fish grow, becoming the large specimens more piscivorous (Bahou *et al*., 2007; Faulatano *et al*., 2007). Thus, the differences of the levels of infection of didymozoids between the small fish from the Gulf of Valencia and the adults from La Azohía and Tarifa can be due to the different feeding strategy of these groups, or to differences in their feeding grounds. Moreover, the host length can also influence the abundance of the ectoparasites; in fact, the higher mean abundance of *C. bonito* and *P. appendiculatus* of the large specimens from la Azohía and Tarifa in relation to the small specimens from the Gulf of Valencia can be due to the larger gill surface available for attachment.

The results of the multivariate analyses (CA and NMDS) showed that the samples of the Atlantic Ocean and those of the western Mediterranean Sea are separated, suggesting that the P% of *Didymocystis* sp. 2, *Didymozoon* sp., *L. multisacculatum*, *Melanocystis* sp. and Nematobothriinae sp. 2 is useful to distinguish the didymozoid assemblages from these two areas. Moreover, the ectoparasite assemblage of *E. alletteratus* from the Atlantic Ocean differs

from those of the western Mediterranean Sea, indicating that the P% of several species (*i.e. A. macrova*, *C. bonito*, *C. magronum*, *C. pelamydis*, *H. keokeo*, *H. lintoni*, *H. thunninae* and *M. ventrosicula*) could be useful to distinguish these localities. Nevertheless, the lack of some tropical Atlantic monogeneans in the Mediterranean fish cannot totally exclude the migration of the fish from the tropical Atlantic areas to the Mediterranean Sea, because these parasites can be lost during the migration, as indicated for other tunas from the tropical and temperate areas (Lester *et al*., 1985; Jones, 1991).

CHAPTER 5. METAZOAN PARASITES OF THE GILLS OF THE SKIPJACK TUNA *KATSUWONUS PELAMIS* (OSTEICHTHYES: SCOMBRIDAE) FROM THE WESTERN MEDITERRANEAN SEA

5.1. INTRODUCTION

The skipjack tuna *Katsuwonus pelamis* is an opportunistic predator that inhabits the tropical and warm-temperate waters of all oceans (Collette and Nauen, 1983). The range of this fish in the Atlantic Ocean is from 40°N to 32°S, from the surface to about 260 m depth. Its distribution is influenced by water temperature (optimum range from 15 to 30° C; Evans *et al.*, 1981). This tuna spawns when the surface temperature is ≥ 24° C, and its spawning season varies according to locality (Cayré and Farrugio, 1986). In the eastern Atlantic Ocean, it spawns year round over a wide area on both sides of the equator, from the Gulf of Guinea to 20–30°W, while only in the warm season in the tropical area. In the Mediterranean Sea the skipjack tuna has been traditionally considered occasional (Postel, 1963), but Macías *et al.* (2010) reported its presence in the western Mediterranean Sea throughout the summer, where it can also spawn (Alemany *et al.*, 2010). In the western Atlantic Ocean, the skipjack tuna spawns in two main subtropical areas off the coast of South America, and its migration is influenced by season (Andrade and Santos, 2004).

The skipjack tuna fishery is the fourth largest in the world (2.5 million t in 2010, 191 thousand t of which is from the Atlantic Ocean, and only 66 t from the Mediterranean; FAO, 2011). The International Commission for the Conservation of Atlantic Tunas (ICCAT) manages this fishery, dividing it into western and eastern (including Mediterranean Sea) stocks, with the boundary at 30°W (ICCAT, 2006).

Despite its great economic importance and that the several studies on this species have been carried out worldwide, little is known about its biology or migrations in the Mediterranean Sea (Di Natale *et al*., 2009).

Salvatore Mele. Gill metazoan parasites of tunas (Scombridae: Thunnini) from the western Mediterranean Sea: systematics, assemblages and use as biological tags. Tesi di dottorato in Riproduzione, Produzione, Benessere Animale e Sicurezza degli Alimenti di Origine Animale. Università degli studi di Sassari.

As described in the chapter 1.6, parasitological studies are useful to investigate the biology, ecology, migration and population structure of marine organisms (Mackenzie 2002). The parasite fauna of the head of the skipjack tuna has been investigated by several authors in the Indian and Pacific Oceans (Silas, 1962; Silas and Ummerkutty, 1967; Yamaguti, 1970; Lester *et al*., 1985), and in the Atlantic Ocean (Bussiéras and Baudin-Laurencin, 1973; Watertor, 1973; Lester *et al*., 1985; Alves and Luque, 2006), but no parasitological data is available for the Mediterranean Sea.

The aim of the present study was to investigate the metazoan gill parasites of the skipjack tuna from the Alboran Sea (western Mediterranean Sea) and to evaluate their possible use as biological tags.

5.2. MATERIALS AND METHODS

The gills of 35 specimens of skipjack tuna, caught by trolling in the western Mediterranean Sea in 2008, were examined for parasites (Table 2.2.1). Among them, 31 specimens were caught in the Alboran Sea (western Mediterranean Sea), area F of the Fig. 2.2.1. Immediately after landing fish were measured (FL range 58-68 cm), weighed (total weight range 4.2–6.4 kg) and sexed (14 males and 17 females). In addition, four skipjack tuna (2 males and 2 females) from the Balearic Sea (western Mediterranean Sea), area B of the Fig. 2.2.1, with FL range 58- 67 cm and total weight range 4.0-6.3 kg (Table 2.2.1), were analysed for comparative purposes.

The gills of all specimens were excised and processed as described in the Chapter 2.2. The references cited in the Chapter 2.4 were used to identify the parasite species found.

The parasitological terms used are as defined in the Chapter 2.5; prevalence (P%), mean abundance (MA) and mean intensity (MI) of each parasite species and their confidence intervals were calculated according to the methods described in the Chapter 2.5.

Possible correlations between parasite abundance and host size were assessed using the Spearman rank correlation coefficient.

The differences of the parasite assemblages between the host sexes were evaluated using the Fisher's exact test for prevalence and the bootstrap t-test for mean abundance and mean intensity (Ròzsa *et al.*, 2000).

Component community parameters (species richness; Berger-Parker index, d) were calculated according to Magurran (2005).

The results were compared with previously published quantitative data of *K. pelamis* from the Brazilian waters of the south-western Atlantic Ocean, published by Alves and Luque (2006).

A datasheet of presence/absence was created on the basis of the published records of parasites of the gills of *K. pelamis* according to geographical region. These data served to

evaluate the dissimilarity between the parasite fauna of the gills of *K. pelamis* from the Alboran Sea (Alb) and the Atlantic Ocean (Car, Caribbean Sea; CeA, central-eastern Atlantic Ocean; SwA, south-western Atlantic Ocean) (Table 5.2.1), according to the methods described in the Chapter 2.5.

Table 5.2.1. Published data of the parasites of the gills of *Katsuwonus pelamis* according to locality: Car, Caribbean Sea; CeA, central-eastern Atlantic Ocean; SwA, south-western Atlantic Ocean (no data for the Mediterranean Sea). Numbers represent literature sources: 1, Lester *et al*. (1985); 2, Justo and Kohn (2005); 3, Alves and Luque (2006); 4. Cissé *et al*. (2007).

Parasite species	SwA	Car	CeA
Monogenea			
Allopseudaxine macrova (Unnithan, 1957)	3		4
Capsalidae gen. sp.	3		
Didymozoidae			
Atalostrophion cf. *biovarium* Skrjabin, 1955			
Didymocylindrus filiformis Ishii, 1935		1	
Didymocylindrus simplex (Ishii, 1935)		1	
Didymocystis reniformis Ariola, 1902			
Didymoproblema fusiforme Ishii, 1935	2	1	
Didymozoon sp.	3		
Didymozoon longicolle Ishii, 1935			
Diplotrema pelamydis Yamaguti, 1938	2		
Koellikeria sp.			
Lobatozoum multisacculatum Ishii, 1935	3		
Copepoda			
Caligus bonito Wilson, 1905	3		
Caligus pelamydis Krøyer, 1863			4
Caligus productus Dana, 1852	3	1	
Pseudocycnus appendiculatus Heller, 1865	3		

5.3. RESULTS

A total of nine parasite species/taxa were found in the gills of the skipjack tuna from the Alboran Sea (Table 5.3.1, Figs. 5.3.1-8).

All the fish from the Alboran Sea were infected by at least one parasite species (Table 5.3.2). Most of the parasites (79.2% of all specimens) were adult didymozoids, represented by *Atalostrophion* cf. *biovarium*; *Didymocylindrus filiformis*; *Didymocylindrus simplex*; *Didymocystis reniformis*; *Didymoproblema fusiforme*; *Didymozoon longicolle*; *Koellikeria* sp.; and *Lobatozoum multisacculatum*. Copepods (20.8% of all specimens) were represented only by the caligid *Caligus bonito* (chalimi and adults). *Didymozoon longicolle* was the dominant species

(d = 0.64), showing the highest prevalence, mean abundance (MA) and mean intensity (P% = 94%, MA = 8.6, MI = 9.2), followed by *C. bonito* (P% = 77%, MA = 2.8, MI = 3.6).

No significant differences of P%, MA and MI were found between host sexes, and no significant correlation was found between the abundance of infection and the host size. The parasites of the 4 specimens caught off Majorca are given in Table 5.3.1; *Koellikeria* sp. and *L. multisacculatum* were not recorded in the Balearic Sea. The MA of *C. bonito* was significantly higher in the Alboran Sea than that of Balearic Sea (p-value ≤ 0.05).

The comparison of the levels of infection in the fish from the Alboran Sea with those from the south-western Atlantic Ocean (Justo and Kohn, 2005; Alves and Luque, 2006) is shown in Table 5.3.2. Two species had higher P% (*D. simplex* and *D. longicolle*) and two lower P% (*A. macrova* and Capsalidae gen. sp.) in the Alboran Sea than in the south-western Atlantic Ocean.

The cluster analysis of the parasite assemblages according to the indices of dissimilarity based on the presence/absence data of parasite species in *K. pelamis* from the Mediterranean Sea (present study) and from other areas of the world (Table 5.2.1) are shown in the Fig. 5.3.9. The graphs showed that the parasite assemblage of *K. pelamis* from the Alboran Sea (Alb) is more similar to that from the Caribbean Sea (Car) than those from the central-eastern and south-western Atlantic Ocean (CeA and SwA).

Table 5.3.1. Prevalence P (%), mean abundance (MA) and mean intensity (MI) of the parasites of the gills of *Katsuwonus pelamis* from the western Mediterranean Sea according to locality and location; (95% confidence intervals in parentheses). Alb: Alboran Sea; Bal: Balearic Sea. *, new geographical record; #, new host record. α, significant differences ($p \leq 0.05$).

Parasite / Host group	Alb			Bal			
Parameter	P%	MA	MI	P%	MA	MI	Location
Didymozoidae							
A. cf. *biovarium* *	16 (7–34)	0.4 (0.1–0.8)	2 (1.4–3.2)	50 (10–90)	0.8 (0.0–1.5)	1.5 (1.0-1.5)	Gill arch
D. filiformis	10 (3–26)	0.2 (0.0–0.5)	1.7 (1.0–2.3)	50 (10–90)	2.5 (0.0–3.8)	5.0 (-)	Outer side of gill filaments
D. simplex *	26 (13–44)	0.8 (0.3–1.7)	3.1 (1.9–5.0)	50 (10–90)	1.5 (0.0–3.0)	3.0 (2.0-3.0)	Outer side of gill filaments
D. reniformis #	7 (1–30)	0.1 (0.0–0.2)	1.0 (-)	25 (1–75)	0.3 (0.0–0.5)	1.0 (-)	Gill arch
D. fusiforme *	29 (16–47)	0.5 (0.2–0.9)	1.8 (1.2–2.2)	25 (1–75)	2.8 (0.0–5.5)	11.0 (-)	Inner side of gill filaments
D. longicolle	94 (79–99)	8.6 (6.1–12.7)	9.2 (6.7–13.6)	75 (25–99)	6.0 (0.3–11.5)	8.0 (1.0-12.3)	Inner side of gill filaments
Koellikeria sp. *#	3 (0–17)	0.0 (0.0–0.1)	1.0 (-)	0 (0–53)	0 (-)	0.0(-)	Gill arch
L. multisacculatum *	3 (0–17)	0.0 (0.0–0.1)	1.0 (-)	0 (0–53)	0 (-)	0.0(-)	Outer side of gill filaments
Copepods							
C. bonito *	77 (60–89)	2.8 (1.9–4.0) α	3.6 (2.7–5.0)	25 (1–75)	0.5 (0.0–1.0) α	2.0 (-)	Gill arch and filaments

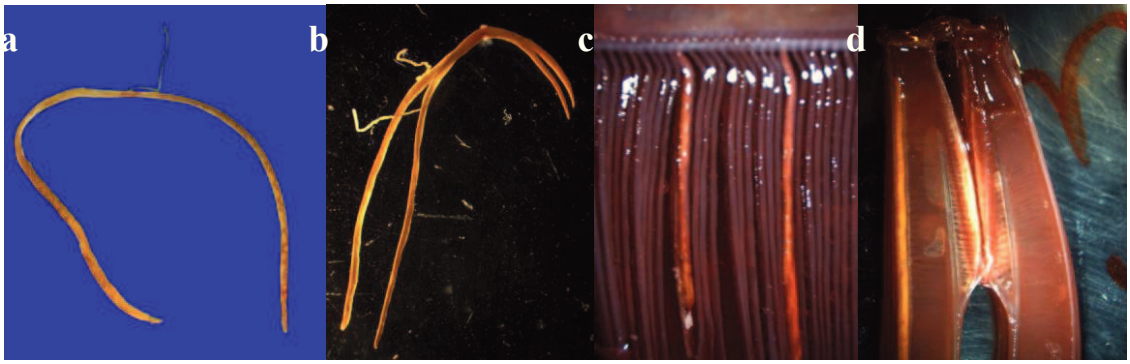

Figure 5.3.1. a-b, *Didymocylindrus filiformis ex* the gills of *Katsuwonus pelamis*. **c-d**, *D. filiformis in situ*.

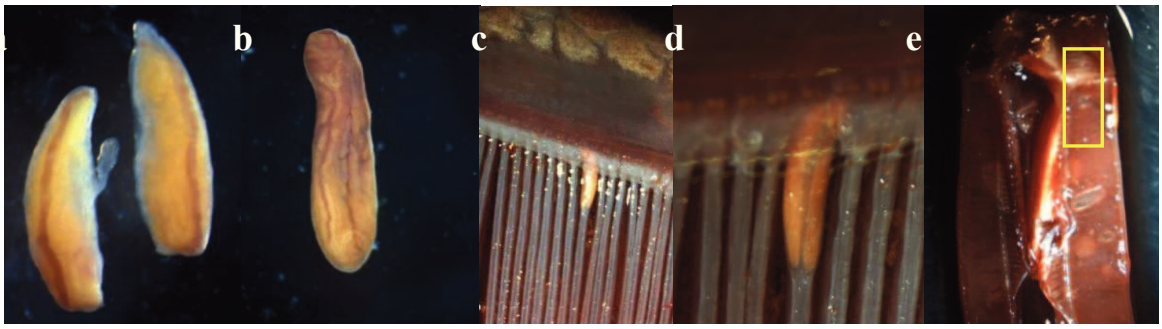

Figure 5.3.2. **a-b**, *Didymocylindrus simplex ex* the gills of *Katsuwonus pelamis*. **c-e**, *D. simplex in situ*.

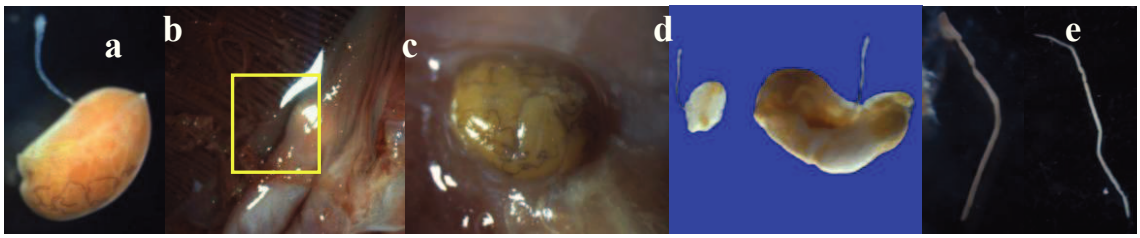

Figure 5.3.3. Didymozoid species *ex* the gills of *Katsuwonus pelamis*: **a**, *Didymocystis reniformis*; **b-c**, *D. reniformis in situ*. **d**, *Koellikeria* sp.; **e**, *Atalostrophion* cf. *biovarium*.

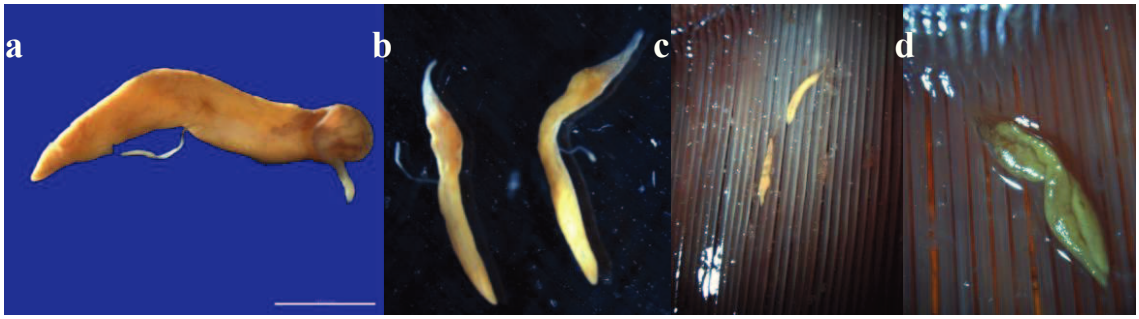

Figure 5.3.4. **a-b**, *Didymoproblema fusiforme ex* the gills of *Katsuwonus pelamis*. **c-d**, *D. fusiforme in situ*.

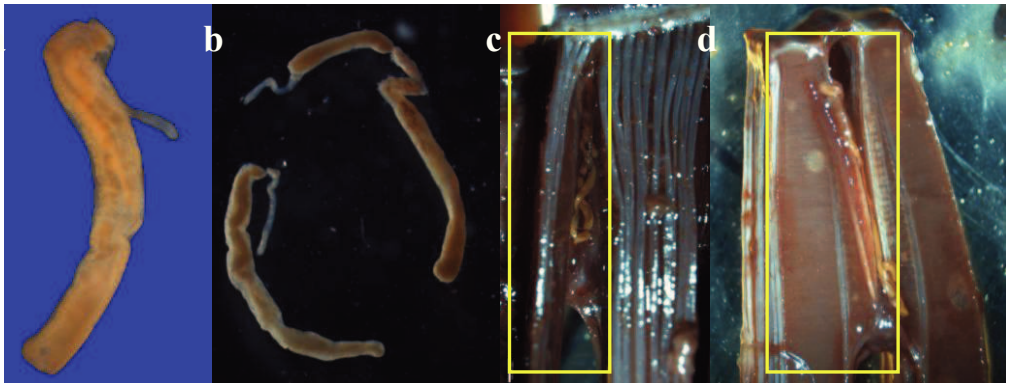

Figure 5.3.5. a-b, *Didymozoon longicolle ex* the gills of *Katsuwonus pelamis*. **c-d**, *D. longicolle in situ*.

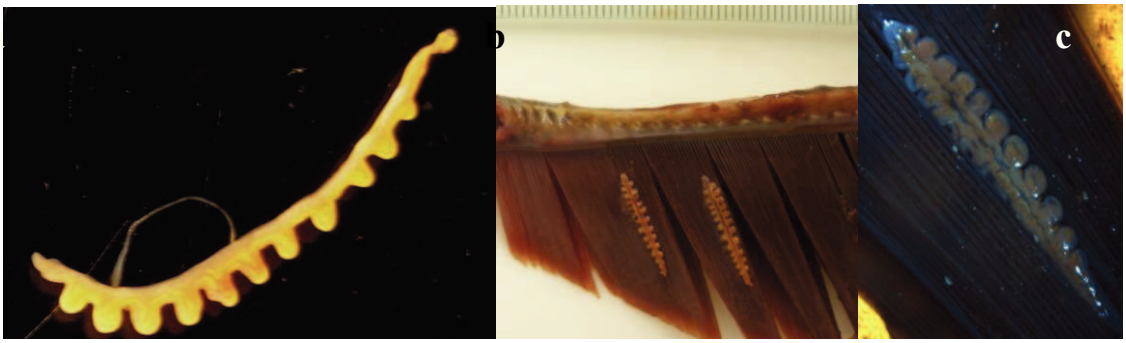

Figure 5.3.6. a, *Lobatozoum multisacculatum ex* the gills of *Katsuwonus pelamis*. **b-c**, *L. multisacculatum in situ.*

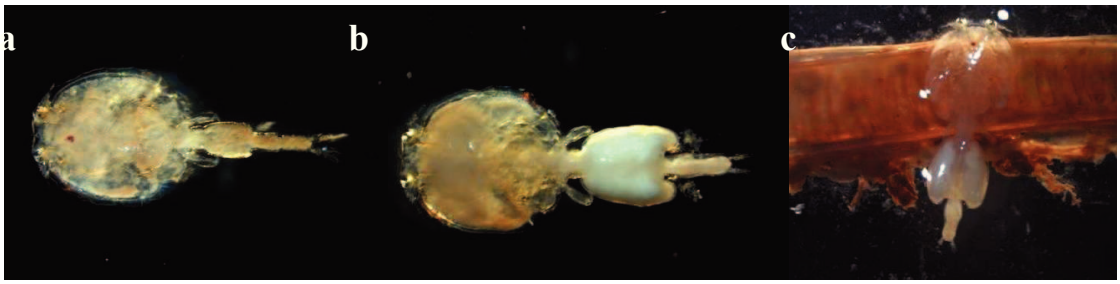

Figure 5.3.7. **a- b**, male and female specimens of *Caligus bonito ex* the gills of *Katsuwonus pelamis*. **c**, female of *C. bonito in situ*.

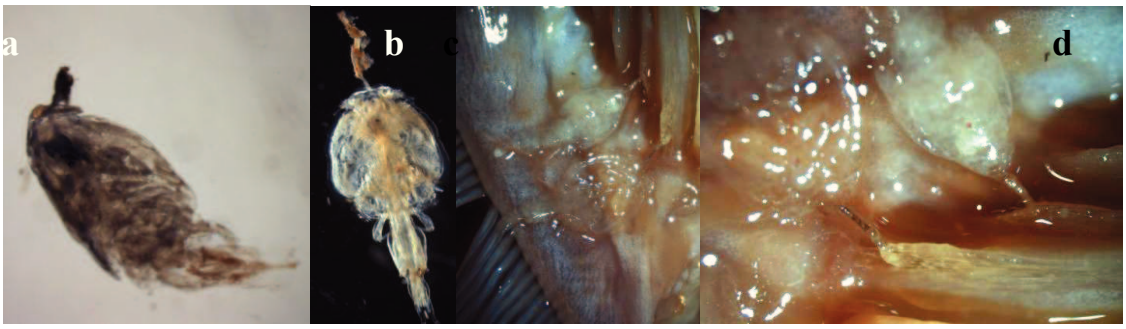

Figure 5.3.8. a-b, chalimus of *Caligus bonito ex* the gills of *Katsuwonus pelamis*. **c-d**, chalimus of *C. bonito in situ.*

Salvatore Mele. Gill metazoan parasites of tunas (Scombridae: Thunnini) from the western Mediterranean Sea: systematics, assemblages and use as biological tags. Tesi di dottorato in Riproduzione, Produzione, Benessere Animale e Sicurezza degli Alimenti di Origine Animale. Università degli studi di Sassari.

Table 5.3.2. Prevalence P (%) of the parasites of the gills of *Katsuwonus pelamis* from the Alboran Sea (Alb, present results) and from the south-western Atlantic Ocean (SwA, Justo and Kohn, 2005; Alves and Luque 2006; 95% confidence intervals in parentheses). *, significant differences ($p \leq 0.05$).

Parasite / Host group	Alb	SwA
Monogeneans		
A. macrova	0 (0–11)*	27*
Capsalidae gen. sp.	0 (0–11)*	13*
Didymozoids		
A. cf. *biovarium*	16 (7–34)	0
D. pelamydis	0 (0–11)	13
D. filiformis	10 (3–26)	0
D. simplex	26 (13–44)*	0*
D. reniformis	7 (1–30)	0
D. fusiforme	29 (16–47)	38
Didymozoon sp.	0 (0–11)	27
D. longicolle	94 (79–99)*	0*
Koellikeria sp.	3 (0–17)	0
L. multisacculatum	3 (0–17)	13
Copepods		
C. bonito	77 (60–89)	80
C. pelamydis	0 (0–11)	0
C. productus	0 (0–11)	13
P. appendiculatus	0 (0–11)	13

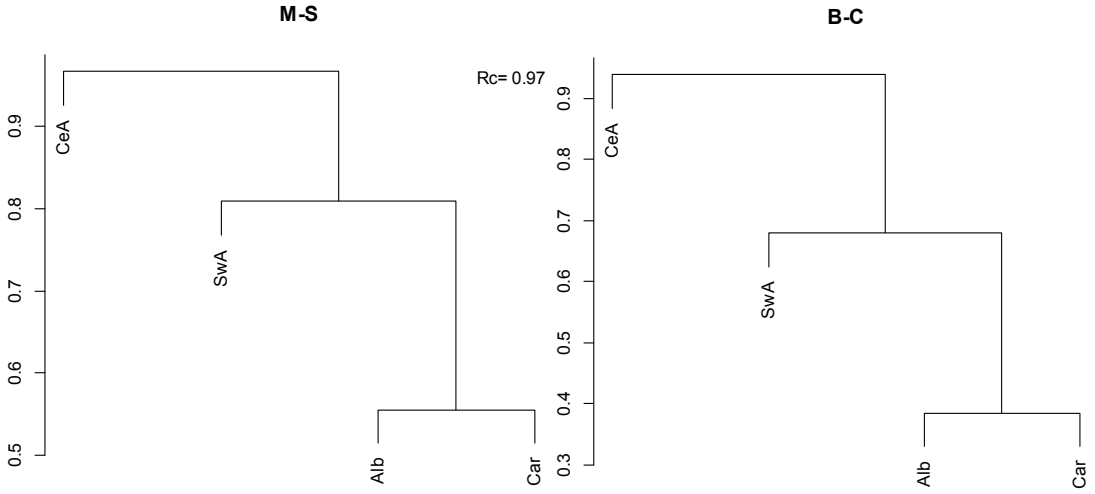

Figure 5.3.9. Cluster dendograms (group-average linkage) of the parasites of the gills of *Katsuwonus pelamis* from different localities, using the Marczewski-Steinhaus distance (M-S) and the Bray-Curtis index (B-C). Alb, Alboran Sea; Car, Caribbean Sea; CeA, central-eastern Atlantic Ocean; SwA, south-western Atlantic Ocean.

5.4. DISCUSSION

The metazoan parasite fauna of the gills of the skipjack tuna from the Atlantic Ocean and the Mediterranean Sea includes a total of 16 species/taxa. Among them, *Atalostrophion* cf.

biovarium, *Diplotrema pelamydis* and *Didymocylindrus simplex* may be considered specialists, being reported only in this host (Skrjabin, 1955; Lester *et al.*, 1985; Justo and Kohn, 2005, present results).

Most of the parasites of the skipjack tuna have a wide geographical range, being reported from the equator to the temperate latitudes of the Atlantic and Pacific Oceans, and they infect more than one host species: *Didymocylindrus filiformis* has been reported in the Pacific *Katsuwonus pelamis* and *Thunnus orientalis* (Ishii, 1935), in the Caribbean *K. pelamis* (Lester *et al*., 1985), and in the Mediterranean *Thunnus thynnus* (Mladineo *et al.*, 2008). The copepods *Caligus bonito*, *Caligus pelamydis*, and *Pseudocycnus appendiculatus* have been found in several scombrids from all oceans (Cressey and Cressey 1980).

Other parasites are limited to the tropical regions. *Allopseudaxine macrova* has been found in some fish from tropical areas of the Pacific and Indian Oceans, and from the central-eastern and south-western Atlantic Ocean (Bussiéras and Baudin-Laurencin, 1973; Mogrovejo *et al*., 2004). Bussiéras and Baudin-Laurencin (1973) collected many specimens of Didymozoidae, belonging to the genera *Didymozoon*, *Koellikeria*, *Didymocystis*, *Nematobothrium* and *Lobatozoum*, from four tuna species (including *K. pelamis*) from the central-eastern Atlantic Ocean. *Didymoproblema fusiforme* has been recorded in the Pacific *K. pelamis* and *Thunnus orientalis* (Ishii, 1935) and in the south-western Atlantic *K. pelamis* (Justo and Kohn, 2005). *Lobatozoum multisacculatum* has been found in the Pacific *K. pelamis* and *T. orientalis* (Ishii, 1935) and in the south-western Atlantic *Euthynnus alletteratus* and *K. pelamis* (Alves and Luque, 2006). *Caligus productus* has been found in several tropical scombrids from all oceans (Cressey and Cressey, 1980).

Other species seem to have a more narrow range. For example, *Didymocystis reniformis* has been found only in *T. thynnus* from the Mediterranean Sea and in *Thunnus alalunga* from the Gulf of Biscay (Dollfus, 1926, 1952); *K. pelamis* is a new host for this parasite. *Didymozoon longicolle* has been reported in several scombrids from the Pacific Ocean (Ishii, 1935), but in the Atlantic area it has been collected only in *T. thynnus* from the Mediterranean Sea (Mladineo *et al*., 2008), and in *Thunnus albacares* from the Gulf of Mexico (Nikolaeva and Parukhin, 1968).

The present study is the first contribution to knowledge of the gill metazoan parasites of *K. pelamis* from the Mediterranean Sea; this is also the first time that *A.* cf. *biovarium*, *D. simplex*, *D. fusiforme* and *L. multisacculatum* are reported from the Mediterranean Sea. Most of the gill parasites of *K. pelamis* from the Alboran Sea are didymozoids, with *D. longicolle* as dominant species. A clear dominance of didymozoids was also observed in *Auxis rochei* (Chapter 3.3), *E. alletteratus* (Chapter 4.3), *T. alalunga* (Jones, 1991; Chapter 6.3), *T. albacares* (Lardeaux, 1982)

and *T. thynnus* (Rodríguez-Marín *et al*., 2008, Chapter 7.3), suggesting that tunas are among the preferred hosts for didymozoids (Nikolaeva, 1985).

The parasite assemblage from the Alboran Sea is more similar to that of the Caribbean Sea than those of other Atlantic areas. *Didymoproblema fusiforme* and *L. multisacculatum* were found in both Alboran and south-western Atlantic skipjack tuna, and they are the only two didymozoids found in both places. This may be due to the occurrence of these parasites only in the tropical Atlantic areas (central-eastern and south-western Atlantic Ocean), and in tuna from the Alboran Sea only after the host migration; this hypothesis is supported by the record of these parasites in other tropical Atlantic tunas (Bussiéras and Baudin-Laurencin, 1973; Justo and Kohn, 2005; Alves and Luque, 2006) and their absence in any other Mediterranean host (Dollfus, 1926; Mladineo *et al*., 2008 and the present results, see Chapters 3, 4, 6, 7).

The significant differences in prevalence of *D. simplex* and *D. longicolle* (recorded only in the Alboran hosts) and of *A. macrova* and Capsalidae gen. sp. (only in the south-western Atlantic hosts) suggest that the presence or absence of these species could be distinctive of each locality. However, the presence of unidentified *Didymozoon* sp. in both the central-eastern and south-western Atlantic Ocean (Bussiéras and Baudin-Laurencin, 1973; Alves and Luque, 2006) suggests caution, because these parasites might belong to the species *D. longicolle*. The absence of *A. macrova* and Capsalidae gen. sp. in the skipjack tuna from the Alboran Sea could be due to their loss when fish migrate from the endemic area of these parasites (*i.e.* the tropical Atlantic Ocean) to the higher latitudes, where the conditions could be unsuitable for the parasites (MacKenzie and Abaunza, 1998). Likely, the lower mean abundance of *C. bonito* in the hosts from the Balearic Sea than in the Alboran ones could be due to the further lost of this parasite during the migration from the Alboran to the Balearic Sea. Also the parasitic copepods of *T. thynnus*, *Euryphorus brachypterus* (Gerstaecker, 1853) and *P. appendiculatus* have higher levels of infection in the Atlantic Ocean (Rodríguez-Marín *et al*., 2008) than in the Mediterranean Sea (Mladineo *et al*., 2008, 2011), confirming that copepods are vulnerable to the change of the environmental conditions.

Because the size of the fish sampled in the Alboran Sea and in the south-western Atlantic Ocean (Lester *et al*., 1985; Alves and Luque, 2006) is similar, the size should have not to be considered to explain the differences between areas.

Lester *et al*. (1985) observed differences in the parasite assemblages of the skipjack tuna from tropical Pacific waters to New Zealand and suggested the gill parasites *D. filiformis*, *D. simplex*, *D. fusiforme* and *L. multisacculatum* as tags to show the trophic migration from tropical Pacific waters to New Zealand, and to distinguish the skipjack tuna populations of the two areas.

The record of *D. fusiforme* and *L. multisacculatum* in the Alboran Sea supports the hypothesis of migration of the skipjack tuna from the Atlantic Ocean into the Mediterranean Sea, as assumed by the current stock management (ICCAT, 2006). Moreover, the absence of significant differences between the prevalence of *D. fusiforme* and *L. multisacculatum* between the tropical and the Alboran samples suggests that the lifespan of these parasites could be longer than the duration of the host migration. The high P% and MI of *D. longicolle* in *K. pelamis* from the Alboran Sea, which was also found in *T. alalunga* and *T. thynnus* from the Mediterranean Sea (Mladineo *et al*., 2008; Chapter 6.3) and in *T. albacares* from the Gulf of Mexico (Nikolaeva and Parukhin, 1968), suggests that this didymozoid could be useful to follow the specimens of *K. pelamis* that leave these areas. Nevertheless, the location of *D. longicolle* (inside of the gill tissue) makes its detection difficult, and perhaps it could explain the lack of its record in some studies.

CHAPTHER 6. METAZOAN PARASITES OF THE GILLS OF THE ALBACORE *THUNNUS ALALUNGA* (OSTEICHTHYES: SCOMBRIDAE) FROM THE WESTERN MEDITERRANEAN SEA

6.1. INTRODUCTION

The albacore *Thunnus alalunga* is a migratory cosmopolitan tuna distributed throughout the tropical and temperate areas of all oceans, including the Mediterranean Sea (Collette and Nauen, 1983). It is a top level predator, and its diet varies according to size and availability of prey, *e.g*. pilchard, anchovy, mackerel, squid and crustaceans (Consoli *et al*., 2008). The populations of this fish from different oceans are managed as separate stocks, based on the evidence of geographical separation and distinct spawning areas and seasons (Joseph, 2003; Alonso *et al*., 2005). Therefore, the North and South Atlantic populations (separated by the parallel 5° North), and the Mediterranean one are considered as three distinct units (ICCAT, 1996). The Mediterranean populations have been separated from the North Atlantic ones by genetics (Nakadate *et al*., 2005), spawning areas (Dicenta *et al*., 1975; Duclerc *et al*., 1973), growth (Megalofonou, 2000), and size and age at first maturity (Arena *et al*., 1980). Despite this separation for management purposes, the knowledge of the extent of the transzonal migrations of this fish is little (Arrizabalaga *et al*., 2003).

As described in the Chapter 1.6, parasitological studies can be useful to investigate the biology, ecology, migration and population structure of marine organisms (Mackenzie, 2002). The parasite fauna of the gills of the albacore has been investigated in the Pacific Ocean by Podznjakov (1990) and Jones (1991); in the Atlantic Ocean by Guiart (1940), Legendre (1940), Priol (1944), Dollfus (1952), Postel (1963, 1964) and Aloncle and Delaporte (1974); no parasitological data is available for the Mediterranean Sea.

The aim of this work is to describe the metazoan parasites of the gills of *T. alalunga* from the Balearic Sea (western Mediterranean Sea) and to evaluate their possible use as biological

tags.

6.2. MATERIALS AND METHODS

Thirty albacore caught in July 2008 by trolling in the Balearic Sea (western Mediterranean Sea), area B of the Fig. 2.2.1, were examined for parasites (Table 2.2.1). Immediately after landing fish were measured (FL range 50-96 cm), weighed (TW range 3.8-16.2 kg) and sexed (15 males and 15 females).

The gills of all specimens were excised and processed as described in the Chapter 2.2.

In addition to the general references cited in the Chapter 2.4, the following specific literature was used for the identification of the parasite species: for monogeneans, Bussieras (1972); for didymozoids, Ariola (1902), Guiart (1940), Legendre (1940), Pozdnyakov (1990); for copepods, Hewitt (1969).

The parasitological terms used are as defined in the Chapter 2.5; prevalence (P%), mean abundance (MA) and mean intensity (MI) of each parasite species and their confidence intervals were calculated according to the methods described in the Chapter 2.5.

Possible correlations between parasite abundance and host size were assessed using the Spearman rank correlation coefficient.

The levels of infection of each parasite species were calculated according to host size, dividing hosts into three groups (Table 6.2.1): small (FL = 50-66 cm), medium (FL = 67-74 cm), large (FL = 75-96 cm). The differences between the parasite assemblages of the three groups and between host sexes were evaluated using the Fisher's exact test for prevalence and the bootstrap t-test for mean abundance and mean intensity (Ròzsa *et al*. 2000).

Component community parameters (species richness; Berger-Parker index, d) were calculated according to Magurran (2005).

Table 6.2.1. Sampling data of *Thunnus alalunga* according to size. N, number of specimens; W = total weight.

Group of size	N	Mean FL ± s.d. (cm)	FL range (cm)	Mean W ± s.d. (kg)
Small	16	62.2 (4.1)	FL 50-66	4.9 (0.8)
Medium	6	69.8 (1.1)	FL 67-74	6.3 (0.7)
Large	8	81.7 (6.8)	FL 75-96	10.4 (2.6)

A datasheet of presence/absence was created on the basis of the published records of parasites of the gills of *T. alalunga* according to geographical region. These data served to evaluate the dissimilarity between the parasite fauna of the gills of *T. alalunga* from the western

Mediterranean Sea and the North Atlantic Ocean (Table 6.2.2), according to the methods described in the Chapter 2.5.

Table 6.2.2. Published data of the parasites of the gills of *Thunnus alalunga* from the North Atlantic Ocean.

Parasite	Reference
Monogenea	
Capsala thynni (Guiart, 1938)	Dollfus (1952)
Didymozoidae	
Didymocystis alalongae Yamaguti 1938	Dollfus (1952)
Didymocystis lanceolata Guiart, 1938	Guiart (1940)
Didymocystis macrorchis Guiart, 1938	Guiart (1940)
Didymocystis reniformis Ariola, 1902	Dollfus (1952)
Nematobothrium. latum Guiart, 1938	Guiart (1940)
Wedlia bipartita (Wedl, 1855)	Guiart (1940)
Copepoda	
Caligus productus Dana, 1852	Cressey *et* Cressey (1980)
Euryphorus brachypterus (Gerstaecker, 1853)	Dollfus (1952)
Pseudocycnus appendiculatus Heller, 1865	Cressey *et* Cressey (1980)

6.3. RESULTS

A total of nine parasite species/taxa were found in the gills of the albacore from the Balearic Sea (Table 6.3.1, Figs. 6.3.1-8). Most of the parasites were didymozoids (95.8% of all specimens), with six species: *Didymosulcus aahi*; *Didymosulcus dimidiatus*; *Didymozoon longicolle*; *Didymozoon pretiosus*; *Nematobothrium latum*; *Wedlia bipartita;* followed by crustaceans (2.9%), with two species: *Pseudocycnus appendiculatus*; *Rocinela* sp.; and one capsalid monogenean *Capsala paucispinosa* (1.3%). *D. longicolle* was the dominant species, with 113 specimens (36.1%).

Table 6.3.1. Prevalence P (%), mean abundance (MA), mean intensity (MI) and location of the parasites of the gills of *Thunnus alalunga* from the western Mediterranean Sea (95% confidence intervals in parentheses). *, new geographical record; #, new host record.

Parasite / Parameter	P%	MA	MI	Location
Monogenea				
Capsala paucispinosa (Mamaev, 1968) *#	7 (1-21)	0.1 (0.0-0.3)	2.0 (-)	Gill arch
Didymozoidae				
Didymosulcus aahi Pozdnyakov, 1990 *	30 (16-48)	0.6 (0.3-1.0)	2.0 (1.4-2.6)	Outer margin of gill filaments
Didymosulcus dimidiatus Pozdnyakov, 1990 *	53 (35-70)	2.6 (1.5-4.2)	4.9 (3.3-7.1)	Gill arch
Didymozoon longicolle Ishii, 1935 *#	63 (45-79)	3.8 (1.7-10.6)	6.0 (2.8-16.0)	Inner margin of gill filaments
Didymozoon pretiosus Ariola, 1902 *#	10 (3-26)	0.6 (0.0-2.0)	5.7 (1.0-9.0)	Inner margin of gill filaments
Nematobothrium. latum Guiart, 1938 *	37 (21-55)	1.6 (0.7-3.1)	4.5 (2.5-7.2)	Gill arch
Wedlia bipartita (Wedl, 1855) *	27 (13-45)	0.8 (0.3-1.8)	3.1 (1.5-5.4)	Gill arch
Crustacea				
Pseudocycnus appendiculatus Heller, 1868 *	20 (9-38)	0.3 (0.1-0-6)	1.3 (1.0-1.7)	Gill filaments
Rocinela sp. *#	3 (0-18)	0.0 (0.0-0.1)	1.0 (-)	Gill filaments

Figure 6.3.1. *Capsala paucispinosa ex* the gills of *Thunnus alalunga*.

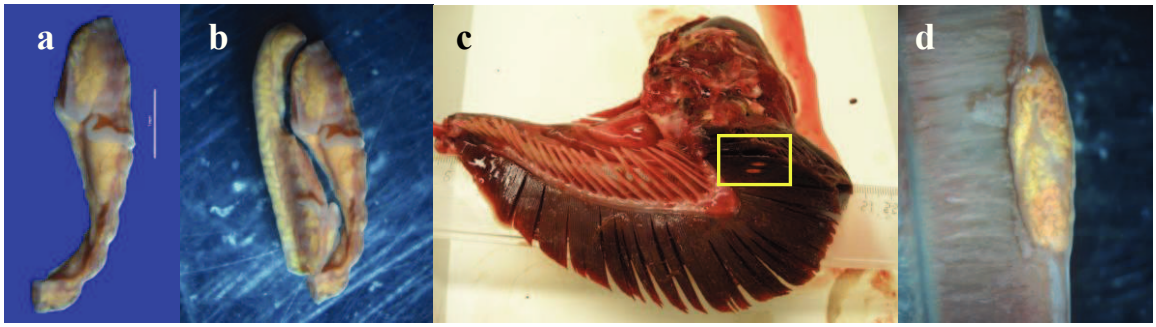

Figure 6.3.2. a-b, *Didymosulcus aahi ex* the gills of *Thunnus alalunga*. **c-d**, *D. aahi in situ*.

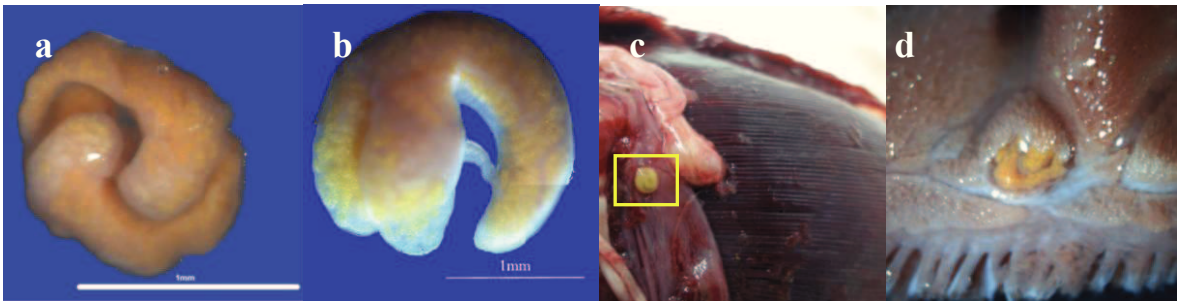

Figure 6.3.3. a-b, *Didymosulcus dimidiatus ex* the gills of *Thunnus alalunga*. **c-d**, *D. dimidiatus in situ*.

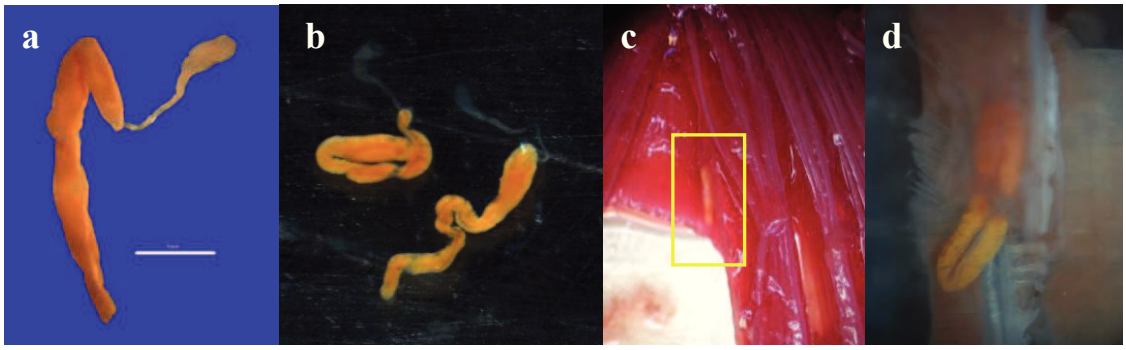

Figure 6.3.4. a-b, *Didymozoon longicolle ex* the gills of *Thunnus alalunga.* **c-d**, *D. longicolle in situ*.

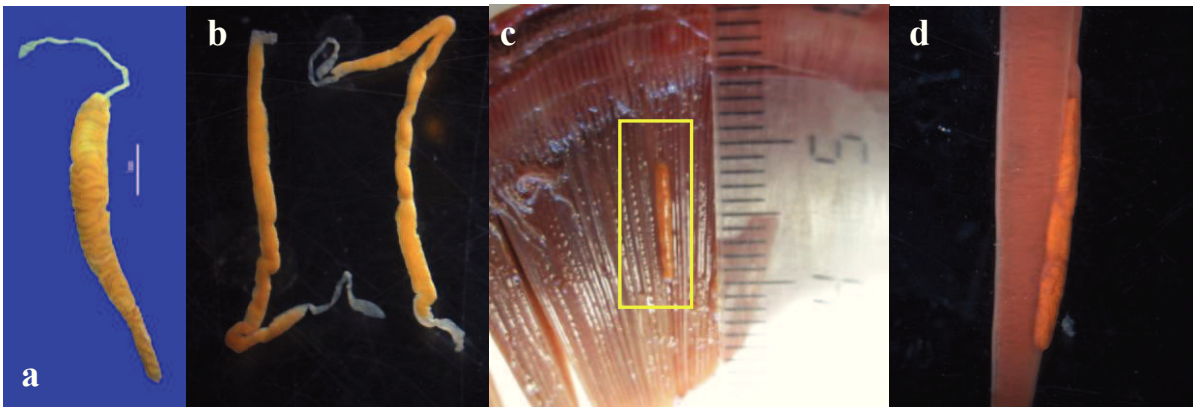

Fig. 6.3.5. a-b, *Didymozoon pretiosus ex* the gills of *Thunnus alalunga*. **c-d**, *D. pretiosusin situ*.

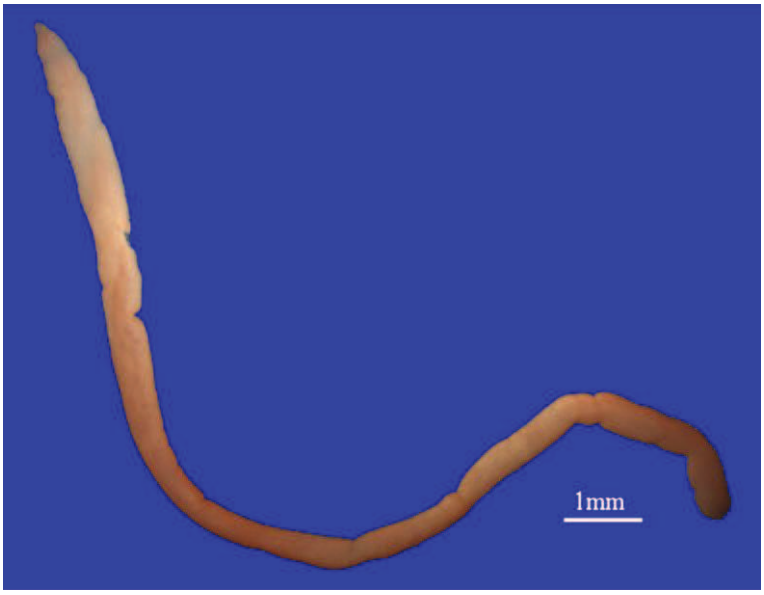

Figure 6.3.6. *Nematobothrium latum ex* the gills of *Thunnus alalunga*.

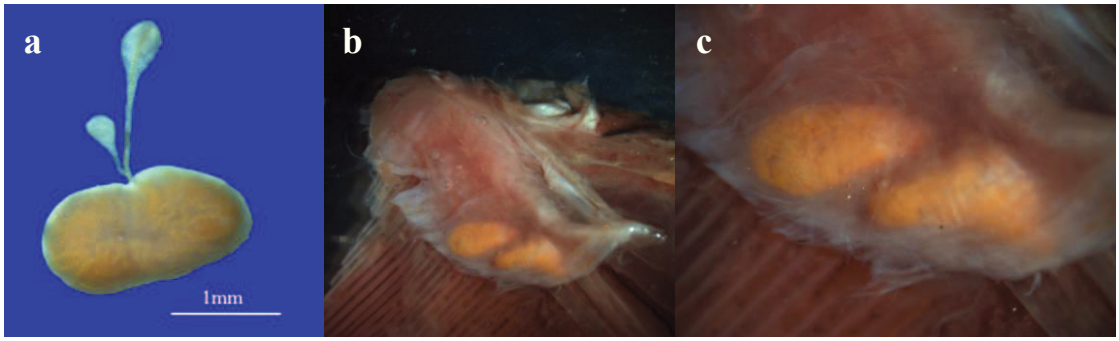

Fig. 6.3.7. a, *Wedlia bipartita ex* the gills of *Thunnus alalunga*; . **b-c**, *W. bipartita in situ*.

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Fig. 6.3.8. Crustacean parasites *ex* the gills of *Thunnus alalunga*: **a**, *Pseudocycnus appendiculatus*; **b**, *Rocinela* sp.

The Table 6.3.1 shows the levels of infection in the hosts according to size and the results of the statistical analyses. No significant difference in P%, MA and MI of each parasite species was found between host sexes and between host size groups. Regarding the possible correlations between the abundance and the host size no significant relationship was found. In general, the lowest values of infection were recorded in the large hosts, and two species (*D. pretiosus* and *P. appendiculatus*) were not recorded in this group. *C. paucispinosa* was only found on two specimens of the small fish.

The high values of the Marczewski-Steinhaus distance (0.77) and of the Bray-Curtis index (0.63) showed high dissimilarity between the parasite assemblages of *T. alalunga* from the Mediterranean Sea and that from the North Atlantic Ocean.

Table 6.3.2. Prevalence P (%), mean abundance (MA) and mean intensity (MI) of the parasites of the gills of *Thunnus alalunga* from the western Mediterranean Sea according to host size (95% confidence intervals in parentheses). No significant differences in P%, MA and MI were found.

Parasite / Host group	Small			Medium			Large		
Parameter	P%	MA	MI	P%	MA	MI	P%	MA	MI
Monogenea									
C. paucispinosa	13 (2-37)	0 (-)	2.0 (N/A)	0 (0-41)	0.0 (-)	0.0 (-)	0 (0-36)	0.0 (-)	0.0 (-)
Didymozoidae									
D. aahi	44 (21–70)	2.6 (1.4-4.0)	2.0 (-)	17 (0–59)	0.3 (0.0-0.7)	2.0 (-)	13 (1–50)	0.3 (0.0-0.5)	2.0 (-)
D. dimidiatus	70 (44–87)	0.9 (0.4-1.6)	3.7 (1.3-2.7)	50 (15–85)	4.3 (0.7-9.8)	8.7 (4.0-12.3)	25 (46–64)	2.8 (0.1-6.6)	5.5 (-)
D. longicolle	70 (44–87)	5.1 (1.4-17.5)	7.4 (2.5-5.4)	50 (15–85)	4.0 (0.5-8.3)	8.0 (3.0-11.3)	63 (29–89)	2.0 (0.9-4.4)	1.6 (1.0-1.8)
D. pretiosus	13 (2-37)	1.0 80.0-3.1)	8.0 (2.3-25.6)	17 (1–59)	0.2 (0.0-0.3)	1.0 (-)	0 (0-36)	0.0 (-)	0.0 (-)
N. latum	50 (27–73)	2.1 (0.8-4.6)	4.3 (5.0-8.0)	33 (6–73)	2.2 (0.0-6.0)	6.5 (3.0-6.5)	13 (1–50)	0.3 (0.0-0.5)	2.0 (-)
W. bipartita	31 (13–56)	0.9 (0.3-2.3)	3.0 (1.8-7.6)	17 (1–59)	1.3 (0.0-2.7)	8.0 (-)	25 (46–64)	0.5 (0.0-1.0)	1.0 (-)
Crustacea									
P. appendiculatus	25 (9–50)	0.4 (0.1-0.9)	1.5 (1.2-5.2)	33 (6–73)	0.2 (0.0-0.3)	1.0 (-)	0 (0-36)	0.0 (-)	0.0 (-)
Rocinela sp.	0 (0-21)	0.0 (-)	0.0 (-)	0 (0-41)	0.0 (-)	0.0 (-)	13 (1-50)	0.1 (0.0-0.4)	1.0 (-)

6.4. DISCUSSION

Nine species of metazoan parasites were found in the gills of the Mediterranean albacore. This species is a new host record for *Capsala paucispinosa* and *Didymozoon pretiosus*. *Didymosulcus dimidiatus* and *Nematobothrium latum* have been reported only in *Thunnus alalunga* (Guiart, 1940; Pozdnyakov, 1990), while the other species have been already recorded in several hosts: *Capsala paucispinosa* in *Euthynnus affinis*, *Thunnus albacares*, *Thunnus obesus* and *Thunnus orientalis* (Chisholm and Whittington, 2007); *Didymosulcus aahi* in *T. alalunga* and *Thunnus albacares* (Pozdnyakov, 1990); *Didymozoon longicolle* in several scombrids from the Pacific and Atlantic Oceans (Ishii, 1935, Munday *et al*., 2003); *Didymozoon pretiosus* in *Thunnus thynnus* (Chapter 7.3, Ariola, 1902; Mariniello *et al*., 2000); *Wedlia bipartita* in *T. alalunga*, *T. thynnus* and *Seriola dumerili* (Risso, 1810) (Ariola, 1902; Grau *et al*., 1999; Guiart, 1940); and *Pseudocycnus appendiculatus* in *Euthynnus* spp., *Katsuwonus pelamis* and *Thunnus* spp. from all oceans (Chapter 7.3, Cressey *et al*., 1983). This study is the first contribution to the knowledge of the gill parasites of *T. alalunga* from the Mediterranean Sea. *Capsala paucispinosa*, *D. aahi*, *D. dimidiatus*, *N. latum* and *Rocinela* sp. are for the first time reported in this area (Junoy and Castelló, 2003; Munday *et al*., 2003; Chisholm and Whittington, 2007).

Among the 16 species previously reported in the albacore from the Atlantic Ocean (9 of which in the gills) only three (*N. latum*, *W. bipartita* and *P. appendiculatus*) overlap with the parasites found in the present study, but on the basis of the descriptions and drawings given by Guiart (1940), it is likely that the new species there described as *Didymocystis macrorchis* and *Didymocystis lanceolata* could be synonyms of *D. aahi* and *D. dimidiatus*, respectively. On the other hand, Jones (1991) reported quantitative data on the parasites of the albacore from the southern Pacific Ocean, but only the crustaceans were identified at the species level, while didymozoids where simply listed with labels according to morphological types.

According to the criteria proposed by MacKenzie and Abaunza (1998), several parasites can distinguish the albacore from the Mediterranean Sea. *Didymozoon longicolle*, only found in the Mediterranean albacore, *K. pelamis* (see Chapter 5.3) and *T. thynnus* (Maldineo *et al*., 2008), could be used to discriminate the Mediterranean and the north-eastern Atlantic albacore, although it was also described in *T. albacares* from the Gulf of Mexico (Nikolaeva and Parukhin, 1968) and the Pacific Ocean (Ishii, 1935). *Wedlia bipartita*, only recorded in the Mediterranean albacore and in the Atlantic bluefin tuna from the Mediterranean Sea and the Gulf of Biscay, could be useful to study the migrations of the albacore from the Mediterranean Sea or the north-eastern Atlantic Ocean to other areas.

CHAPTER 7. METAZOAN PARASITES OF THE GILLS OF THE ATLANTIC BLUEFIN TUNA *THUNNUS THYNNUS* (OSTEICHTHYES: SCOMBRIDAE) FROM THE WESTERN AND EASTERN MEDITERRANEAN SEA

7.1. INTRODUCTION

The Atlantic bluefin tuna *Thunnus thynnus* is a pelagic fish inhabiting the central and north-eastern Atlantic Ocean, including the Mediterranean Sea, and the central and north-western Atlantic Ocean, from the Gulf of Mexico to Newfoundland (Fromentin and Powers, 2005). The International Commission for the Conservation of Atlantic Tunas (ICCAT) manages this species as two distinct stocks, separated by the 45th meridian west. The Atlantic bluefin tuna is one of the most important and valuable finfish worldwide, with 32244 t landed in 2007 (about 490 million of US $), of which 26059 t (80%) from the Mediterranean Sea (FAO, 2011). The ecology of this species is complex, because of its migratory nature, wide distribution, natal homing behaviour, and other features such as the variety of diet and the wide range of depth and temperature it tolerates (Rooker *et al*., 2007; Anon., 2011). At present, *T. thynnus* is considered overexploited, and it is included in a multiannual recovery plan by ICCAT (ICCAT rec. 08–05/2008 and EC n. 1559/2007). For all these reasons, this species is one of the most studied fish (Rooker *et al*., 2007), but some of its biological and ecological traits are still poorly known (Fromentin and Powers, 2005).

Parasitological studies of the Atlantic bluefin tuna and other bluefin tunas, *i.e.* the southern bluefin tuna *Thunnus maccoyii* and the Pacific bluefin tuna *Thunnus orientalis*, deal mainly with taxonomy (Ariola, 1902; Ishii, 1935; Cressey and Cressey, 1980), with the effects of parasitic infections in cage-reared fish (Mladineo *et al*., 2008, 2011; Ruiz de Ybañez *et al*., 2011), and with the use of parasites as biological tags (MacKenzie, 1983; Rodríguez-Marín *et al*., 2008; Mladineo *et al*., 2010). However, the parasite assemblages of *T. thynnus* from the Mediterranean Sea have been poorly investigated, despite its economic importance and the ecological relevance

of this species as apical predator in the Mediterranean marine ecosystem (Rooker *et al*., 2007).

The aim of this study is to describe the metazoan parasites of the gills of *T. thynnus* from the western and eastern Mediterranean Sea, and to evaluate their possible use as biological tags to improve the knowledge of the bio-ecological aspects of this fish.

7.2. MATERIALS AND METHODS

Sixty-three Atlantic bluefin tunas, caught in the Mediterranean Sea between 2006 and 2007, were examined for parasites (Table 7.2.1). Among them, 53 specimens were from two localities off Sardinia (western Mediterranean Sea): 49 adults caught by traditional trap fishery "tonnara" off the Isle of San Pietro (Sardinia Sea), area C of Fig. 2.2.1 (39°11' N : 8°18' E), and 4 young of the year caught by trolling off the Isle of Tavolara (Tyrrhenian Sea), area D of the Fig. 2.2.1 (40°50' N : 9°57' E). In addition, 10 specimens of juveniles were caught by trolling in the Levantine Sea (eastern Mediterranean Sea), area H of the Fig. 2.2.1 (Table 7.2.1). After landing, fish were measured and weighed (Table 7.2.1). The gills of all specimens were dissected and processed as described in the Chapter 2.2.

Table 7.2.1. Sampling data of *Thunnus thynnus* according to locality.

Locality	N	FL range (cm)	Total Weight range (kg)
Tyrrhenian Sea	4	27 – 31	0-1
Levantine Sea	10	51 – 100	9-19
Sardinian Sea	49	106 – 207	23-192

In addition to the general references cited in the Chapter 2.4, the following specific literature was used for species identification of didymozoids: Guiart (1940), Pozdnyakov (1990).

The parasitological terms used are as defined in the Chapter 2.5; prevalence (P%), mean abundance (MA) and mean intensity (MI) of each parasite species and their confidence intervals were calculated according to the methods described in the Chapter 2.5.

Possible correlations between the parasite abundance and the host size were assessed using the Spearman rank correlation coefficient (Chapter 2.5).

The levels of infection of each parasite species were calculated dividing hosts into four groups according to size (S, Fork Length < 50cm; M, FL =51-100 cm; L, FL=101-150 cm; XL, FL = 151-230 cm), and locality (E Med, eastern Mediterranean Sea; W Med, western Mediterranean Sea): S-W-Med; M-E-Med; L-W-Med; XL-W-Med (Table 7.2.2). The results were compared with the data on the gill parasites of the Atlantic bluefin tuna from the Adriatic Sea (Mladineo *et al*., 2008) and the north-eastern Atlantic Ocean (Rodríguez-Marín *et al*., 2008)

(Table 7.2.2).

Table 7.2.2. Groups of *Thunnus thynnus* according to size class and locality. S, FL < 50 cm; M, FL = 51-100 cm; L, FL = 101-150 cm; XL, FL = 151-230 cm. Adr, Adriatic Sea (§); E Med, eastern Mediterranean Sea; W Med, western Mediterranean Sea; NE Atl, north-eastern Atlantic Ocean (♯).

Group	N	Locality	Size group	FL (cm)	Weight (kg)
S W Med	4	W Mediterranean	Small	27-31	0-1
M Adr §	183	Adriatic Sea	Medium		5-50
M E Med	10	E Mediterranean	Medium	78-100	9-19
M NE Atl ♯	228	NE Atlantic Ocean	Medium	56-90	
L W Med	44	W Mediterranean	Large	106-150	23-60
XL W Med	5	W Mediterranean	Extra Large	172-227	88-192

§, Mladineo *et al*. (2008); ♯, Rodríguez-Marín *et al*. (2008)

The differences between the parasite assemblages of the six groups and between sexes were evaluated with the Fisher exact test for prevalence and the bootstrap t-test for mean abundance and mean intensity (Ròzsa *et al*., 2000).

Non-Metric Multidimensional Scaling (NMDS) and Cluster Analysis (CA) were performed as described in the Chapter 2.5, considering prevalence, mean abundance and mean intensity of each parasite species to identify possible differences between the six groups.

Component community parameters (species richness; Berger-Parker index, d) were calculated according to Magurran (2005).

The dissimilarity between the parasite assemblages of the six groups were evaluated according to the methods described in the Chapter 2.5.

A datasheet of presence/absence was created on the basis of the published records of the gills parasites of the three bluefin tuna species (*Thunnus maccoyii*, *Thunnus orientalis* and *T. thynnus*) according to the geographical region (Table 7.2.3): *T. thynnus* from the Mediterranean Sea: central Mediterranean Sea (BFTCM), eastern Mediterranean Sea (BFTEM) and western Mediterranean Sea (BFTWM); the north-eastern Atlantic Ocean (BFTEA); and the north-western Atlantic Ocean (BFTWA); *T. maccoyii* from the Indian Ocean (SBT); *T. orientalis* from the Pacific Ocean (PBT). These data, pooled with the present results and elaborated according to the methods described in the Chapter 2.5, allowed to evaluate the dissimilarity between the parasite fauna of the gills of the three bluefin tuna species from the different localities.

Table 7.2.3. Published data of the gill parasites of the bluefin tunas according to locality. SBT, *Thunnus maccoyii* (Indian Ocean); PBT, *Thunnus orientalis* (Pacific Ocean); BFTEA, BFTWA, BFTWM, BFTWM, *Thunnus thynnus* (north-eastern Atlantic Ocean, north-western Atlantic Ocean, central Mediterranean Sea, western Mediterranean Sea, respectively); no data from the eastern Mediterranean Sea (BFTEM). Data from: 1, Silas (1962); 2, Silas and Ummerkutty (1967); 3, Cressey and Cressey (1980); 4, Arru and Garippa (1995); 5, Mariniello *et al*. (2000); 6, Momoyama and Kobayashi (2004); 7, Chisholm and Whittington (2007); 8, Hayward *et al*. (2007); 9, Mladineo *et al*. (2008); 10, Rodríguez-Marín *et al*. (2008); 11, Mladineo *et al*. (2011).

Parasite	SBT	PBT	BFTEA	BFTWA	BFTWM	BFTCM
Monogenea						
Capsala interrupta (Monticelli, 1891)					7	
Capsala magronum (Ishii, 1936)		7	1			
Capsala maccallumi Price, 1939				7		
Capsala onchidiocotyle (Setti, 1899)	8		10		7	
Capsala paucispinosa (Mamaev, 1968)	7					
Hexostoma grossum (Goto, 1894)		1				
Hexostoma thynni Rafinesque, 1815	8		10		1, 4α	9, 11
Digenea						
Copiatestes thyrsitae Crowcroft, 1948			10			
Dyidymocylindrus filiformis Ishii, 1935		1				9, 11
Didymocystis reniformis Ariola, 1902 §		1, 6	10		1, 5	9, 11
Didymocystis sp. 3 *sensu* Rodríguez-Marín *et al*., 2008			10			
Didymoproblema fusiforme Ishii, 1935		1				
Didymosulcus crassa (Ishii, 1935)		1				
Didymosulcus sp. 2 *sensu* Rodríguez-Marín *et al*., 2008			10			
Didymosulcus wedli (Ariola, 1902)		1, 6	10		1, 4, 5	9, 11
Didymosulcus soleiformis Ishii, 1935		1				
Didymozoon longicolle Ishii, 1935		1				9, 11
Didymozoon pretiosus Ariola, 1902 ♯		1	10		1, 5	
Kollikerioides apicalis Yamaguti, 1970						9, 11
Lobatozoum multisacculatum Ishii, 1935		1,				9, 11
Wedlia bipartita Ariola, 1902			1		1	1
Wedlia reniformis Ishii, 1935		1, 6				
Crustacea Copepoda						
Caligus bonito Wilson, 1905		2		3		
Caligus coryphaenae Steenstrup *et* Lütken, 1861		2		3		
Caligus chiastos Lin et Ho, 2003	8					
Caligus productus Dana, 1852		2, 3		3		
Euryphorus brachypterus (Gerstaecker, 1853)	8	2, 3	10	2, 3	4	11
Pseudocycnus appendiculatus Heller, 1865	8		10			

§, synonymised with *Didymocystis semiglobularis* Ishii, 1935; ♯, synonymised with *Didymozoon filicolle* Ishii, 1935

7.3. RESULTS

In the present study 11 parasite species/taxa were found in the gills of *T. thynnus* from the eastern and western Mediterranean Sea (Tables 7.3.1-3; Figs. 7.3.1-11). Most of the parasites were didymozoids (88.2% of all specimens) with five species: *Didymocystis reniformis*, *Didymosulcus wedli*, *Didymosulcus* sp. 2 *sensu* Rodríguez–Marín *et al*. 2008, *Didymozoon pretiosus* and *Wedlia bipartita*; followed by monogeneans (9.6%) with four species: *Capsala magronum*, *Capsala onchidiocotyle*, *Capsala paucispinosa* and *Hexostoma thynni*; and copepods (2.2%) with two species: C*aligus coryphaenae* and *Pseudocycnus appendiculatus*.

All parasite species were found in the large western Mediterranean group (L-W-Med); in the extra large western Mediterranean group (XL-W-Med) were found three species: *D. wedli*, *H. thynni* and *P. appendiculatus*; and in the small western Mediterranean tunas (S-W-Med) only *Didymosulcus* sp. 2. Four didymozoids (*Didymosulcus* sp. 2, *D. wedli*, *D. pretiosus*, *W. bipartita*) were recorded in the medium eastern Mediterranean group (M-E-Med). *Didymosulcus wedli* was the dominant species in all the M-XL groups (M-E-Med, $d = 0.54$; L-W-Med, $d = 0.83$; XL-W-Med, $d = 0.81$); while *Didymosulcus* sp. 2 was dominant in the small group (S-W-Med, $d = 1.00$).

No significant difference in P%, MA and MI of each parasite species was found between host sexes. A positive correlation of the abundance with the host size was found for *H. thynni* ($\rho = 0.296$, $p = 0.02$) and *D. wedli* ($\rho = 0.437$, $p = 0.00$), while the abundance of *Didymosulcus* sp. 2 was negatively correlated with the host size ($\rho = -0.435$, $p = 0.00$).

The Table 7.3.1 shows the P% of the four Mediterranean host groups (present results), compared with the Adriatic Sea (Mladineo *et al*., 2008) and the north-eastern Atlantic Ocean (Rodríguez–Marín *et al.*, 2008) data. The P% of *D. wedli* and *H. thynni* in the north-eastern Atlantic Ocean (M-NE-Atl) were higher than those of the Mediterranean Sea, except the XL group (XL-W-Med). The P% of *Didymosulcus* sp. 2 of the small western Mediterranean hosts (S-W-Med) was higher than in any of the other groups. The P% of *D. reniformis* was higher in the medium size hosts from the Adriatic Sea (M-Adr) and the Atlantic Ocean (M-NE-Atl) than in the large fish from the western Mediterranean Sea (L-W-Med); the P% of *D. pretiosus* was higher in the medium size hosts from the eastern Mediterranean Sea (M-E-Med) and the Atlantic Ocean (M -NE-Atl) and the large hosts from the western Mediterranean Sea (L-W-Med) than in the medium size group from the Adriatic Sea (M-Adr). The P% of *K. apicalis* in the Adriatic Sea (M-Adr) was higher than in the Atlantic hosts (M-NE-Atl) and in the large hosts from the western Mediterranean Sea (L-W-Med); the P% of *W. bipartita* was higher in the medium

eastern Mediterranean hosts (M-E-Med) than in those from the Adriatic Sea (M-Adr) and the Atlantic Ocean (M-NE-Atl). The P% of *P. appendiculatus* was higher in the medium hosts from the Atlantic Ocean (M-NE-Atl) and in the large and extra large fish from the western Mediterranean Sea (L-W-Med and XL-W-Med) than in those from the Adriatic Sea (M-Adr).

Table 7.3.1. Prevalence (%) of the gill parasites of *Thunnus thynnus* (95% confidence intervals in parentheses) according to host size (S, M, L, XL) and locality (E-Med and W-Med, eastern and western Mediterranean Sea, present results); Adr, Adriatic Sea (Mladineo *et al.*, 2008); NE Atl, north-eastern Atlantic Ocean (Rodríguez–Marín *et al.*, 2008). *, new geographical record. Greek letters, significant differences ($p \leq 0.05$).

Parasite	S–W-Med	M-Adr §	M-E-Med	M-NE-Atl ♯	L-W-Med	XL-W-Med	Location
Monogenea							
C. magronum *	0 (0-53)	0	0 (0-29)	0	2 (0-10)	0 (0-50)	Gill arch
C. onchidiocotyle	0 (0-53)	0 $^{\alpha}$	0 (0-29)	5 $^{\alpha}$	2 (0-10)	0 (0-50)	Gill arch
C. paucispinosa *#	0 (0-53)	0	0 (0-29)	0	2 (0-10)	0 (0-50)	Gill arch
H. thynni	0 (0-53) $^{\alpha}$	2 $^{\gamma, \varepsilon, \eta}$	0 (0-29) $^{\beta, \zeta}$	61 $^{\alpha, \beta, \gamma, \delta}$	27 (16-42) $^{\delta, \varepsilon}$	60 (19-92) $^{\zeta, \eta}$	Gill filaments
Digenea							
C.thyrsitae	0 (0-53)	0	0 (0-29)	2	0 (0-9)	0 (0-50)	
D.filiformis	0 (0-53)	1	0 (0-29)	0	0 (0-9)	0 (0-50)	
D. reniformis	0 (0-53)	24 $^{\alpha}$	0 (0-29)	18 $^{\beta}$	2 (0-9) $^{\alpha, \beta}$	0 (0-50)	Gill arch
Didymocystis sp. 3	0 (0-53)	0 $^{\alpha}$	0 (0-29)	19 $^{\alpha, \beta}$	0 (0-9) $^{\beta}$	0 (0-50)	
Didymosulcus sp. 2 *	100 (47-100) $^{\alpha, \beta, \gamma, \delta, \varepsilon}$	0 $^{\beta, \zeta, \eta, \iota}$	20 (4-55) $^{\alpha, \zeta}$	23 $^{\gamma, \eta, \theta}$	5 (1-16) $^{\delta, \theta, \iota}$	0 (0-50) $^{\varepsilon}$	Gill arch
D. wedli	0 (0-53) $^{\alpha, \beta, \gamma, \delta}$	62 $^{\alpha, \zeta}$	50 (22-78) $^{\varepsilon}$	92 $^{\beta, \varepsilon, \zeta, \eta}$	81 (68-90) $^{\gamma, \eta}$	100 (50-100) $^{\delta}$	Outer margin of gill filaments
D. longicolle	0 (0-53)	1	0 (0-29)	0	0 (0-9)	0 (0-50)	
D. pretiosus	0 (0-53)	0 $^{\alpha, \beta, \delta}$	20 (5-55) $^{\alpha}$	19 $^{\beta, \gamma}$	7 (2-19) $^{\gamma, \delta}$	0 (0-50)	Inner margin of gill filaments
K. apicalis	0 (0-53)	11 $^{\alpha, \beta}$	0 (0-29)	0 $^{\alpha}$	0 (0-9) $^{\beta}$	0 (0-50)	
W. bipartita	0 (0-53)	0 $^{\alpha}$	10 (1-45) $^{\alpha, \beta}$	0 $^{\beta}$	2 (0-10)	0 (0-50)	Gill rakers
Copepoda							
C. coryphaenae	0 (0-53)	0	0 (0-29)	0	2 (0-10)	0 (0-50)	Gill filaments
E. brachypterus	0 (0-53)	0	0 (0-29)	2	0 (0-9)	0 (0-50)	
P. appendiculatus	0 (0-53)	0 $^{\alpha, \gamma, \delta}$	0 (0-29)	29 $^{\alpha, \beta}$	11 (5-23) $^{\beta, \gamma}$	40 (8-81) $^{\delta}$	Gill filaments

§ Mladineo et al. (2008); ♯ Rodríguez-Marín et al. (2008)

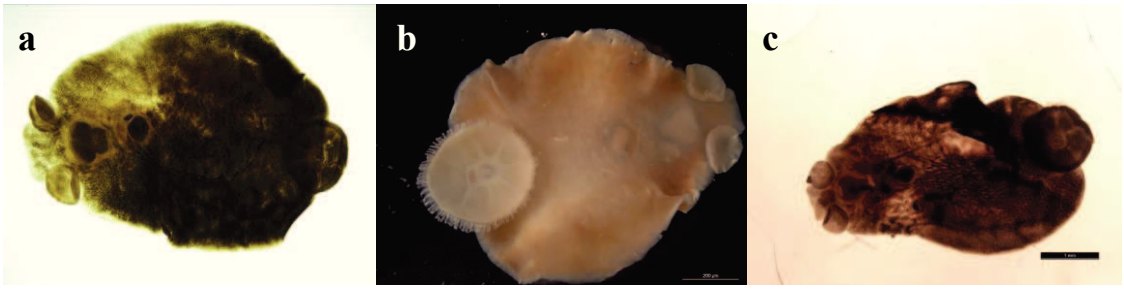

Figure 7.3.1. Capsalid monogeneans *ex* the gills of *Thunnus thynnus*: **a**, *Capsala magronum*; **b**, *Capsala onchidiocotyle*; **c**, *Capsala paucispinosa.*

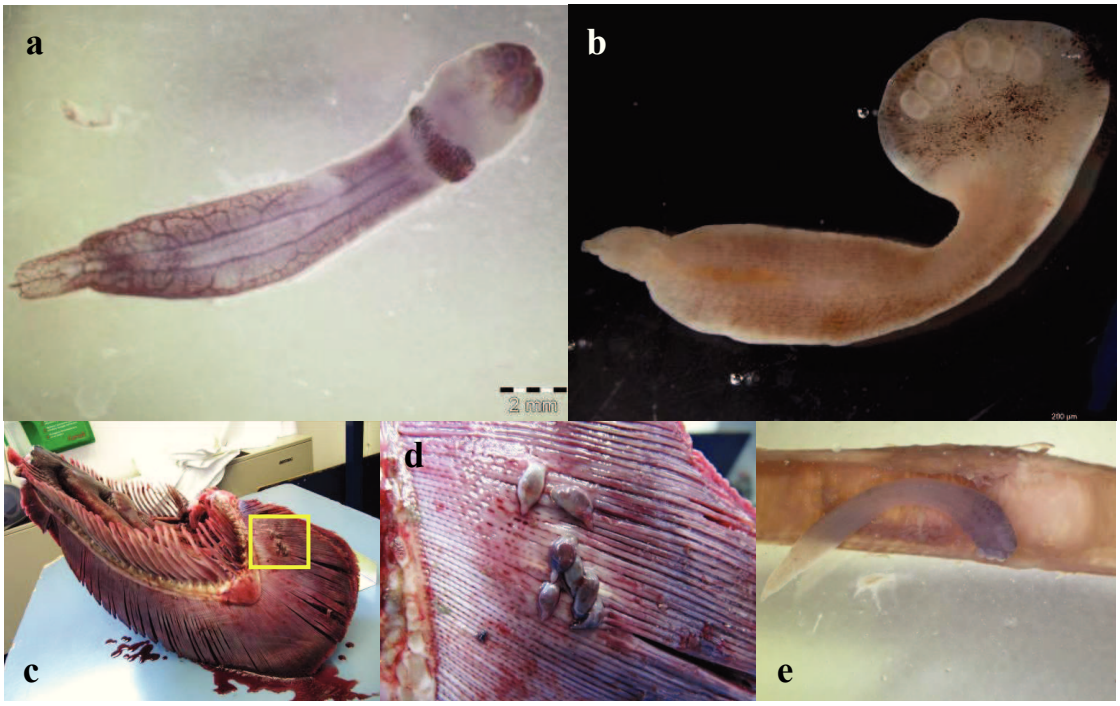

Figure 7.3.2. a-b, *Hexostoma thynni ex* the gills of *Thunnus thynnus.* **c-e**, *H. thynni in situ.*

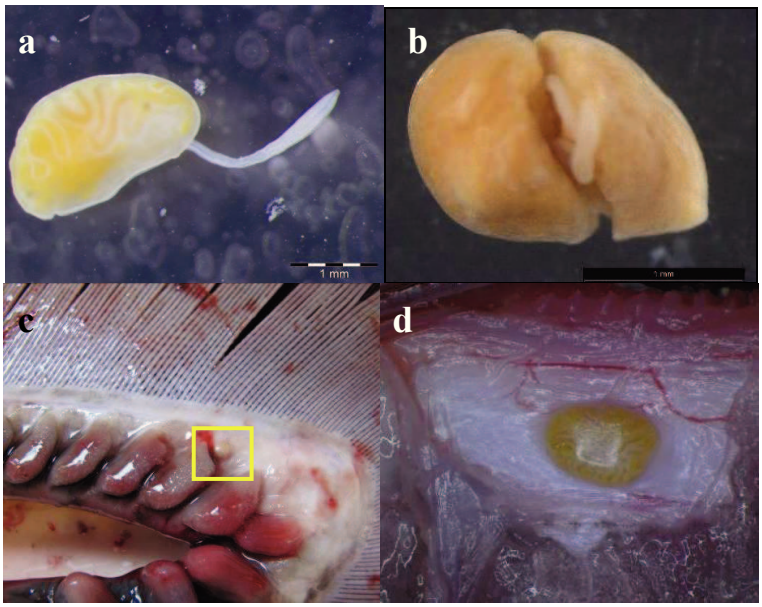

Figure 7.3.3. a-b, *Didymocystis reniformis ex* the gills of *Thunnus thynnus.* **c-d**, *D. reniformis in situ.*

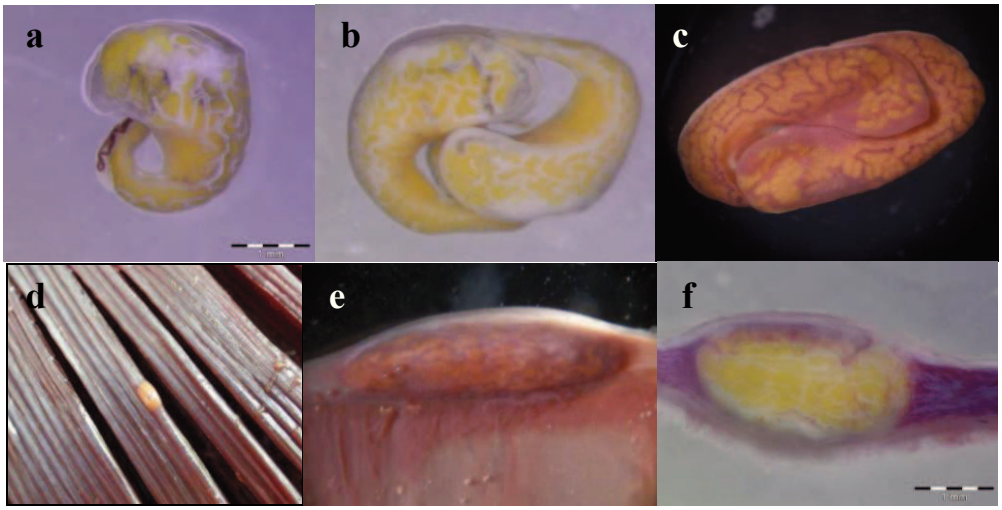

Figure 7.3.4. a-c, *Didymosulcus wedli ex* the gills of *Thunnus thynnus*.**d-f**, *D. wedli in situ.*

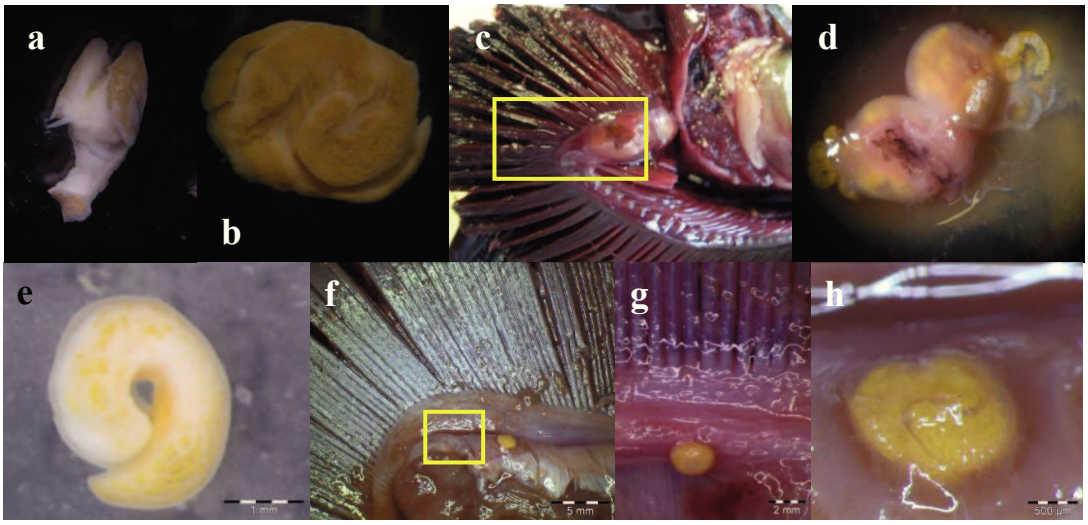

Figure 7.3.5. a, **b**, **e**, *Didymosulcus* sp. 2. *ex* the gills of *Thunnus thynnus*. **c-d**, *Didymosulcus* sp. 2 of juvenile *T. thynnus in situ.* **f-h**, *Didymosulcus* sp. 2 of adult *T. thynnus in situ*.

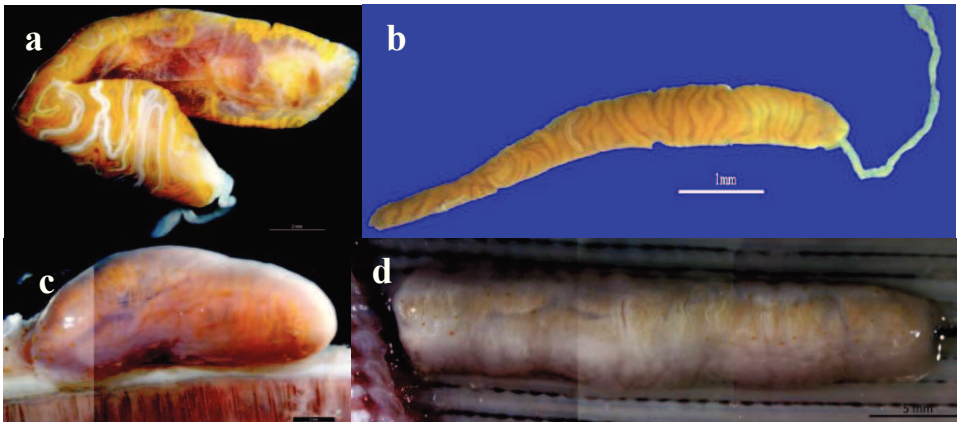

Figure 7.3.6. a, b, *Didymozoon pretiosus ex* the gills of *Thunnus thynnus*. **c-d**, *D. pretiosus in situ.*

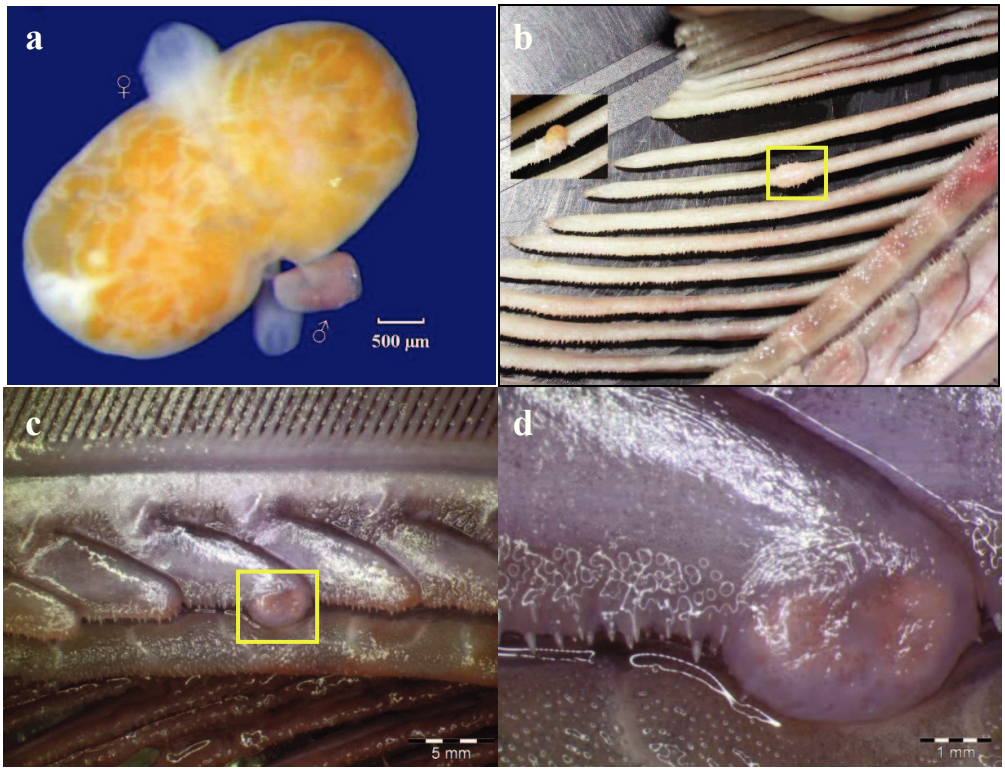

Figure 7.3.7. a, *Wedlia bipartita ex* the gills of *Thunnus thynnus.* **b-d**, *W. bipartita in situ.* **a** and **b** are a courtesy of Jacopo Culurgioni.

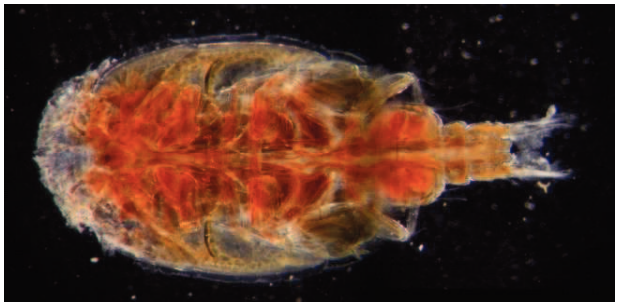

Figure 7.3.8. Male of *Caligus coryphaenae ex* the gills of *Thunnus thynnus.*

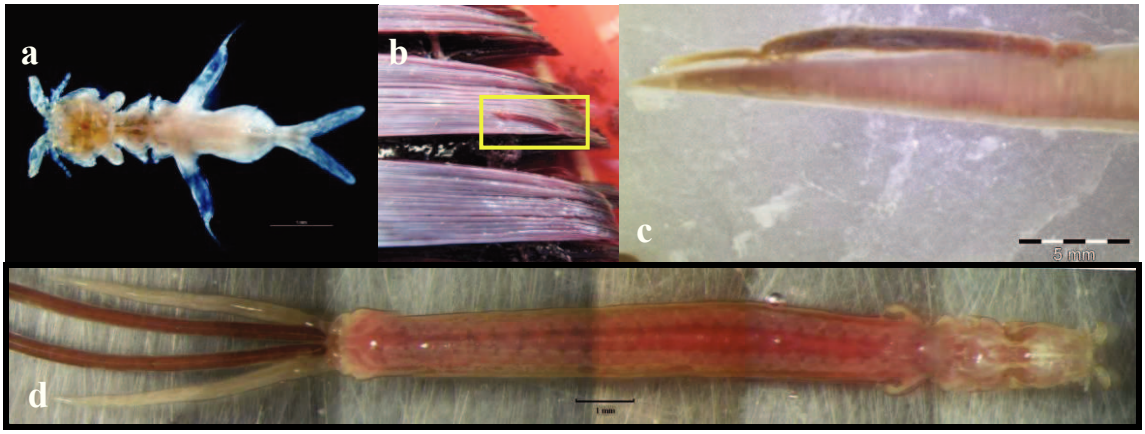

Figure 7.3.9. *Pseudocycnus appendiculatus ex* the gills of *Thunnus thynnus*: **a,** male; **b-c**, female specimen *in situ*; **d**, female (courtesy of Jacopo Culurgioni).

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The Table 7.3.2-3 shows the MA and the MI of the four Mediterranean host groups (present results). The MA of *Didymosulcus* sp. 2 of the small fish (S-W-Med) was higher than that of the eastern Mediterranean ones (M-E-Med), and the MA of *D. wedli* was lower in the small group (S-W-Med) than in the large one (L-W-Med). No significant differences in MI were found.

Table 7.3.2. Mean abundance of the gill parasites of *Thunnus thynnus* (95% confidence intervals in parentheses), according to host size (S, M, L, XL) and locality (E-Med, eastern Mediterranean Sea; W-Med, western Mediterranean Sea). Greek letters, significant differences ($p \leq 0.05$).

Parasite species	S-W-Med	M-E-Med	L-W-Med	XL-W-Med
Monogenea				
C. magronum	0 (-)	0 (-)	0.1 (0.0-0.1)	0 (-)
C. onchidiocotyle	0 (-)	0 (-)	0.1 (0.0-0.1)	0 (-)
C. paucispinosa	0 (-)	0 (-)	0.1 (0.0-0.1)	0 (-)
H. thynni	0 (-)	0 (-)	0.8 (0.4-1.7)	2.7 (1.0-4.0)
Digenea				
D. reniformis	0 (-)	0 (-)	0.1 (0.0-0.3)	0 (-)
Didymosulcus sp. 2	10.5 (9.3-12.3) $^{\alpha}$	1.7 (0.0-6.5) $^{\alpha}$	0.1 (0.0-0.1)	0 (-)
D. wedli	0 (-) $^{\alpha}$	2.8 (0.8-8.0)	6.2 (4.3-9.0) $^{\alpha}$	12.0 (2.8-21.4)
D. pretiosus	0 (-)	0.6 (0.0-2.1)	0.2 (0.1-0.5)	0 (-)
W. bipartita	0 (-)	0.1 (0.0-0.3)	0.0 (0.0-0.1)	0 (-)
Copepoda				
C. coryphaenae	0 (-)	0 (-)	0.0 (0.0-0.1)	0 (-)
P. appendiculatus	0 (-)	0 (-)	0.1 (0.0-0.2)	1.2 (0.0-2.4)

Table 7.3.3. Mean intensity of the gill parasites of *Thunnus thynnus* (95% confidence intervals in parentheses), according to host size (S, M, L, XL) and locality (E-Med, eastern Mediterranean Sea; W-Med, western Mediterranean Sea). Greek letters, significant differences ($p \leq 0.05$).

Parasite species	S-W-Med	M-E-Med	L-W-Med	XL-W-Med
Monogenea				
C. magronum	0 (-)	0 (-)	1 (-)	0 (-)
C. onchidiocotyle	0 (-)	0 (-)	1 (-)	0 (-)
C. paucispinosa	0 (-)	0 (-)	1 (-)	0 (-)
H. thynni	0 (-)	0 (-)	3.1 (1.8-5.3)	2.7 (1.0-4.0)
Digenea				
D. reniformis	0 (-)	0 (-)	5 (-)	0 (-)
Didymosulcus sp. 2	10.5 (9.3-12.3)	8.5 (1.0-8.5)	1.0(-)	0 (-)
D. wedli	0 (-)	5.6 (2.2-14.0)	8.2(5.9-11.7)	12.0 (2.8-21.4)
D. pretiosus	0 (-)	3.0 (1.0-3.0)	3.0 (2.0-3.7)	0 (-)
W. bipartita	0 (-)	1 (-)	1 (-)	0 (-)
Copepoda				
C. coryphaenae	0 (-)	0 (-)	1 (-)	0 (-)
P. appendiculatus	0 (-)	0 (-)	1.0 (-)	3.0 (2.0-3.0)

The non-metric Multidimensional scaling (NMDS) plots and the cluster analysis (CA)

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diagrams of the species that had significant differences of prevalence between almost two of the six groups (the four Mediterranean and the Adriatic and Atlantic ones) are shown in Fig. 7.3.10. The cophenetic index of the dendrograms indicate that the graph of didymozoids and ectoparasites are both highly representative (Rc = 0.97 and 0.94). The CA and NMDS of didymozoids shows that the P% of *Didymosulcus* sp. 2 and *D. wedli* separate the small Mediterranean tunas from all the other ones; the P% of *Didymocystis* sp. 3 and *K. apicalis* separate the Atlantic group (M-NE-Atl) from the Adriatic one (M-Adr). The CA and NMDS of the prevalence of the ectoparasites showed that the P% of *H. thynni* separates three group of hosts: one including the small and medium Mediterranean hosts (S-W-Med, M-Adr, M-E-Med), one with the large fish from the western Mediterranean Sea (L-W-Med), and one with the Atlantic (M-NE-Atl) and the extra large western Mediterranean (XL-W-Med) groups.

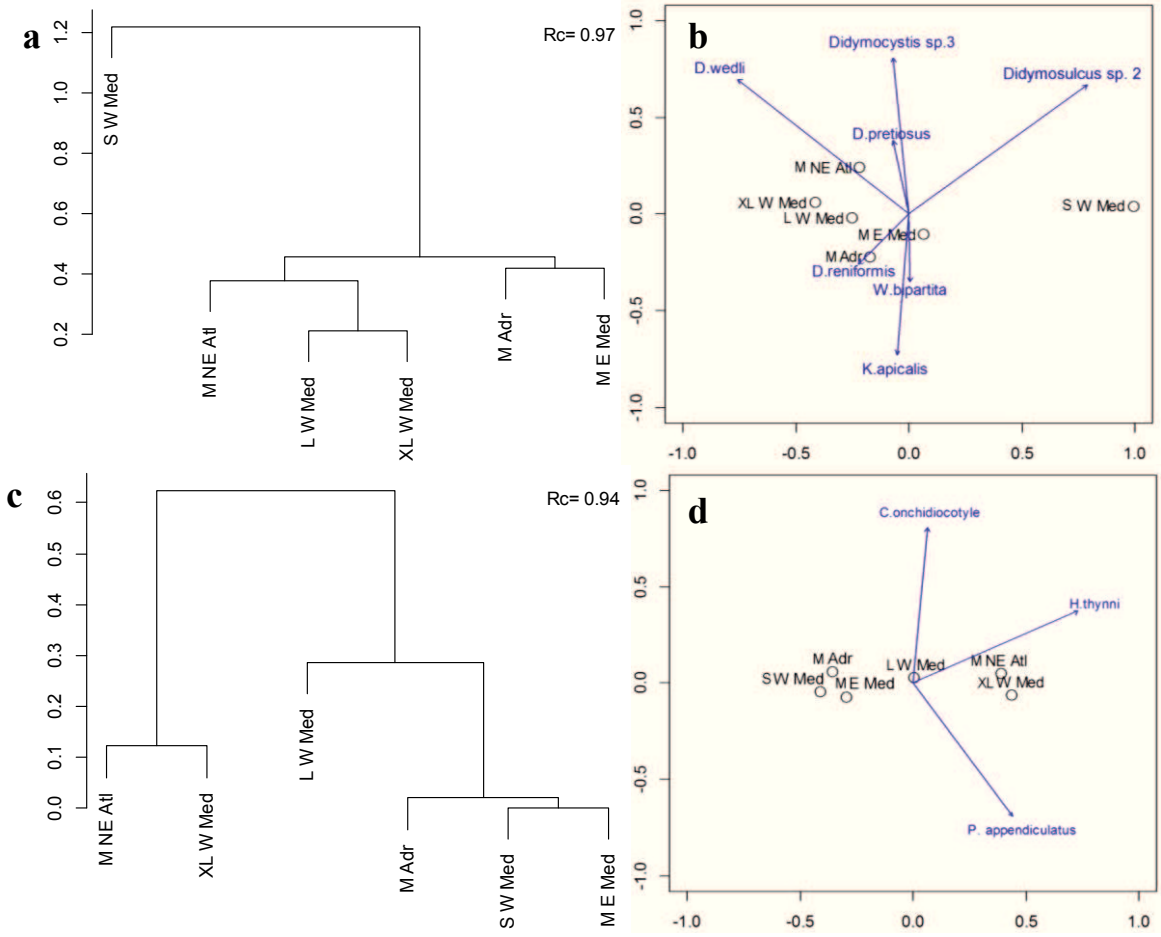

Figure 7.3.10. Cluster dendrograms (**a**, **b**) and NMDS plots (**c**, **d**) based on the Bray-Curtis distance of the data of the species that showed differences of prevalence between at least one pairwise of host groups according to host size (S, M, L, XL) and locality (Adr, Adriatic Sea; E-Med, eastern Mediterranean Sea; W-Med, western Mediterranean Sea; NE-Atl, north-eastern Atlantic Ocean). **a-b**, CA and NMDS of the prevalence of didymozoids, **c-d**, CA and NMDS of the prevalence of ectoparasites.

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The NMDS plot and the CA diagrams of the didymozoid species that had significant differences of the MA between almost two of the four Mediterranean groups are shown in Fig. 7.3.11. The cophenetic index of the dendrograms is less representative than that of prevalences (Rc = 0.83). The MA of *Didymosulcus* sp. 2 and *D. wedli* separates three groups: the small fish (S-W-Med), the extra large ones (XL-W-Med), and the medium and large ones (M-E-Med and L-W-Med).

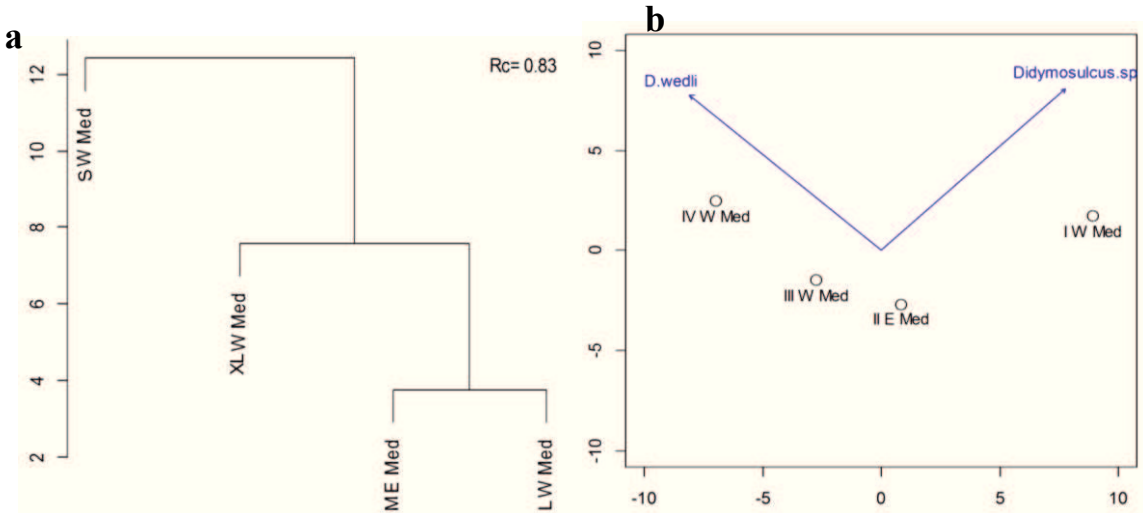

Figure 7.3.11. Cluster dendrograms (**a)** and NMDS plots (**b**) based on the Bray-Curtis distance of the data of the species that showed differences of mean abundance between at least one pairwise of host groups according to host size group (S, M, L, XL) and locality (E-Med, eastern Mediterranean Sea; W-Med, western Mediterranean Sea).

The cluster analysis of the parasite assemblages according to the indices of dissimilarity based on the presence/absence data species in the parasites assemblages of *T. thynnus* from the eastern, central and western Mediterranean Sea (BFTEM, BFTCM, BFTWM, data from literature and present results), and from the north-eastern and north-western Atlantic Ocean (BFTEA, BFTWA); of *T. maccoyii* from the Indian Ocean (SBT); and of *T. orientalis* from the Pacific Ocean (PBT) (Table 4.2.2) are shown in Fig. 4.3.12. Only two groups cluster, *T. thynnus* from the north-eastern Atlantic Ocean and the western Mediterranean Sea (BFTEA and BFTWM), while all the other five groups are well separated.

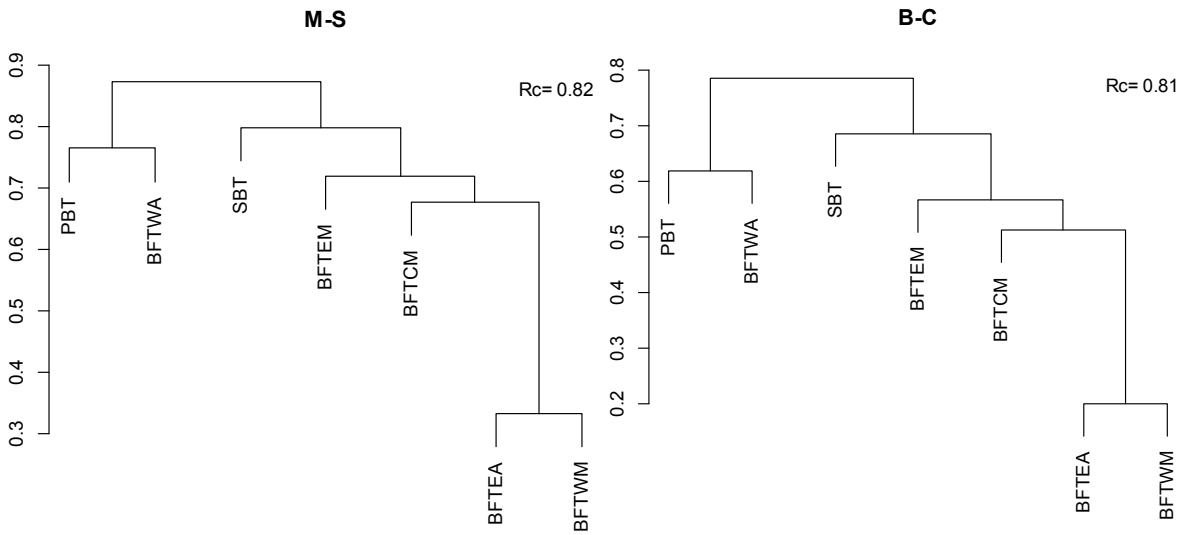

Figure 7.3.12. Cluster dendrograms (group-average linkage) of the gill parasites of *Thunnus thynnus* from the eastern Mediterranean Sea (BFTEM, present results), central Mediterranean Sea (BFTCM, data from literature), western Mediterranean Sea (BFTWM, data from literature and present results), and eastern and western Atlantic Ocean (BFTEA and BFTWA), *Thunnus maccoyii* from the indian Ocean (SBT), and *Thunnus orientalis* from the Pacific Ocean (PBT), using Marczewski-Steinhaus (M-S) and Bray-Curtis (B-C) dissimilarity measures based on the presence/absence of parasites (see Table 4.2.2 and present results).

7.4. DISCUSSION

The whole metazoan parasite fauna of the gills of *Thunnus thynnus* includes a total of 20 species/taxa. The richest parasite fauna of this species is in the north-eastern Atlantic Ocean (12 species), followed by the western Mediterranean Sea, with 11, five here reported for the first time in this area: *Caligus coryphaenae*, *Capsala magronum*, *Capsala paucispinosa*, *Didymosulcus* sp. 2, *Pseudocycnus appendiculatus* (*T. thynnus* is a new host record for *C. paucispinosa*). Among the western Mediterranean parasite fauna, *Capsala interrupta* (Monticelli, 1891) and *Euryphorus brachypterus* (Gerstaecker, 1853) were not found in this study. The parasite fauna of the western Mediterranean bluefin tuna shares almost all species with the north-eastern Atlantic one, except *Copiatestes thyrsitae* Crowcroft, (1948) and *Didymocystis* sp. 3 *sensu* Rodríguez-Marín *et al*. (2008) (found only in the north-eastern Atlantic Ocean), and *C. interrupta* (reported only in the western Mediterranean Sea).

The parasite faunas of *T. thynnus* from the north-western Atlantic Ocean and the eastern Mediterranean Sea have the lowest richness (5 and 4 parasites, respectively), no parasites shared with the Adriatic and eastern Mediterranean tunas, and only two crustaceans (*C. coryphaenae* and *E. brachypterus*) with the north-eastern Atlantic and the western Mediterranean ones. The low richness reported in these localities can also be due to the scarcity of studies on the gill parasites of *T. thynnus* from these areas, *e.g. C. magronum* has been reported in other tuna

species from the north-western Atlantic Ocean (Silas, 1962).

The Atlantic bluefin tuna shares five parasite species with the southern bluefin tuna, and 11 with the Pacific bluefin tuna. Because the three bluefin tuna species are geographically isolated, the presence of common parasites could be due to a common extinct ancestor (Huyse *et al*., 2005), but also to the presence of other tuna/scombrid species in the range of at least two bluefin tunas, that could allow the exchange of parasites (Aiken *et al*., 2007). In fact, most of the parasites of bluefin tunas have also been recorded in several other tuna species, *e.g*. *C. paucispinosa*, *P. appendiculatus, Didymocylindrus filiformis*, *Didymoproblema fusiforme*, *Didymosulcus wedli*, *Lobatozoum multisacculatum*, *E. brachypterus*, and many caligid copepods worldwide distributed (Silas, 1962; Cressey and Cressey, 1980). On the other hand, other parasites seem to be strictly specific to *Thunnus orientalis*: *Hexostoma grossum*, *Didymosulcus crassa*, and to *T. thynnus*: *C. interrupta*, *Didymocystis* sp. 3, *Didymosulcus* sp. 2. Nevertheless, the taxonomic status of a number of helminths here reported is still unclear, and some of them have been suggested as synonyms of other species with similar morphological features (*e.g*., the capsalids *C. interrupta*, *Capsala maccallumi* and *C. paucispinosa*; the hexostomids *H. grossum* and *H. thynni*; the didymozoids *Didymosulcus soleiformis* and *Didymosulcus* sp. 2; and *W.bipartita* and *Wedlia reniformis*) requiring a taxonomic revision of these taxa (Chisholm and Whittington, 2007; Podznyakov and Gibson, 2008).

The parasite assemblages of the Atlantic bluefin tuna vary according to the host size, probably as a consequence of migration at different age and/or of a change of feeding habits. The lack of monogeneans and copepods in the small and medium Mediterranean tunas (S-W-Med and M-E-Med) and their lower presence in the Adriatic group and in the large western Mediterranean tuna (L-W-Med) than in the Atlantic (M-NE-Atl) and in the extra large Mediterranean (XL-W-Med) fish could be due to the different environmental conditions between the Atlantic and Mediterranean areas, being the Mediterranean waters unsuitable for these parasite species. On the other hand, the infection by parasites with complex life cycle, such as the didymozoids, is related with the host diet (Williams *et al*., 1992). Although juvenile and adult Atlantic bluefin tunas are opportunistic feeders (Fromentin and Powers 2005), the diet of the large specimens includes a wider spectrum of preys (Sará and Sará, 2007). Thus, the lack of some didymozoids (*e.g. D. reniformis*, *D. wedli*, W. *bipartita*) in the small fish could be related to the lack of intermediate hosts in their diet (Williams *et al*. 1992), or also to the absence of infected intermediate hosts in their feeding areas.

Tagging results indicated that *T. thynnus* is a migratory fish, able to cross large geographical distances with the increase of size, the giant tunas being able to cross the Atlantic

Ocean (Rooker *et al*., 2007). Juvenile tunas (*i.e.* the small and medium size classes) seem to stay around their own breeding areas (Balearic, Ionian and Levantine Seas) with local migrations to the feeding grounds of the Gulf of Lion (Sorell, 2011), Adriatic Sea (Tudela *et al*., 2011) and Aegean Sea (Karakulak and Oray, 2009), respectively, until reaching the size to migrate to the Atlantic Ocean (Tudela *et al*., 2011). These basins are characterised by different environmental conditions (Garibaldi and Caddy, 1998) that can allow the presence of different parasite faunas, and this can explain the differences between the Adriatic and the eastern Mediterranean groups. Likely, the different environmental conditions in the Mediterranean Sea and in the north-eastern Atlantic Ocean could explain the differences between the parasite assemblages of the two groups from the Mediterranean Sea (M-Adr and M-E-Med) and that of the juveniles from the Atlantic Ocean (M-NE-Atl). Once adults (*i.e.,* the L and XL size classes), during the warm season tunas go back from the Atlantic and Mediterranean feeding grounds to the Mediterranean spawning areas (Anon., 2011).

The multivariate analyses (CA and NMDS) of the P% of didymozoids differed from that of the MA. According to the graphs of the P% the large and extra large western Mediterranean tunas (L-W-Med and XL-W-Med) cluster together, well separated from all the other Mediterranean groups, and they have a similar didymozoid infection to the Atlantic ones. This fact suggests that the large and extra large Mediterranean tunas in some moment of their life history migrated to the Atlantic feeding areas. On the other hand, the multivariate analyses of the didymozoid MA of the Mediterranean hosts showed that the large Mediterranean tunas (L-W-Med) cluster with the small and medium ones (S-W-Med and M-E-Med), and they are separated from the extra large group, that has infections similar to the Atlantic tunas (Rodríguez-Marín *et al*., 2008). The analyses of the P% of the ectoparasites confirmed the separation between L-W-Med and XL-W-Med. The discrepancy between the results of P% and MA of the large group from the western Mediterranean Sea (L-W-Med) suggests that during the spawning season the schools of this group may have a multiple structure: mainly composed by Mediterranean resident tunas (poorly infected by didymozoids, and with levels of infection similar to the juvenile S-W-Med and M-E-Med groups) and by a lower number of tunas migrating from the Atlantic Ocean (highly infected by didymozoids, that harbour a richest parasite fauna than the Mediterranean groups). The high P% and MA of some parasites (*D. wedli*, *H. thynni*, *P. appendiculatus*) in the XL-W-Med group, more similar to those of the M-NE-Atl tunas than to the L-W-Med ones can be due to a higher proportion in the XL-W-Med group of tunas migrating from the north-eastern Atlantic area, but the low number of XL-W-Med hosts sampled could have biased the results, and does not allow to estimate the significance of the results.

CONCLUSIONS

The whole point of view: metapopulations and metassemblages of the gill metazoan parasites of tunas and use of the parasites as biological tags

The concept of metapopulation was introduced to emphasise that populations are not simple homogeneous collections of individuals, but they are often composed by groups, or local populations, spatially separated but linked by exchanges of individuals (Rohde, 2005). The local populations may become extinct and re-establish through migration of individuals from extant local populations (Rohde, 2005; Simkova and Morand, 2005). The tunas inhabiting the western Mediterranean Sea migrate inside of this sea, but also to the adjacent areas of the Mediterranean Sea (Adriatic Sea, Eastern Mediterranean Sea, etc.) and to the Atlantic Ocean, although with great differences according to species and size. This fact allows the dispersal of the parasites among the various geographical areas. Hence, as stated by Simkova and Morand (2005) the study of the parasite metapopulations can be used to know as a whole the parasites of the tunas from the western Mediterranean Sea, and to estimate which host is the reservoir of which parasite, and where are the endemic areas of the parasite infections.

In the previous chapters the parasite assemblages of tunas have been analysed at the infra- and component community levels. The whole check-list of the metazoan gill parasites of the five tuna species considered in this study (based on the present and published data), in the Atlantic and Mediterranean areas, is reported in the Table 8.1.1. Over a total of 50 species, 38 have been described in the western Mediterranean Sea, 32 of which found in the present study, adding 20 new records for this area, 11 new host records, and reporting six species identified only at the genus or family level because they are likely new species to the science.

The Table 8.1.1 shows that the parasite assemblages of these tunas are not discrete units, but they are part of a wide metassemblage. In fact, the hosts share a number of parasite species, and the set of the parasite populations of one parasite species in each host forms the parasite metapopulation. In particular, 16 species are shared between two (or more) of the five tuna

species, and of these eight occur in the Mediterranean Sea. Two parasites species shared between Atlantic hosts are not present in the same Mediterranean host species (*i.e. Caligus pelamydis* and *Caligus productus*), and eight are found only in some category of them (size class, locality, etc.) (*i.e. Allopseudaxine macrova*, *Caligus bonito*, *Caligus coryphaenae*, *Capsala magronum*, *Euryphorus brachypterus*, *Hexostoma thynni*, *Lobatozoum multisacculatum*, *Pseudocycnus appendiculatus*).

The dissimilarity analysis of the whole Atlantic and Mediterranean gill parasite faunas of the five tuna species (literature data and present results, see Tables 3.2.2, 4.2.2, 5.2.2, 6.2.2, 7.2.2 and Tables 3.3.1, 4.3.1, 5.3.1, 6.3.1, 7.3.1) shows that *Auxis rochei* has the most dissimilar parasite fauna (with the lower number of shared species), while the other four species are divided into two groups, one including *Euthynnus alletteratus* and *Katsuwonus pelamis* and the other *Thunnus alalunga* and *Thunnus thynnus* (Fig 8.1.1). These results, apart from the ecological affinity between the host species, reflect the taxonomic relationships between them (Collette 2001), indicating that the host-parasite coevolution is an important factor for the parasites of tunas. These findings are similar to those of Cressey *et al*. (1983), that compared the phylogenetic tree of some scombrid fish (based on the morphological characteristics) with that of their parasitic copepod fauna, showing that the scombrid fish and the copepods are co-evolved.

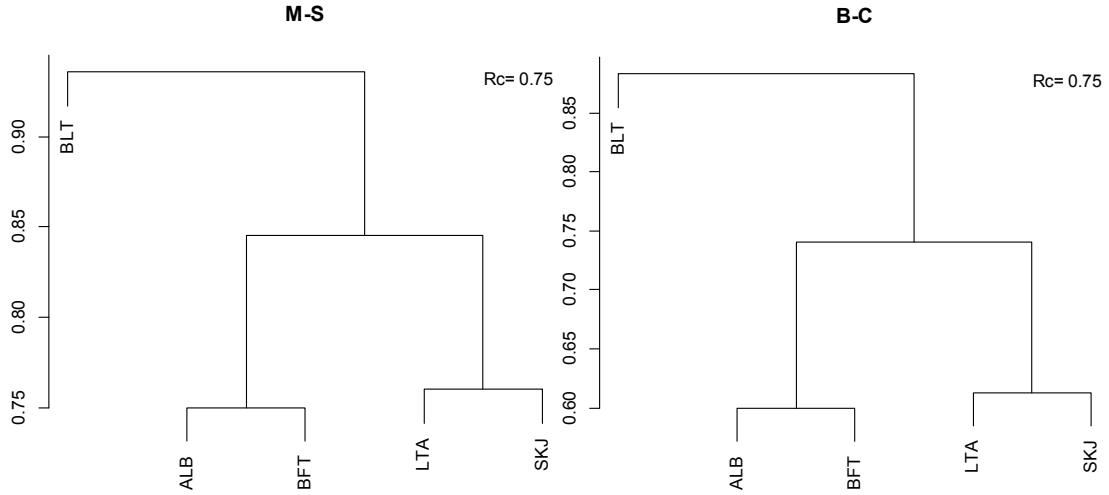

Figure 8.1.1. Dissimilarity of the whole metazoan parasite fauna of the gills of five tuna species from the Mediterranean Sea and the Atlantic Ocean, using the Marczewski-Steinhaus distance (M-S) and the Bray-Curtis index (B-C). ALB, *Thunnus alalunga*; BLT, *Auxis rochei*; BFT, *Thunnus thynnus*; LTA, *Euthynnus alletteratus*; SKJ, *Katsuwonus pelamis*.

According to Dove (2006) the occurrence of one parasite species in multiple component assemblages depends on the spatial dynamic of the host populations. The different species of Mediterranean tunas are competitors for the same ecological niche and have the same gregarious and migratory behaviours that the tunas from other areas of the world, and these features can

influence the sharing of parasites between host species. Concerning feeding habits, the studies on the diet of the five Mediterranean tunas show that most of the food items are shared among species, and this fact explains the sharing of many foodborne parasites among tunas. Regarding the spatial dynamic, diverse species of tunas can school together and also with other pelagic fish species according to size. This allows the transmission of non specific monoxenous parasites (*e.g*. monogeneans and crustaceans) between different host species in the different areas where these species migrate and form schools.

The structure and dynamics of the different metapoulations of the gill parasites of tunas are hereafter analysed in relation to the bio-ecological features of both hosts and parasites, and this information is considered to evaluate the use of the parasites as biological tags.

The only foodborne parasites found in the gills of tunas are the several species of didymozoid trematodes. The numerous species of gill didymozoids shared between the tunas of the Atlantic Ocean and the Mediterranean Sea indicate an overlapping of food items among tuna species, and it is related to the presence of infected intermediate hosts in the tuna diets and/or feeding grounds. These parasites dominate the parasitofauna of tunas from the western Mediterranean Sea, as previously reported for the same or other species from other localities, *e.g. T. alalunga* and *K. pelamis* from the Pacific Ocean (Lester *et al*., 1985; Jones, 1991), *T. thynnus* from the Adriatic Sea and the north-eastern Atlantic Ocean (Mladineo *et al*., 2008; Rodríguez-Marín *et al*., 2008), and other tunas from the tropical areas of the oceans (Lardeaux, 1982; Nikolaeva, 1985; Madhavi and Ram 2000), although with several differences according to host species and locality. In general the abundance and richness of the didymozoids of tunas from the western Mediterranean Sea is lower than those of the central, north-eastern and south-western Atlantic Ocean (see Chapters 3.3, 4.3, 5.3, 7.3), and higher than those of the eastern Mediterranean Sea and north-western Atlantic Ocean. This finding agrees with the results of Lester *et al*. (1985), Nikolaeva (1985) and Jones (1991), who reported a high richness and level of infection of didymozoids in fish from the tropical areas, suggesting that tunas are mainly infected by these parasites in the tropical regions of the Oceans. Nevertheless, Nikolaeva (1985) could have underestimated the richness of the Mediterranean didymozoids; in fact, this author reported a total of 23 species in all the Mediterranean fish, whereas in the present study 25 species have been found just in the gills (or head) of five tuna species, twelve of them reported for the first time in the Mediterranean Sea (six of wich likely new species), increasing significantly the didymozoid richness in this area.

Didymozoon longicolle infects *K. pelamis*, *T. alalunga*, *Thunnus albacares* and *T. thynnus*. In the western Mediterranean Sea it was found in *K. pelamis* and *T. alalunga* (Chapters 5.3 and

6.3) with similar levels of infection in *T. albacares* in the Atlantic tropical areas (Nikolaeva and Parukhin, 1968), whereas it is not reported in the north-eastern Atlantic Ocean. No data are available for the central and eastern Mediterranean Sea, except the record of one specimen in *T. thynnus* from the Adriatic Sea (Mladineo *et al*., 2008). These data indicate that this parasite is widespread in the central and southern Atlantic Ocean and western Mediterranean Sea, and that probably lacks in the temperate areas of the North Atlantic Ocean.

Didymocylindrus filiformis is shared between *K. pelamis* (Chapter 5.3) and *T. thynnus* (one specimen recorded in the Adriatic Sea, Mladineo *et al*., 2008). The higher values of infection in the skipjack tuna suggest that this parasite is specific to *K. pelamis* although it can occasionally infect other tunas.

Lobatozoum multisacculatum infects *Euthynnus alletteratus* and *K. pelamis* with low levels of infection worldwide (Lester *et al*., 1985, Alves and Luque 2006, present results). In the central Atlantic Ocean it infects both *E. alletteratus* and *K. pelamis*, while in the western Mediterranean Sea only *K. pelamis*. The absence of this parasite in the western Mediterranean *E. alletteratus* (a non migratory species) suggests its absence in the Mediterranean Sea, and that *K. pelamis* get infected in the tropical Atlantic area before migrating to the Mediterranean Sea.

Didymozoon pretiosus and *Wedlia bipartita* are shared between *T. alalunga* and *T. thynnus*. The first species is specific to the genus *Thunnus*, whereas second one has also been found occasionally in *Seriola dumerilii* from the western Mediterranean Sea (Grau *et al*., 1999), a pelagic fish that can share several food items with the two tuna species (Badalamenti *et al*., 1995) as confirmed by this parasitological data.

Although the heteroxenous didymozoids dominate the gill parasites assemblages of the Mediterranean tunas, a rich set of monoxenous parasites (axinid, capsalid, gastrocotilid, hexostomid monogeneans, and copepod crustaceans) also characterise these assemblages.

The axinid monogenean *A. macrova* infects *Auxis thazard*, *E. alletteratus*, *K. pelamis* and *T. albacares* in the tropical central and south-western Atlantic Ocean (Bussiéras and Baudin-Laurencin, 1973; Mogrovejo and Santos, 2004; Alves and Luque, 2006; Cissé *et al.*, 2007), while in the western Mediterranean Sea it infects only *A. rochei* (Chapter 3.3), with P% similar to the tunas of the tropical Atlantic areas. The absence of this parasite in other Mediterranean tunas (particularly in *E. alletteratus* and *K. pelamis*) suggests that this parasite is not resident in the Mediterranean Sea, but it is acquired by *Auxis rochei* in the tropical Atlantic feeding areas, and then it is introduced in the western Mediterranean Sea seasonally with host migration.

Nine capsalid monogeneans infect the Atlantic and Mediterranean tunas, with two of them recorded only one time in the tuna host (*i.e.*, *Capsala interrupta* and *Capsala maccallumi* in *T. thynnus* from the western Mediterranean Sea and the north-western Atlantic Ocean, respectively). Among the five capsalid species recorded in the western Mediterranean Sea, only *Capsala paucispinosa* is shared between two hosts (*T. alalunga* and *T. thynnus*, present results) with low levels of infection in both hosts. This species infects also *T. albacares* from the central-eastern Atlantic Ocean (Bussiéras, 1972), whereas is not described in *T. alalunga* and *T. thynnus* from the eastern Mediterranean Sea and the north-eastern Atlantic Ocean (present results), suggesting that it infects tunas in the tropical Atlantic areas and in the western Mediterranean Sea. The parasite populations of these two areas can be linked by the migrations of *T. thynnus* between the Atlantic western Sahara feeding grounds (shared with *T. albacares*) and the Mediterranean spawning areas (shared with *T. alalunga*). Analogously, Aiken *et al.* (2007) described another monogenean shared among tunas from separate regions as a consequence of the migration of another host species; these authors indicated that *Hexostoma thynni* infects both *T. thynnus* from the north Atlantic Ocean and *T. maccoyii* from the Indian Ocean because it also infects *T. albacares*, that partially overlaps its distribution with the two other species.

Concerning the Mediterranean copepods of tunas, *C. bonito* has been described in several scombrids worldwide (Cressey and Cressey, 1980), and in the present study it was found with higher P% in *K. pelamis* than in *E. alletteratus* and *A. rochei*. These results are similar to those reported in the same hosts from the south-western Atlantic Ocean (Alves and Luque, 2006), suggesting that *K. pelamis* is an important host for this parasite. *Pseudocycnus appendiculatus* is a specific parasite of tunas distributed worldwide (Cressey and Cressey, 1980); in the Mediterranean Sea (Chapters 4.3, 6.3, 7.3) it was found with higher P% in *E. alletteratus* than in *T. alalunga* and *T. thynnus*, suggesting *E. alletteratus* is a preferred host for this parasite. Both *C. bonito* and *P. appendiculatus* infect almost all size groups of tunas from the different areas of the western Mediterranean Sea, suggesting that the environmental conditions of this area (see chapter 1.2) are suitable for these parasites, and they can be considered part of the resident western Mediterranean fauna. On the other hand, *P. appendiculatus* was not found in *T. thynnus* from the Adriatic and eastern Mediterranean Seas (Chapter 7.3; Mladineo *et al*., 2008), likely because in these localities there are not favourable environmental conditions for this species.

The analysis of the parasite assemblages were used to evaluate the use of the parasites as biological tags, and mainly the following criteria were applied:

A parasite shared between several tuna species in the Atlantic Ocean, and occurring only in some of them (or just in one size group) in the Mediterranean Sea, indicates the migration of the

infected Mediterranean host species (or size group) from the Atlantic Ocean.

A parasite with significant differences of infection between Atlantic and Mediterranean fish of to the same size group indicates a geographical separation of these fish.

A parasite infecting two host species geographically isolated indicates the migration of a third host species between the two localities, this third species must partially overlap the range of distribution of both species and must be infected by the same parasite in both areas.

A foodborne parasite shared among two or more tuna species indicates that some food items are shared among these host species.

Concluding, the present study showed that the gills of tunas are infected by a rich metazoan parasitofauna, that seems to be a promising tool to infer information on the host biology and ecology. Didymozoids are likely the most useful group to be used as tags, because they are permanent parasites, and becasue of their host and site specifity, but also capsalid and axiniid monogeneans can be used as tags with some caution.

The most important drawback in the use of these parasites as tags is the limited knowledge of the taxonomy of the parasites of the gills of tunas; in fact, there are still many doubts regarding the nomenclature of almost all didymozoids and in the taxonomy of many capsalid and axiniid monogeneans. Therefore, the first step necessary to allow effective these parasites as tags is to deepen the knowledge on the taxonomy, biology, and ecology of the single parasite species living in the gills of tunas.

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Table 8.1.1. Check list (parasite-host list) of the gill parasites of *Auxis rochei* (BLT), *Euthynnus alletteratus* (LTA), *Katsuwonus pelamis* (SKJ), *Thunnus alalunga* (ALB) and *Thunnus thynnus* (BFT) from the Atlantic Ocean (NEA, north-eastern Atlantic Ocean; NWA, north-western Atlantic Ocean; CEA, central-eastern Atlantic Ocean; CWA, central-western Atlantic Ocean; SEA, south-eastern Atlantic Ocean; SWA, south-western Atlantic Ocean) and the Mediterranean Sea (CM, central Mediterranean Sea; EM, eastern Mediterranean Sea; WM, western Mediterranean Sea). OTH, other host species than tunas; TUN, other tunas species than the five of this study. *, present results.

Species	Host species	Locality		Source
Monogenea				
Allopseudaxine macrova (Unnithan, 1957)	BLT	WM	*	
		SWA		Alves and Luque (2006)
	SKJ	CEA		Cissé *et al.* (2007)
		SWA		Alves and Luque (2006)
	TUN	CEA		Bussiéras and Baudin-Laurencin (1973)
		SWA		Alves and Luque (2006)
Capsalidae gen. sp.	SKJ	SWA		Alves and Luque (2006)
Capsala gouri (Chauhan, 1951)	LTA	NWA		Chisholm and Whittington (2007)
Capsala laevis (Verrill, 1874)	BFT	CM		Mladineo *et al.* (2008)
	OTH	NWA		Chisholm and Whittington (2007)
Capsala interrupta (Monticelli, 1891)	BFT	WM		Palombi (1949)
Capsala maccallumi Price, 1939	BFT	NWA		Chisholm and Whittington (2007)
Capsala magronum (Ishii, 1936)	BFT	WM	*	
		NEA		Chisholm and Whittington (2007)
	LTA	SWA		Chisholm and Whittington (2007)
	TUN	CEA		Chisholm and Whittington (2007)
		CWA		Chisholm and Whittington (2007)
Capsala manteri (Price, 1951)	LTA	WM	*	
		CEA		Chisholm and Whittington (2007)
		CWA		Chisholm and Whittington (2007)
		SWA		Chisholm and Whittington (2007)
	TUN	CEA		Chisholm and Whittington (2007)
		CWA		Chisholm and Whittington (2007)
		SWA		Chisholm and Whittington (2007)
Capsala onchidiocotyle (Setti, 1899)	BFT	WM	*	Palombi (1949)
		NEA		Rodríguez-Marín *et al.* 2008
	LTA	NWA		Chisholm and Whittington (2007)
Capsala paucispinosa (Mamaev,	ALB	WM	*	

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Species	Host species	Locality		Source
1968)	BFT	WM	*	
		CWA		Chisholm and Whittington (2007)
	TUN	CEA		Bussiéras (1972)
Capsala thynni (Guiart, 1938)	ALB	NEA		Dollfus (1952)
Hexostoma auxisi Palombi, 1943	BLT	WM	*	
	TUN	WM		Palombi (1949)
		SWA		Alves and Luque (2006)
Hexostoma euthynni Meserve, 1938	LTA	SWA		Alves and Luque (2006)
Hexostoma thunninae Parona *et* Perugia, 1889	LTA	WM	*	Palombi (1949)
		CM		Palombi (1949)
		SWA		Alves and Luque (2006)
Hexostoma thynni Rafinesque, 1815	BFT	WM	*	Palombi (1949)
		CM		Mladineo *et al.* (2008)
		NEA		Rodríguez-Marín *et al.* 2008
		NWA		Hendrix (1994)
	TUN	CEA		Hayward et al. (2005)
Metapseudaxine ventrosicula Mamaev, 1967	LTA	SWA		Alves and Luque (2006)
Neohexostoma mochimae Zambrano, 1997				Fuentes Zambrano (1997)
Udonella caligorum Johnston, 1835	LTA	NWA		Hendrix (1994)
Digenea				
Atalostrophion cf. *biovarium* Skrjabin, 1955	SKJ	WM	*	
Copiatestes thyrsitae Crowcroft, 1948	BFT	NEA		Rodríguez-Marín *et al*. 2008
Didymocylindrus filiformis Ishii, 1935	BFT	CM		Mladineo *et al*. (2008)
	SKJ	WM	*	
		CWA		Lester *et al*. (1985)
Didymocylindrus simplex (Ishii, 1935)	SKJ	WM	*	
		CWA		Lester *et al*. (1985)
Didymocystis lanceolata Guiart, 1938	ALB	NEA		Guiart (1940)

Species	Host species	Locality		Source
Didymocystis macrorchis Guiart, 1938	ALB	NEA		Guiart (1940)
Didymocystis reniformis Ariola, 1902	ALB	NEA		Guiart (1940)
	BFT	WM	*	Mariniello *et al*. (2000)
		CM		Mladineo *et al*. (2008)
		NEA		Rodríguez-Marín *et al*. 2008
	SKJ	WM	*	
Didymocystis sp. 1	LTA	WM	*	
Didymocystis sp. 2	LTA	WM	*	
Didymocystis sp. 3 *sensu* Rodríguez-Marín et al., 2008	BFT	NEA		Rodríguez-Marín *et al*. 2008
Didymoproblema fusiforme Ishii, 1935	SKJ	WM	*	
		CWA		Lester *et al*. (1985)
		SWA		Justo and Kohn (2005)
Didymosulcus aahi Pozdnyakov, 1990	ALB	WM	*	
Didymosulcus dimidiatus Pozdnyakov, 1990	ALB	WM	*	
Didymosulcus sp. 2 *sensu* Rodríguez-Marín et al., 2008	BFT	WM	*	
		EM	*	
		NEA		Rodríguez-Marín *et al*. 2008
Didymosulcus wedli (Ariola, 1902)	BFT	WM	*	Mariniello *et al*. (2000)
		CM		Mladineo *et al*. (2008)
		EM	*	
		NEA		Rodríguez-Marín *et al*. 2008
Didymozoinii gen. sp.	LTA	WM	*	
Didymozoon sp. *sensu* Alves *et* Luque 2006	SKJ	SWA		Alves and Luque (2006)
Didymozoon sp.	LTA	WM	*	
Didymozoon auxis Taschenberg, 1879	BLT	WM	*	
	TUN	WM		Dollfus (1926)
		SWA		
Didymozoon longicolle Ishii, 1935	ALB	WM	*	
	BFT	CM		Mladineo *et al*. (2008)

Species	Host species	Locality		Source
	SKJ	WM	*	
	OTH	CWA		Nikolaeva and Parukhin (1968)
Didymozoon pretiosus Ariola, 1902	ALB	WM	*	
	BFT	WM	*	Mariniello *et al*. (2000)
		EM		
		NEA		Rodríguez-Marín *et al*. 2008
Koellikeria sp.	SKJ	WM	*	
Kollikerioides apicalis Yamaguti, 1970	BFT	CM		Mladineo *et al*. (2008)
Lobatozoum multisacculatum Ishii, 1935	LTA	SWA		Alves and Luque (2006)
	SKJ	WM	*	
		CEA		Bussiéras and Baudin-Laurencin (1973)
		SWA		Alves and Luque (2006)
Nematobothrium latum Guiart, 1938	ALB	WM	*	
		NEA		Guiart (1940)
Wedlia bipartita (Wedl, 1855)	ALB	WM	*	
		NEA		Guiart (1940)
	BFT	WM	*	Ariola (1902)
		CM		Ariola (1902)
		EM	*	
		NEA		Rodríguez-Marín *et al*. 2008
	OTH	WM		Grau *et al*. (1999)
Crustacea				
Caligus bonito Wilson, 1905	BLT	WM	*	
	BFT	NWA		Cressey and Cressey (1980)
	LTA		*	
		NWA		Cressey and Cressey (1980)
		SWA		Alves and Luque (2006)
	SKJ		*	
		SWA		Alves and Luque (2006)
	OTH			
		CM		Cressey and Cressey (1980)
		NEA		Cressey and Cressey (1980)
		NWA		Cressey and Cressey (1980)
		CEA		Cressey and Cressey (1980)
		CWA		Cressey and Cressey (1980)
		SWA		Cressey and Cressey (1980)
Caligus coryphaenae Steenstrup *et* Lütken, 1861	BFT	WM	*	
		NWA		Cressey and Cressey (1980)
	LTA	CWA		Cressey and Cressey (1980)

Species	Host species	Locality		Source
	SKJ	CEA		Cressey and Cressey (1980)
		CWA		Cressey and Cressey (1980)
		SWA		Cressey and Cressey (1980)
	TUN	NWA		Cressey and Cressey (1980)
		CEA		Cressey and Cressey (1980)
		CWA		Cressey and Cressey (1980)
		SWA		Cressey and Cressey (1980)
		CEA		Cressey and Cressey (1980)
Caligus pelamydis Krøyer, 1863	LTA	SWA		Alves and Luque (2006)
	SKJ	NWA		Silas and Ummekutty (1967)
	OTH	CM		Cressey and Cressey (1980)
		NWA		Cressey and Cressey (1980)
		CEA		Cressey and Cressey (1980)
		CWA		Cressey and Cressey (1980)
		SEA		Cressey and Cressey (1980)
		SWA		Cressey and Cressey (1980)
Caligus productus Dana, 1852	BFT	NWA		Cressey and Cressey (1980)
		CWA		Cressey and Cressey (1980)
	LTA	NWA		Cressey and Cressey (1980)
	SKJ	NWA		Cressey and Cressey (1980)
		CWA		Cressey and Cressey (1980)
		SWA		Alves and Luque (2006)
	TUN	NWA		Cressey and Cressey (1980)
		CWA		Cressey and Cressey (1980)
		SWA		Cressey and Cressey (1980)
	OTH	NWA		Cressey and Cressey (1980)
		CEA		Cressey and Cressey (1980)
		CWA		Cressey and Cressey (1980)
Euryphorus brachypterus (Gerstaecker, 1853)	ALB	NEA		Dollfus (1952)
		NWA		Cressey and Cressey (1980)
		CEA		Cressey and Cressey (1980)
		SWA		Cressey and Cressey (1980)
	BFT	WM		Arru and Garippa (1995)
		CM		Mladineo *et al.* (20119
		NEA		Rodríguez-Marín *et al*. (2008)
		NWA		Cressey and Cressey (1980)
		SEA		Cressey and Cressey (1980)
	TUN	NWA		Cressey and Cressey (1980)
		CEA		Cressey and Cressey (1980)
		CWA		Cressey and Cressey (1980)
		SWA		Cressey and Cressey (1980)
Pseudocycnus appendiculatus Heller, 1868	ALB	WM	*	
	BFT	WM	*	Arru and Garippa (1995)
		NEA		Rodríguez-Marín *et al*. (2008)

Species	Host species	Locality		Source
	LTA	WM	*	
		NWA		Cressey and Cressey (1980)
		CWA		Cressey and Cressey (1980)
		SWA		Alves and Luque (2006)
	SKJ	CEA		Cressey and Cressey (1980)
		SWA		Alves and Luque (2006)
	TUN	NWA		Cressey and Cressey (1980)
		CEA		Cressey and Cressey (1980)
Isopoda gen. sp.	LTA	SWA		Alves and Luque (2006)
Rocinela sp.	ALB	WM	*	

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ANNEXES

ANNEX 1.

When the biometrical data of hosts were not available the correlation between FL (fork length, Fig. A.1.1) and LLL (length of the lower limb of the first left gill arch, Fig. A.1.2) was used.

Thunnus alalunga: $FL = 7.8076\ LLL + 3.1$;

Thunnus thynnus: $FL = 11.24\ LLL - 4$;

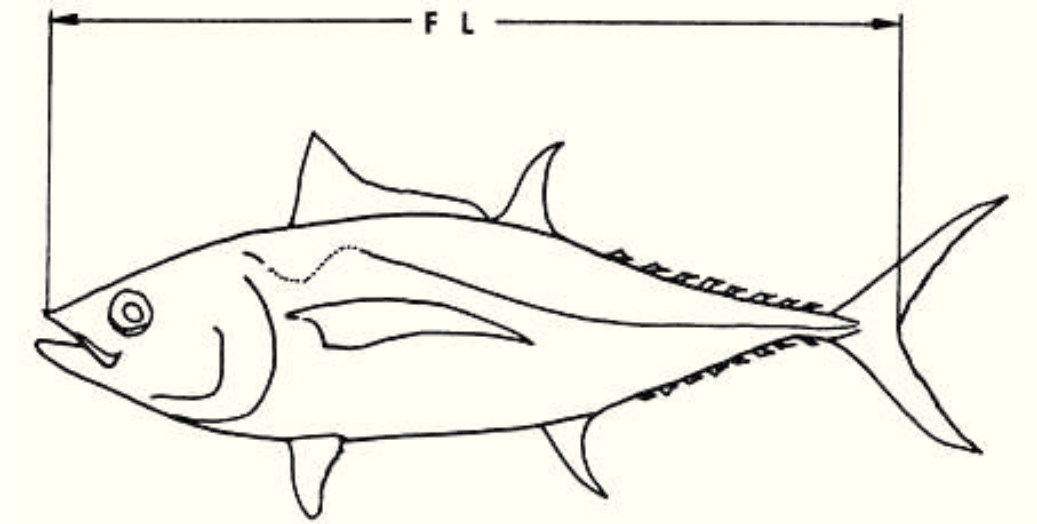


Figure A.1.1. Drawing of a tuna. FL = fork length

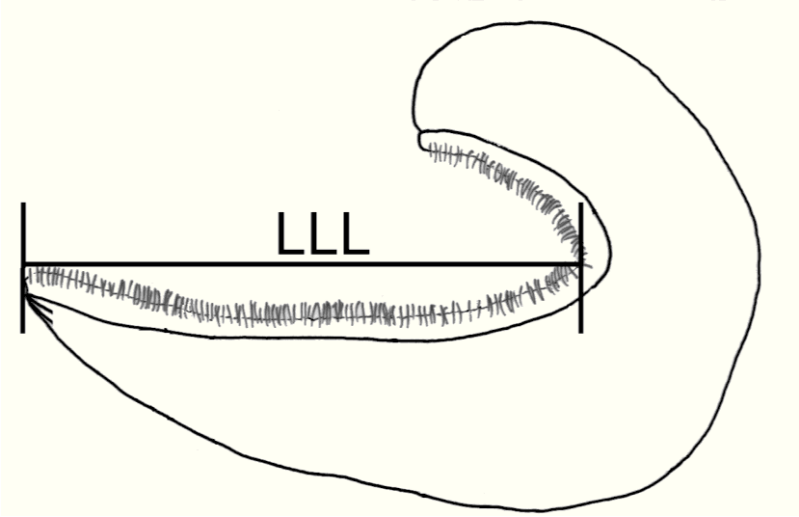


Figure A.1.2. Drawing of a gill of tunas. LLL = length of the lower limb.

Salvatore Mele. Gill metazoan parasites of tunas (Scombridae: Thunnini) from the western Mediterranean Sea: systematics, assemblages and use as biological tags. Tesi di dottorato in Riproduzione, Produzione, Benessere Animale e Sicurezza degli Alimenti di Origine Animale. Università degli studi di Sassari.

ANNEX 2. FORM USED DURING THE PARASITOLOGICAL ANALYSIS

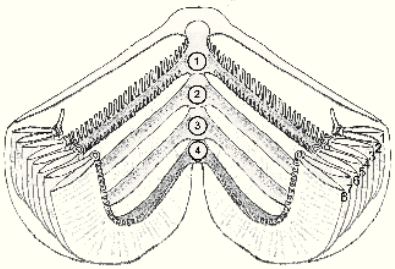
Host	Date of analysis	Date of sampling	Site sampling	Database code

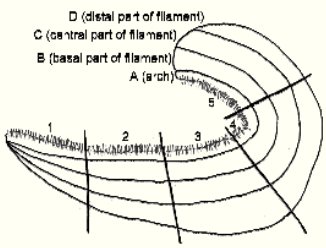
LF (cm)	TW (kg)	LLL* (mm)	Age (years)	Sex	Notes

	Left Holobranchs		Right Holobranchs	
Arch	**Hemibranch**		**Arch**	**Hemibranch**
I	**1** #		**I**	**1** #
	2			**2**
	Holobr. rinse			**Holobr. rinse**
II	**3**		**II**	**3**
	4			**4**
	Holobr. rinse			**Holobr. rinse**
III	**5**		**III**	**5**
	6			**6**
	Holobr. rinse			**Holobr. rinse**
IV	**7**		**IV**	**7**
	8			**8**
	Holobr. rinse			**Holobr. rinse**

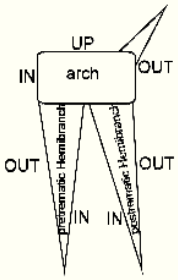
Gill chamber	Operculum	Tongue	Palate
Pharynx	Nostril	Eye	Heart
Total rinse			

Record the intensity as R(I), where R is the region of the side surface of the holobranch and I the intensity. Add In (inner margin of filament) or Out (outer margin), if possible. For ex. B3(2) *H. thynni* = 2 *H. thynni* in the region B3; C2(3)Out *D. wedli* = 3 cysts of *D. wedli* in the region C2 of the outer margin. *LLL=Lower Limb Length





A1	A2	A3	A4	A5
B1	B2	B3	B4	B5
C1	C2	C3	C4	C5
D1	D2	D3	D4	D5



ANNEX 3. STAINING AND MOUNTING TECHNIQUES USED FOR

PLATYHELMINTHES

MALZACHER'S STAINING TECHNIQUE (PRITCHARD AND KRUSE 1982) MODIFIED

STAIN: ASTRA BLUE

Reagents: Astra blue 1 g
Tartaric acid 3 g
Distilled water 100 ml

Procedure: Mix ingredients well (do not use magnetic and ferrous tools to mix).

Notes: Stain can be used immediately.

STAIN: BORAX-CARMINE

Reagents: Carmine 2 g
Borax (decahydrate) 4 g
Distilled water 100 ml
Ethanol 70% 100 ml

Procedure: Dissolve Borax in distilled water and add carmine. Boil for 15 minutes. After 24 hours add 100 ml of ethanol 70% in cooled condition. Filter the solution.

HYDROCLORIDRIC SOLUTION 1M IN ALCOHOL 70%

Reagents Hydrocloridric acid (HCl) concentrate1 ml
Ethanol 70% 99 ml

Procedure: Add HCl to ethanol 70%.

STAINING PROCEDURE

- Transfer flattened specimen from 70% ethanol to borax-carmine for 5-30 minutes (or even more) depending on the size of the specimen.
- Wash in distilled water until parenchyma looks pale.
- Clear in hydrocloridric solution (3-5 minutes).
- Wash in distilled water for 5 minutes.
- Transfer to Astra blue stain for 1-5 minutes.
- Wash in distilled water until the rinse is clear.
- Keep the specimens in ethanol (70%) for almost one day.
- Dehydrate through a series of ethanol solutions (70%, 80%, 90%, 96%, absolute).
- Clear in creosote (better), methyl salicilate or limonene.
- Mount in Canada balsam (good for large Nematobothriinae Ishii, 1935) or other resin.