ALMA MATER STUDIORIUM - UNIVERSITA' DI BOLOGNA

SCUOLA DI SCIENZE

Corso di laurea magistrale in Biologia Marina

The nematode parasite fauna in three odontocete species: *Phocoena Phocoena*, *Lagenorhynchus albirostris*, *Lagenorhynchus acutus* from Northern Scotland and its relationships with diet composition

Tesi di laurea in

Adattamenti degli animali all'ambiente marino

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III sessione

Anno Accademico 2013-2014

Oh, io sono un mezzo fallito. Il poco che so lo devo al mio professore, Albert Sorel. "Cosa vuol diventare?", mi domandò. "Diplomatico." "Ha una grossa fortuna?" "No." "Può con qualche apparenza di legittimità aggiungere al suo cognome un nome celebre?" "No." "E allora rinunci alla diplomazia." "Ma allora cosa posso fare?" "Il curioso." "Non è un mestiere." "Non è ancora un mestiere. Viaggi, scriva, traduca, impari a vivere dovunque, e cominci subito. L'avvenire è dei curiosi di professione.”

(Jules e Jim, Truffaut)

ABSTRACT

Food items and nematode parasites were identified from the stomachs of 42 inviduals of *Phocoena phocoena*, 6 of *Lagenorhynchus acutus* and 8 of *L . albirostris* stranded off the coastal waters of Northern Scotland between 2004 and 2014. Post-mortem examinations have revealed heavy parasitic worm burdens. Four nematode species complex as *Anisakis* spp., *Contracaeucum* spp., *Pseudoterronova* spp., and *Hysterothylacium* spp were recorded. Data on presence of the anisakid species in cetaceans, reported a significative relationship between the presence of *Hysterothylacium* and the month of host stranding; suggesting a decrease of larval *H. aduncum* abundance in the period between April and August due to a seasonal effect related to prey availability. Similarly, the parasite burden of the all anisakid genera was related to the year fraction of stranding, and a relationship statistically significant was found just for *L. albirostris* with an increase between April and October. This finding is explained by a seasonality in occurrence of white-beaked dolphins, with a peak during August, that might be related to movements of shared prey species and competition with other species (*Tursiops truncatus*). Geographical differences were observed in parasites number of all anisakid species, which was the highest in cetaceans from the East area and lowest in the North coast. The parasites number also increased significantly with the length of the animal and during the year, but with a significant seasonal pattern only for *P. phocoena*. Regarding diet composition, through a data set consisting of 34 harbour porpoises and 1 Atlantic white-sided dolphins, we found a positive association between parasite number and the cephalopods genus *Alloteuthis*. This higher level of parasite infection in squid from this area, is probably due to a quantitative distribution of infective forms in squid prey, an abundance of the final host and age or size maturity of squid.

1 INTRODUCTION

1.1 Nematode biology

Parasitic nematodes are known as important pathogens that cause problems for human and animal health. Some of them naturally inhabit the marine environment, where they are widespread and can be found in a variety of different hosts. For most parasites, cetaceans are their final hosts. The sampling of free ranging animals is difficult to perform in the wild thus, studying parasites in cetaceans needs to examine live specimens or fresh carcasses of cetaceans. Researches, therefore, have to rely on unpredictable, occasional strandings or bycatch (Raga *et al.,* 1997; Dhermain *et al.,* 2002). The samples used in this study were just collected by strandings.

Nematoda comprise one of the largest phyla in the animal kingdom, with more than 10,000 described species, about half of them parasitic and numerous species remaining to be described. They affect fish by causing pathological symptoms and possibly mortalities, they reduce the commercial value of fish, and they may infect man producing harmful effects.

Endoparasites can cause various pathological disorders in marine mammals as well.

The disease problems caused by nematodes have to be considered a major factor in the mortality of cetacean stocks. The role that these parasites play in the health of their host and in stranding events is complicated and has been scrutinized by stranding biologists for decades.

Adult nematodes may live in various organs and tissues of their host, but they occur primarily in 2 primary infestation sites: thoracic cavity-head, stomach- intestine. Digestive system parasites can cause internal bleeding, ulceration, obstruction and malnutrition (Dailey 2001).

Nematodes of the family Anisakidae (*Anisakis, Contraceacum, Pseudoterronava*) are composed of several complexes of sibling species that have systematic effect and members of three genera are the most common nematodes of cetaceans, as reported from most marine mammal species examined (Dailey 2001).

In the stomach of cetaceans, anisakid adults are often found in clusters of individuals embedded in the mucosa and submucosa. Ulcers of 5x3 cm associated with anisakids are found in the fundic portion of the stomach (Fig. 1a, b from Abollo *et al.,* 1998). The worms occur in circumscribed, granulomatous nodules as well (Fig. 1c).

Such reaction appears to be a common response to tissue invasion by these parasites. Among the nematodes, the family Anisakidae is probably the most successful in terms of potential for colonizing hosts in many environments.

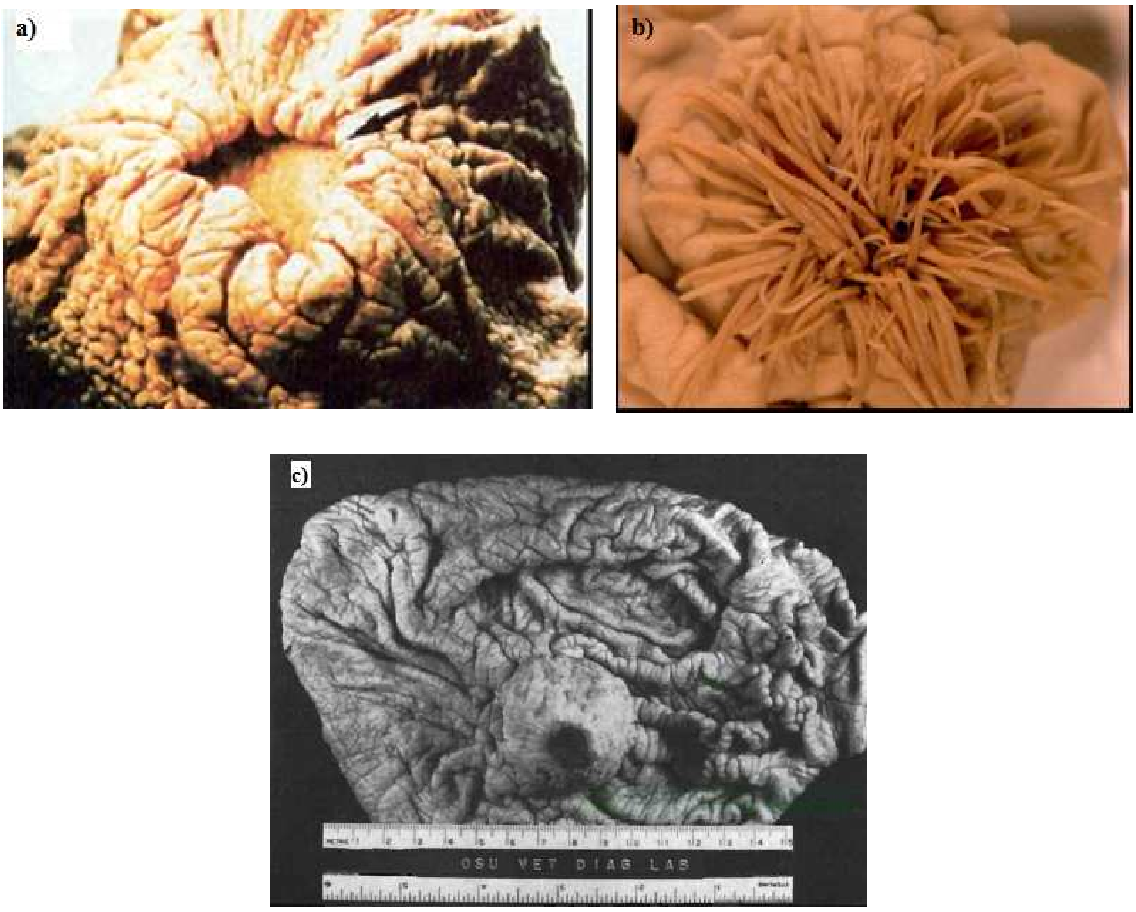


Fig. 1. **a)** stomach wall ulceration (arrow) of *Delphinus delphis;* **b)** cluster of *A. simplex* partially embedded in the inner wall of the stomach of *D. delphis* (After Abollo *et al.,* 1998); **c)** *Phocoena phocoena*. Nematode *(Anisakis simplex)* induced granuloma in first stomach.(After Dailey & Stroud 1978.)

Anisakis has been reported from large numbers of both Odontocetes and Mysticetes (Delyamure, 1955; Dailey & Brownell, 1972). Dans *et al.*, (1999) reported the gastrointestinal helminths of the dusky dolphin *Lagenorhynchus obscurus* off the Patagonian coast. Other studies have described occasional strandings of poorly known cetaceans, such as spectacled porpoises *Phocoena dioptrica*, Burmeister's porpoise

Phocoena spinipinnis, Gray's beaked whale *Mesoplodon grayi*, Cuvier's beaked whale *Ziphius cavirostris*, sperm whale *Physeter macrocephalus* and Hector's beaked whale *Mesoplodon hectori* (Aznar *et al.,* 2003, Berón-Vera *et al.*, 2008, Nikolov *et al.*, 2010). These studies explored parasite transmission within a trophic web, as well as host habitat distribution, reporting new helminth taxa or describing helminth communities of by-caught or stranded dolphins (Romero *et al.,* 2014).

Dailey & Brownell (1972) found nematodes infesting lungs, pulmonary artery, auditory spaces and air sinuses of cetaceans as well. Due to the sharp expirations (in contrast to terrestrial mammals) by cetaceans, the worms must invade the tissue to form 'anchors' through capsule formation. Worms living free in the bronchi would tend to be blown from the host. The posterior portions of the worms are free to mate and discharge larvae.

Parasite infection leads to muco purulent bronchiolitis and pneumonia. Verminous pneumonia with secondary bacterial broncho pneumonia was responsible for the death of 3 subadult *Phocoena phocoena* infested with *Halocercus invaginatus*. This same nematode was found by Dailey & Stroud (1978) throughout the lung parenchyma of a *P. phocoena*, resulting in fibrinous interstitial pneumonia with focal abscesses, and parasites in various stages of calcification.

1.2 Taxonomy of Anisakidae (Ascaridoidea)

DOMAIN: Eukaryota
KINGDOM: Metazoa
PHYLUM: Nematoda
CLASS: Secernentea
SUBCLASS: Ascaridida
ORDER: Ascaridoidea
FAMILY: Anisakidae

The superfamily of Ascaridoidea consists mainly of medium to large-sized nematodes with three lips sometimes separated by interlabia (Hartwick, 1974; Gibson, 1983). This superfamily is divided into five families between them the family Anisakidae is the major group found in mammals, birds, reptiles and fishes. Within Anisakidae, the subfamily Anisakinae Railliet & Henry, 1912, comprise the genera *Anisakis* Dujardin, 1845, *Pseudoterranova* Mozgovoi, 1950, *Contracaecum* Railliet & Henry, 1912, and *Phocascaris* Höst, 1932. The other subfamily Raphidascaridinae Hartwick, 1954 comprises, among others, the genus *Hysterothylacium* Ward & Magath, 1917 (Hartwick, 1974).

Nematodes of the genera *Anisakis, Contraceacum, Pseudoterronava* are composed of several complexes of sibling species that have systematic effect. In fact, anisakid nematodes tend to be very conserved in gross morphology and molecular techniques have shown that many presumed monospecific species consists of several cryptic species (Nascetti *et al.*, 1993; Paggi *et al.*, 1991; Orecchia *et al.*, 1994; Mattiucci *et al.*, 1997).

In literature, a lot of authors discussed difficulties in identifying larval anisakids, and the important characteristics used for generic distinction are the structure of the anterior part of the digestive tract and the position of the excretory pore.

1.2.1 *Hysterothylacium aduncum* (Rudolphi, 1802)

This parasite is one of the most common helminths in marine teleosts, throughout the world, at least in temperate and cold waters (Navone *et al.*, 1998).

The *H. aduncum* third-stage larvae possesses, as the adults, an anterior intestinal caecum and a posterior ventricular appendix. Lips are absent, but a boring tooth is present on the head. These characters are also present in the genera *Phocascaris* and *Contracaecum*: the excretory pore in *Hysterothylacium* is located at the level of the nerve ring, as it is just behind the boring tooth in the two other genera (Fig. 2A). The tail is ‘onion-shaped’, within it the “cactus-tail” of the fourth-stage larva is visible (Fig. 2B, C). The disposition of the future reproductive gonads and ducts are already visible as slender zig-zag structures, one in the male and two in the female. Thus, the future sex is easily identified (Berland, 1989).

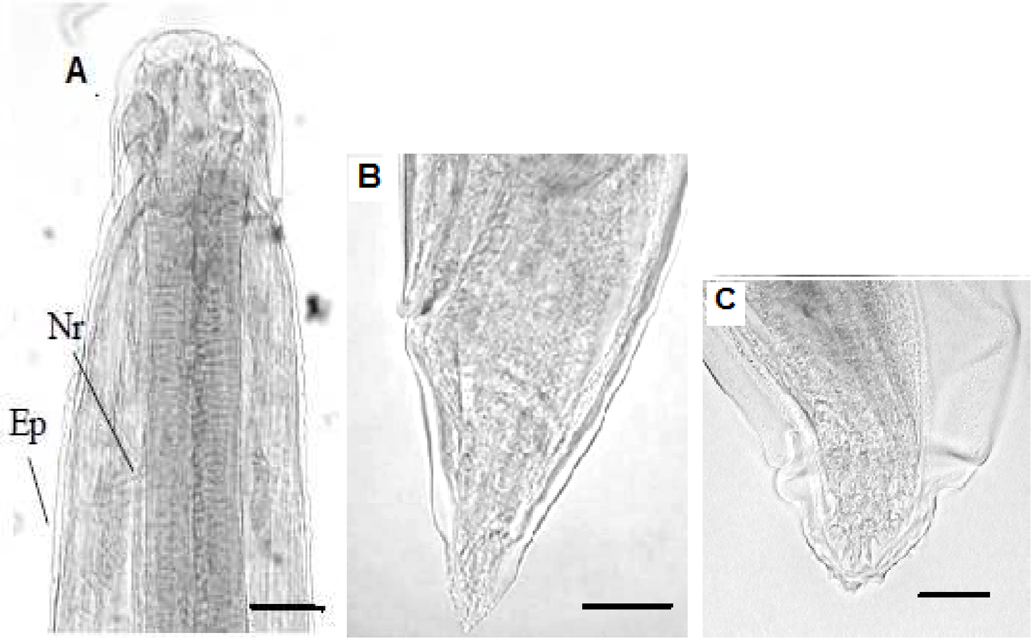


Fig. 2. Fourth-stage *Hysterothylacium aduncum* (A) Anterior part, excretory pore just behind the nerve ring, (B) posterior part, (C) posterior extremity.; Ep, excretory pore; Nr, nerve ring;. Scale bar: 0.1 mm, except 0.025 mm in (C). (After Shih *et al.*, 2002).

1.2.2 *Anisakis simplex* (Rudolphi, 1809)

The adults of *Anisakis* and *Pseudoterranova* have several morphological characters in common: denticulate lips, absence of interlabia, excretory pore on head, and three postanal denticulate plates in males. Both genera possess a well-developed oesophageal ventricle, but an intestinal caecum is only present in *Pseudoterranova*.

The encapsulated *Anisakis* larva forms a tight flat coil, a few mm in diameter. In microscopy, the important characters are the boring tooth (Fig. 3A, B), with the excretory pore just ventral to it, and the slender muscular oesophagus followed by the glandular ventricle. The ventricle- intestine junction is skew. The postanal tail is round, with a terminal mucron (Fig. 3C, D) and the cuticle shows fine transverse striae (Fig. 3E). There is no visible sign of reproductive organs, but the excretory organ is visible as a ventral ribbon behind the ventricle (Berland, 1989).

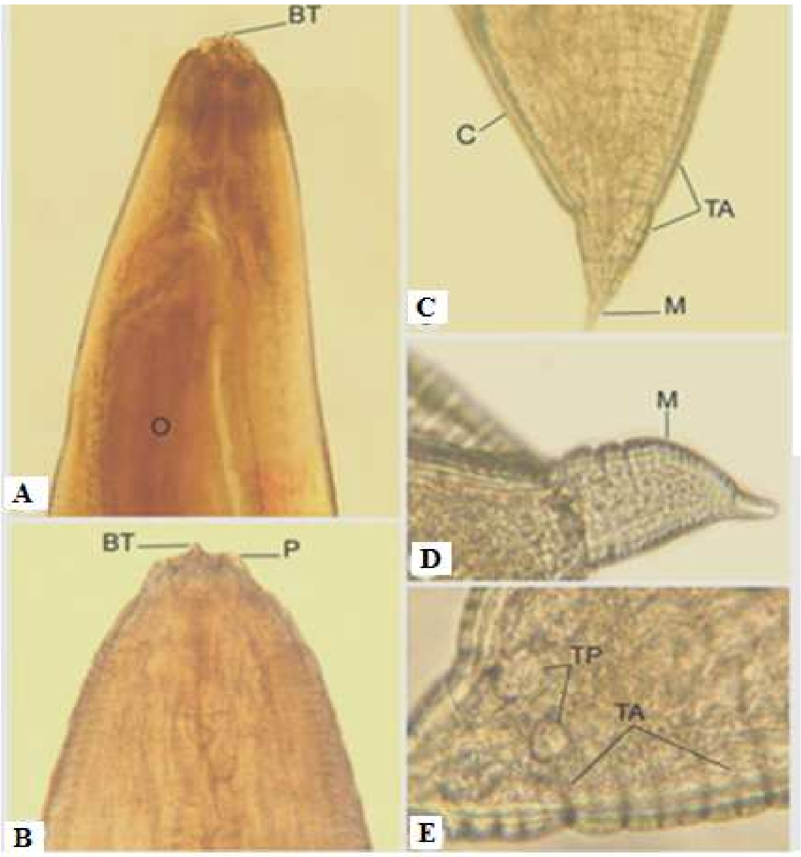


Fig. 3. Anisakid juveniles showing the typical nematode structure. **A,B** the anterior end with the boring tooth (BT), oesophagus (O) and papillae (P). **C** the posterior end showing macron (M), cuticle (C) with its transverse annulations (TA). **D** high magnificationf of mucron (M). **E** high magnification of the posterior end showing the transverse annulations of cuticle (TA) and the tail papillae (TP) (After Morsy 2012).

1.2.3 *Pseudoterranova decipiens* (Krabbe, 1878)

This species was previously known as *Porrocaecum decipiens* and *Terranova decipiens*. The head carries a very small, triangular boring tooth pointing forward (Fig. 4A, C), with the excretory pore just ventral to it. The oesophagus and ventricle are similar to those in *Anisakis*, but a latero-dorsal intestinal caecum runs forward along the ventricle (Fig. 4B, E). It may be necessary to roll or turn the cleared specimen under the coverslip to see this structure clearly. The postanal tail is short and conical with a tiny terminal spine (Fig. 4D). The cuticle on the entire body carries very fine striae; the larva appears to be almost smooth (Berland, 1989).

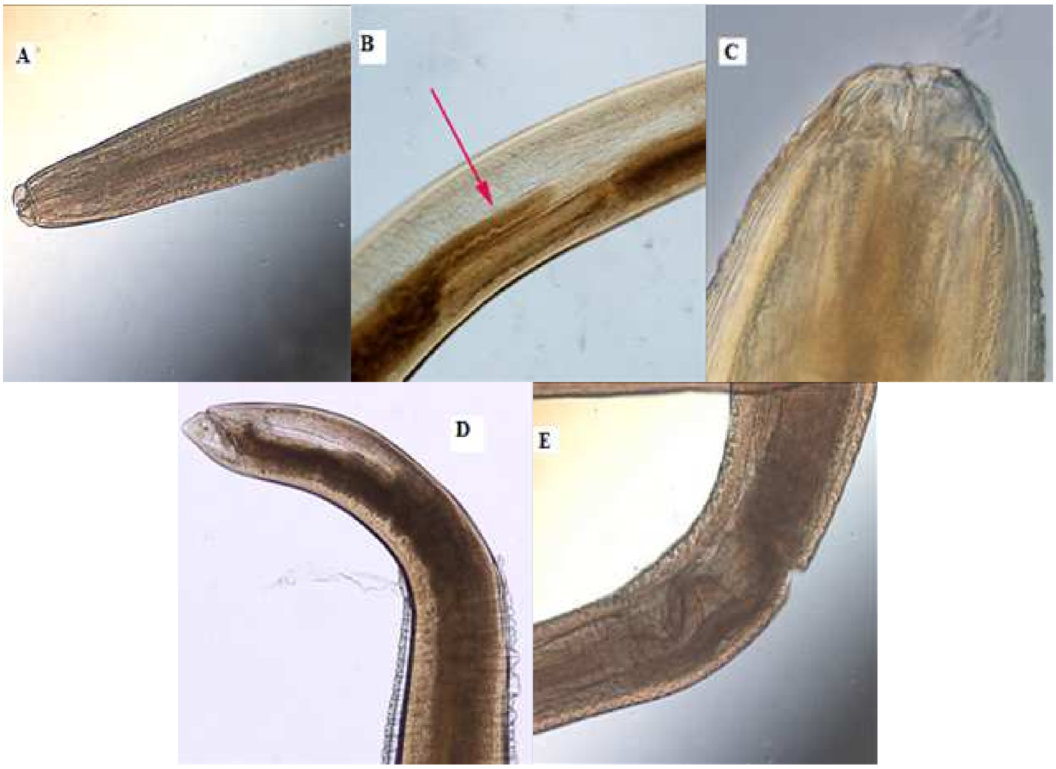


Fig. 4. *Pseudoterranova decipiens* larva**. A**. Anterior end. Image taken at 200x magnification **B**. Close-up of the intestinal caecum. **C**. Head, note small boring tooth. Image taken at 40x magnification **D**. Posterior end. Image taken at 200x magnification **E**. Mid- section, showing the oesophagus and intestine. Image taken at 40x magnification (After National Center for Health Statistics, Health Data Interactive,www.cdc.gov/nchs/hdi.htm. Accessed on [21/10/2014]).

1.2.4 *Contracaecum* (Railliet & Henry, 1912)

Being morphologically very similar, it is practically not possible to identify the third stage larvae of *Contracaecum* and the closely related genus *Phocascaris* (Fagerholm, 1989).

In both these genera, the presence of an intestinal caecum and a posterior appendix give the impression of opposed caeca, thus giving meaning to the name *Contracaecum*. By microscopy, the tapering head of this larva is seen to carry three lip bulges and a fairly prominent rounded boring tooth which is directed anteriorly (Fig. 5A).The nerve ring is located in the anterior portion of the oesophagus and the excretory pore at the base of the boring tooth.

The intestine caecum is slightly smaller than the oesophagus , is directed toward the front end and near the nerve ring (Fig 5B) . The ventricle is small and round with a short ventricular appendix (Fig. 5C). At the far end has a conical , not very long tail, two accessory glands , anus and mucrón (Fig. 5D) (Pardo *et al.*, 2008).

The cuticle in this larva shows transverse striae, which immediately behind the head are closely spaced while, posteriorly the distance between them increases gradually. However, on the anterior body the cuticle carries interrupted longitudinal ridges. These and the tranverse striae give the cuticle a tessellated appearance (Berland, 1989).

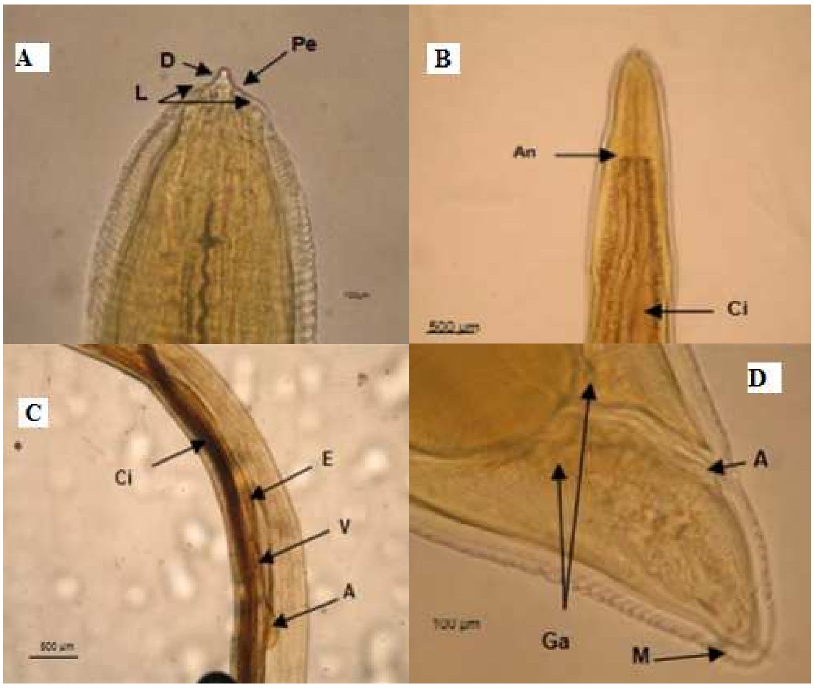


Fig. 5. *Contracaecum* larva. **A**. Anterior part with boring tooth (D), Excretory pore (Pe) and ventrolateral lips (L) note cuticular transverse striae and longitudinal interrupted ridges give the cuticle a tessellated appearance. **B.** Nerve ring (An), intestinal caecum (Ci). **C**.(10x) Digestive tube with intestinal caecum (Ci), oesophagus(E), Ventrículo, V; ventricular appendix (A). **D**. Tail with glans appended (Ga), anus(A) and mucrón (M). .Microfotografías Centro de Investigación Piscícola de la Universidad de Córdoba (CINPIC) (After Pardo, 2008).

1.3 Life cycle ecology of Anisakid Nematodes

The anisakid roundworms include a number of closely related genera within the phylum Nematoda, with a relatively complex life cycle involving a free-living stage and multiple hosts. Life cycle studies of these nematodes have been limited by difficulties in maintaining them alive in the laboratory, culturing sufficient numbers of parasite-free experimental hosts, and creating effective exposure (e.g. Køie & Fagerholm 1995; Køie *et al.,* 1995; Køie 2001; Klimpel *et al.*, 2004, 2008, 2010; Mattiucci & Nascetti 2008). Heteroxeny is the common life cycle pattern of marine ascaridoid nematodes such as *Anisakis*, *Contracaecum*, and *Pseudoterranova*. Transmission pathways are habitat-dependent and usually involve a broad spectrum of invertebrates and intermediate or paratenic fish hosts (McClelland 2005; Klimpel & Ruckert 2005; Palm & Klimpel 2007).

The life cycle of anisakid nematode includes four larval stages (L1–L4), within the eggs (L1–L3) and subsequently in the intermediate or paratenic hosts (L3), and as preadults (L4) and adults in the cetacean final hosts.

Adult *A. Simplex* has been reported in numerous species of cetaceans (*Balaenoptera* spp*., Berardius bairdi, Delphinapterus leucas, Delphinapterus delphis, Globicephalas cammoni, Kogio brevis, Lagenorhynchus spp., Megaptera novaengliae, Tursiops truncatus, Mesoplodon bidens, Monodonmonoceros, Orcinus orca, Phystercatodon, Phocoena phocoena, Phocaeno idesdalli, Pseudorca crassidens, Stenella caeruleoalba,*) and pinnipeds (*Arctocephalus australis, Eumetopias jubatus, Halichoerus grypus, Hydrurga leptonyx, Monachus monachus, Mirounga spp., Odobenus rosmarus, Otaria byronia, Pusa hispida, Zalophus californianus*) (Davey 1971).

The life cycle of *Anisakis simplex* is as follows (Fig. 6). Eggs leave the host in the faeces and develop at temperatures ranging from 2 to 27 °C. At 2 °C they hatch within 40 days. Hatched second-stage larvae are unsheathed in the cuticle of the first larva. Eggs and larvae are slightly heavier than sea water and sink gradually to the bottom. While sinking, they are dispersed by waves and currents. Larvae are eaten by various euphausiids, in which exsheathment occurs within 8 days.

Larger invertebrates (mainly copepods, euphausiids) and smaller fish are thought to be important second intermediate hosts, and various predatory fish species and cephalopods serve as paratenic hosts. During the crustacean’s ingestion by a fish or squid,the larvae migrate into the tissues of this second intermediate host and develop to the advanced third-stage on the viscera or in the muscle. Moulting to the third larval stage occurs before they reach 6 mm in length. Larvae longer than 18 mm can survive in fish and squid when ingested and penetrate through the wall of their digestive tract.

If small fishes are preyed upon by larger piscivorous fishes, the larvae are capable of reinfecting the latter without further moulting. Consequently, piscivorous hosts may accumulate enormous numbers of larvae (Lile 1998).

When an infected fish is eaten by a definitive host such as a marine mammal, the larvae are released into the stomach or intestine where they undergo further moults, developing into fourth-stage larvaeand eventually adults. In the Anisakidae life cycle, adult parasites immigrate to muscles (Grabda 1976).

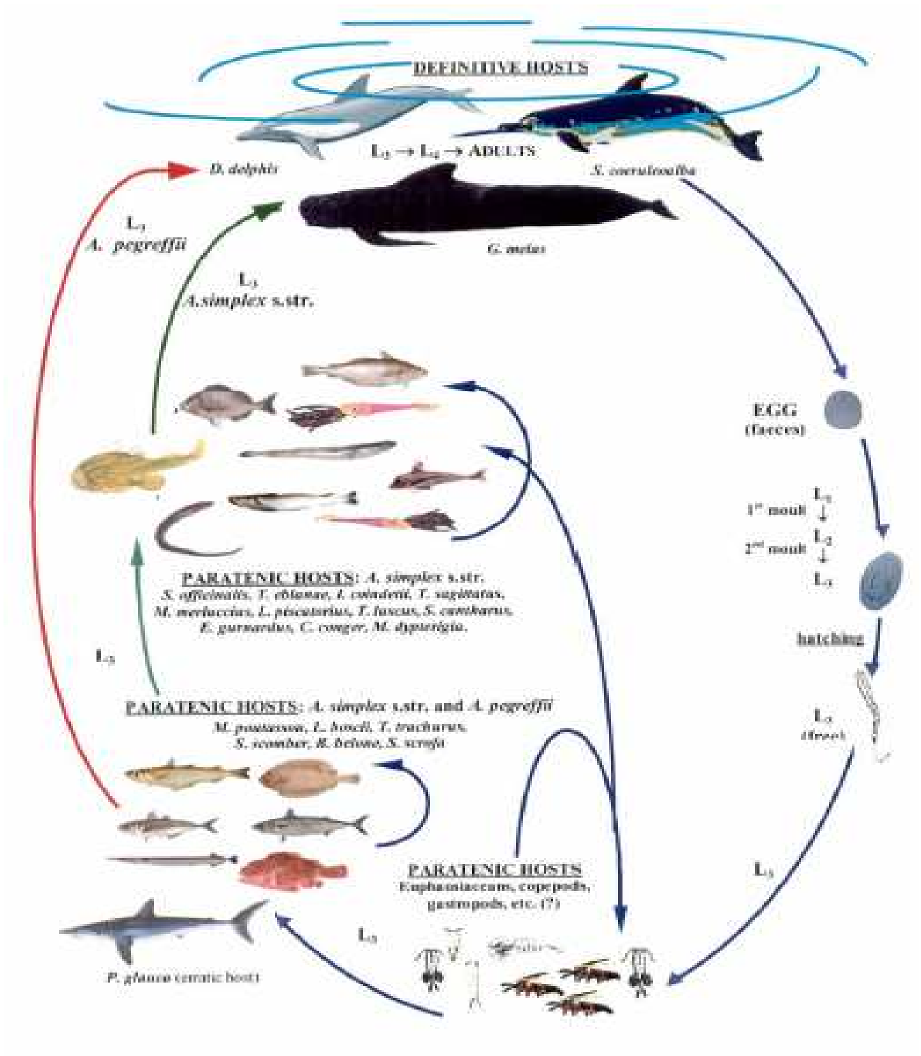


Fig. 6. Schematic life cycle of *Anisakis* species. The pelagic life cycle of Anisakis spp. follows the general nematode life cycle pattern, including four larval stages (L1–L4) and the adults in the cetacean final host (From Abollo, 1999)

Most information on the definitive hosts concerns the harbour porpoise, *Phocoena phocoena*, a small odontocete common in the Northern Hemisphere (Smith, 1989; Brattey & Stenson 1995; Herreras *et al.*, 1997). The data on the population structure of *A. simplex* in this cetacean are based on small sample sizes (Young 1972; Smith 1989) or lack details of the infrapopulation structure (Lick 1991; Brattey & Stenson 1995).

Humans, in the life cycle of this nematode, can only be considered accidental hosts in this life cycle, and have no influence on the transmission of these parasites. Painful infections of the digestive tract in humans originate through consumption of raw or semi-raw fisheries products, for example fish and squid (Klimpel *et al.*, 2011).

Consequently, the population ecology of anisakid nematodes has received more attention than that of any other parasite of marine mammals (Herreras *et al.*, 2004).

The larvae of two anisakid roundworms, *Anisakis simplex* (herring worm), and *Pseudoterranova decipiens* (seal worm), have been implicated in the majority of cases of human anisakiasis. The infective larval stage may be found on the viscera or in the flesh of a number of commercially important fish species, including salmon, cod, herring, and mackerel, as well as squid.

In contrast to the whale worms, the seal worms of the genus *Pseudoterranova* seem to be restricted to a benthic life cycle (Køie *et al.,* 1995; Palm 1999; McClelland 2002) (Fig. 7). Thin-shelled eggs, passed in the faeces of an infected seal, settle on the sea bed where they complete development to the third stage larvae (L3) and hatch. Newly hatched larvae are still ensheathed in the cuticle of the previous second larval stage (L2) and attach to the substrate caudally (Køie *et al.,* 1995; Anderson 2000; McClelland 2002, 2005). When readily predated by copepods including marine benthic harpacticoid and cyclopoids, they exsheath inside the first intermediate host and they penetrate into the haemocoel where they grow rapidly. Numerous amphipods become infected when they feed on copepods containing larvae. Polychaetes, mysids, isopods, cumaceans, decapods and gastropods also acquired larvae from ingesting copepods (McClelland, 1990). These hosts serve to enhance transmission to a larger array of benthic macro-invertebrates as second intermediate hosts, where the larval seal worms growing length (Anderson 2000, McClelland 2002, 2005). At this point they become infective to fish and also to seals. The invertebrate hosts are usually ingested by primary benthic teleosts, including juveniles of larger demersal fish species.

The larvae penetrate the gut wall of the fish and establish themselves in the internal organs or the musculature, where they continue to grow in length. Large, piscivorous fish may serve as second/third fish or paratenic hosts that accumulate the larval worms (Palm 1999; Anderson 2000; McClelland 2002, 2005). Following ingestion by the seal definitive host, infective third stage larvae (L3) escape from the bodies of the fish or invertebrate, embed their anterior part into the gastric mucosa, mature and reproduce.

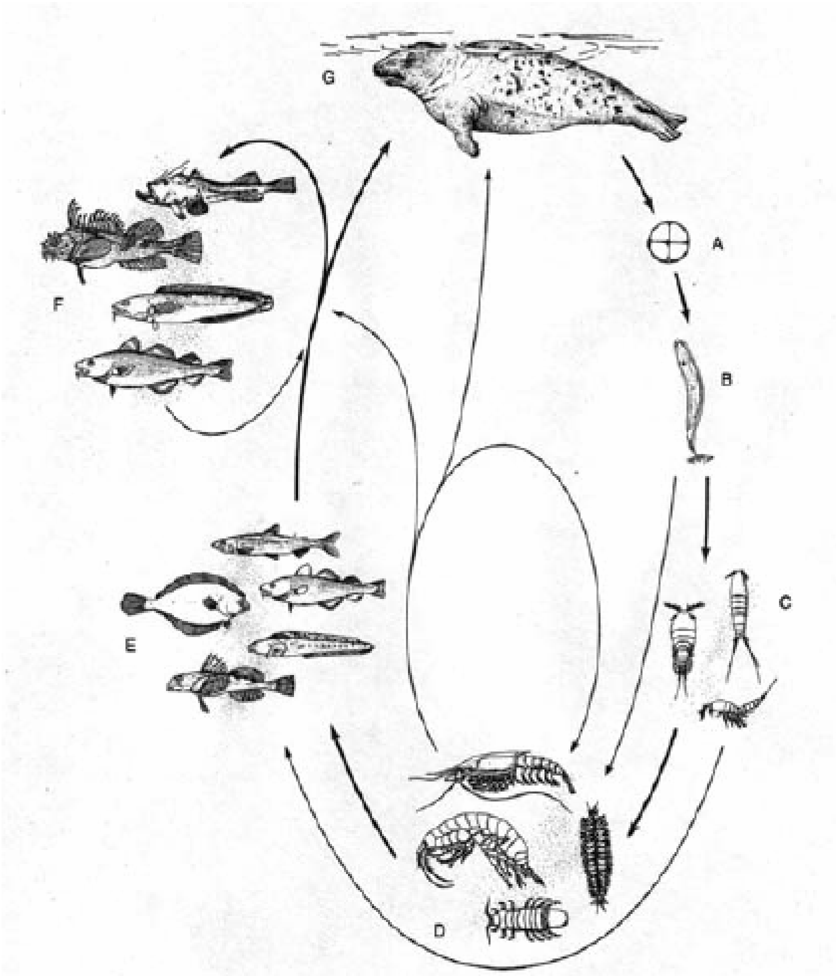


Fig. 7. Life cycle of *Pseudoterranova* species. A. Partly embrionated ova passed into sea water with seal faeces. B. Freshy-hatched, ensheathed second-stage (third-stage?) larva, adhered to substare by caudal extremity. C. Early second-stage larvae in haemocoels of benthic harparticoid and cyclopoid copepods. D. Early to late second-stage larva with possible moult (second moult) to third- (early third) stage larvae in haemocoels of benthic macroinvertebrates (mysids, amphipods, isopods and errant polychaetes). E. Early to late third-stage larvae in body cavities and musculature of benthophagous fish, e.g. (clockwise from top) smelt, juvenile cod, ocean pout, longhorn sculpin and American plaice. F. Late third-stage larvae in body cavities and musculature of demersal piscivorous fish including (top to bottom) monkfish, sea raven and mature cusk and cos. Third (moulting third- stage larva) and fourth (moulting fourth-stage larva) moults and development to adult in stomach of pinniped host. (After G. McClelland *et al.,* 1990) – courtsey Canadian Bulletin of Fisheries and Acquatic Sciences).

Nematodes of the genus *Contracaecum* seem to have equally complex life cycles involving benthic and pelagic invertebrates (Fig. 8) (e.g. crustaceans, squid) and fish (Klöser *et al.,* 1992; Køie & Fagerholm 1995; Køie *et al.*, 1995). The first two moults occur in the egg but, no moult occurs in the crustacean or fish intermediate hosts. The role played by various invertebrates in the natural transmission of *Contracaecum* spp. to fish intermediate hosts is not clear. Copepods are probably important as hosts which carry second- stage larvae to fish intermediate hosts (where development to the third stage occurs) or perhaps to certain invertebrates which consume them along with larvae.

Depending on the relative proportions between the sizes of the third-stage larvae and the potential fish host, a transfer through one fish or more is necessary before the larvae reach a sufficient size to be infective for the final host. The last two moults occur in the final host (Køie, 1995).

Larvae may be found in a great variety of fishes in places where the latter are exposed to the eggs passed in the faeces of pescivorous birds and mammals. It is evident that fish could serve as paratenic host as well. Infective larvae can probably pass from one fish intermediate host to another trough predation and reinvasion of tissues of new host, but without further development.

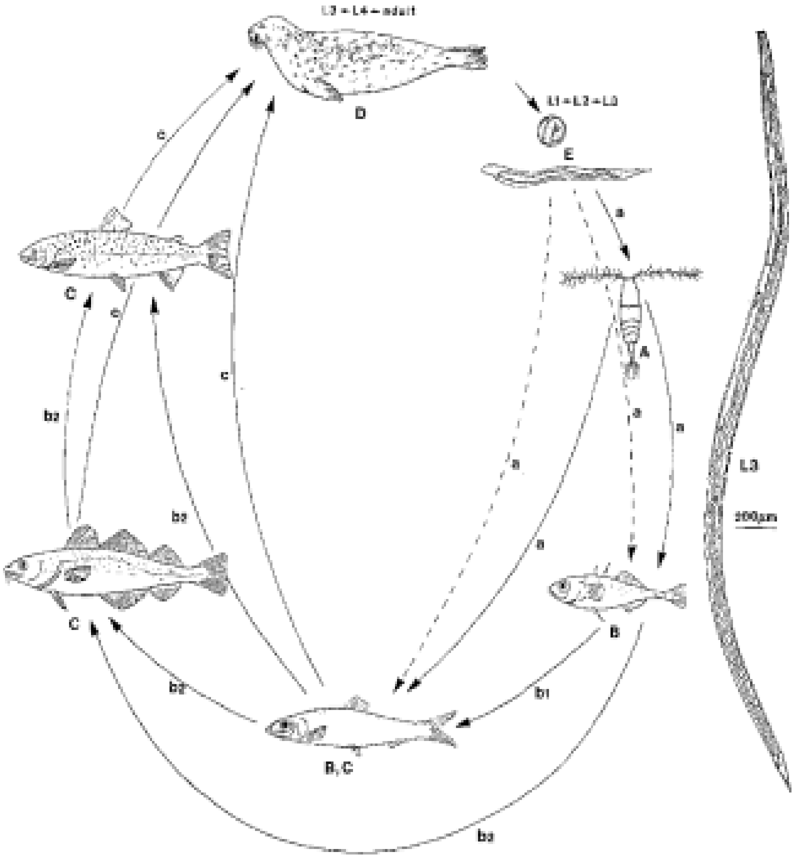


Fig. 8. Life cycle of *Contracaecum* species, is based in the present experimental infections and records of the occurrence of larvae in fish from the literature. Illustration of a third-stage larva from a plaice is shown to the *right.* (A Crustacean paratenic host; B first fish intermediate host; C only fish host or second [third] fish intermediate host; D final host, E free egg [diameter, 65-75 µm] with L1-L3 larvae and free, newly hatched L3 larva [ensheated with L2 cuticle]; an infection of paratenic hosts [A] and direct infection of fish hosts via ingestion of larvae [hypothetically] – third stage larva, >300 gm; *b* transfer of L3 larvae from one fish to another fish host: *bl* third-stage larva, >300 gin; *b2* third stage larva, >1.0 mm; *c* infection of final host – third stage larva, >4 mm) (After M. Køie 1995).

As others anisakid nematodes, the life cycle of *Hysterothylacium* species involves an invertebrate intermediate host.

The development and transmission of *Hysterothylacium*s pecies is incompletely known. Based on their own observations, Norris & Overstreet (1976) outlined the likely life cycle of the species as follows (Fig. 9). Eggs pass out in the faeces of the fish definitive host, embryonate to the second stage which hatches and retains the first- stage cuticle as a sheath. Second stage larvae hatch and are free living, until they are eaten by either an invertebrate or a fish. Within a suitable intermediate host, development proceeds to the third, and occasionally fourth stage, either of which is infective to the definitive host. Certain other invertebrates, apparently unsatisfactory as intermediate hosts, act as transport hosts in which no development occurs. The larvae remain infective for a true intermediate host. These 'transfer' hosts, often copepods, provide the necessary link for infection of plankton-feeders such as herrings and

anchovies. Fishes and invertebrates, after feeding on intermediate hosts, may serve as paratenic hosts, which maintain infective larvae without further development, and in which larvae typically occupy specific sites such as the mesentery, hepatopancreas, liver, or muscle, depending on the species of larva and host. Third or, occasionally, fourth stage larvae mature in the digestive tract of suitable definitive hosts.

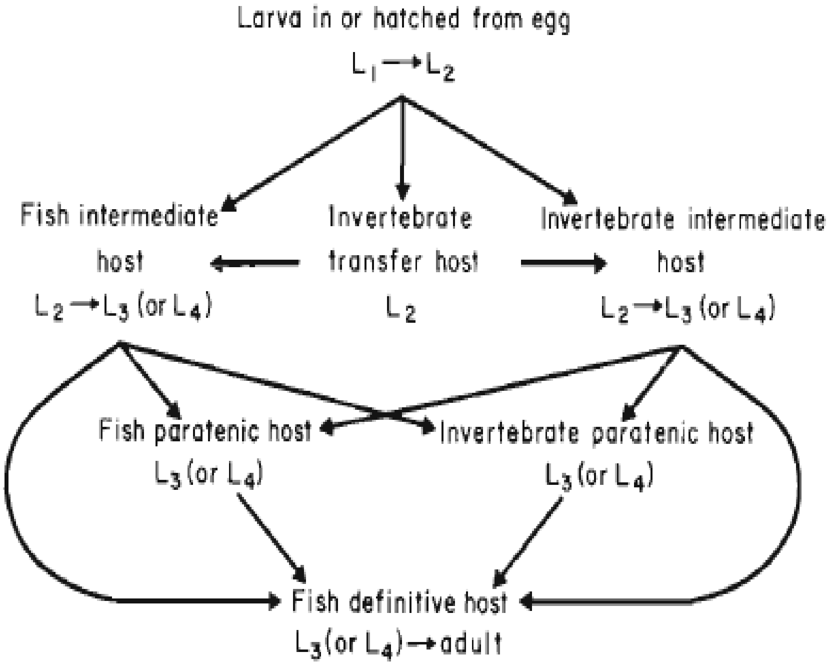


Fig. 9. *Hysterothylacium*. Diagrammatic life-cycle of species belonging to this genus. (After Overstreet, orig. reproduced by permission of Overstreet.)

1.4 *Phocoena phocoena* (Linnaeus, 1758)

1.4.1 Distinctive characteristics

Phocoena phocoena, also known as the "Common" or "Harbour" Porpoise, is a small cetacean, with a blunt, short-beaked head (Fig. 10).

Placed about midway along the back is a short, wide based, triangular dorsal fin, generally with small bumps (often called denticles or tubercles) on the leading edge. The flippers are small and somewhat rounded at the tips. The flukes have a concave trailing edge, and are divided by a prominent median notch; the tips are rounded. The straight mouth line slopes upward towards the eye. The colour pattern of the animal varies from individual to individual, but the most common coloration pattern is a dark dorsal surface that shifts to a lighter coloured hue ventrally. Although the dark colour is usually black or deep grey, albinos have been reported in which the dark segments are completely or partially white. The sides are intermediate, with the border area often splotched with various shades of grey. The flippers and lips are dark; there is a thin, dark grey gape-to-flipper stripe. While the colour pattern is variable, there does not seem to be any consistent difference between the sexes or among populations. Nineteen to 28 small, spatulate, blunt teeth line each tooth row. Adult females reach a mean body length of 160cm and males only 145cm. Mean mass is 60 kg and 50 kg, respectively (Bjorge & Tolley 2009).

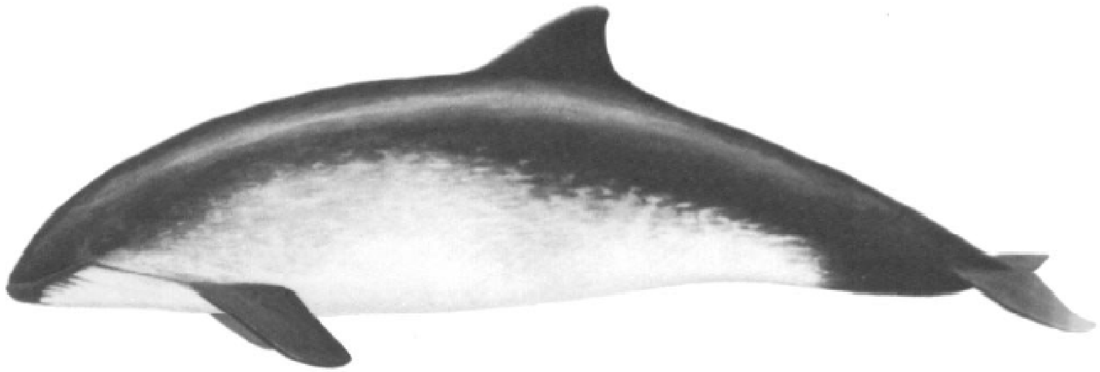


Fig. 10. *Phocoena phocoena* (After Encyclopedia of marine mammals (Perrin, W.F., Würsig, B., Thewissen, J.G.M.))

1.4.2 Geographic distribution

Harbour porpoises are found in cool temperate and subpolar waters of the Northern Hemisphere (Jefferson *et al.,* 1993) (Fig. 11). They are usually found in shallow waters, most often near shore, although they occasionally travel over deeper offshore waters. Their preferred habitats are characterized by a diversity of water depths, substrate types, and prey resources.

In the North Pacific, they range from central California and northern Honshu, Japan, to the southern Beaufort and Chukchi seas. In the North Atlantic, they are found from the south eastern United States to southern Baffin Island (they apparently do not enter Hudson Bay) in the west; and Senegal, West Africa, to Novaya Zemlya in the east. They also occur around southeast and western Greenland, Iceland, and the Faroe Islands. There is also a single stranding record from the Azores. There is a population (or possibly two) in the Black Sea and the Sea of Azov. The species is mostly absent from the Mediterranean, except for former, or sporadic, occurrences in the western part (Strait of Gibraltar, Islas Baleares, Barcelona, and Tunisia; Rice 1998). Major populations in the North Pacific and North Atlantic are isolated from each another, and many provisional stocks have been recognized.

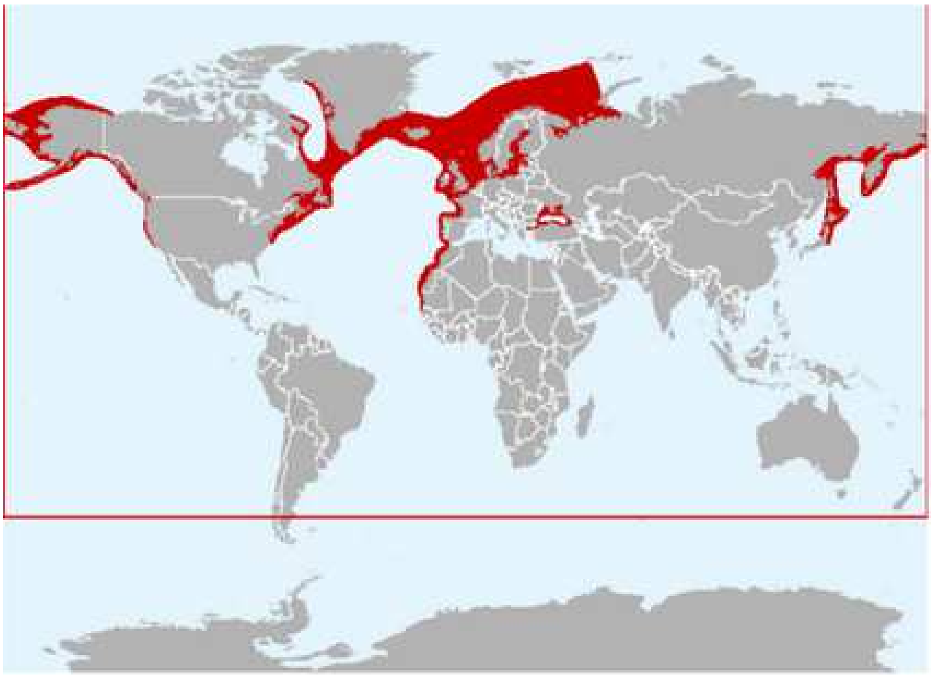


Fig. 11. Distribution of the four subspecies of *Phocoena phocoena:* cold temperate and subarctic waters of the Northern Hemisphere (Hammond *et al.*, 2008a; IUCN)

1.4.3 Diet

Harbour porpoises eat a wide variety of fish and cephalopods, and the main prey items appear to vary on regional and seasonal scales (Jefferson *et al.,*1993). In the North Atlantic, harbour porpoises feed primarily on clupeoids and gadoids, while in the North Pacific they prey largely on engraulids and scorpaenids. Squids and benthic invertebrates have also been recorded. Many prey items are probably taken on, or very close to, the sea bed. Even though a wide range of species has been recorded in the diet, porpoises in any one area tend to feed primarily on two to four main species (e.g. whiting *Merlangius merlangus* and sandeels (Ammodytidae) in Scottish waters). The literature on porpoise diets in the northeast Atlantic suggests that there has been a long term shift from predation on clupeid fish (mainly herring *Clupea harengus*) to predation on sandeels and gadoid fish, possibly related to the decline in herring stocks since the mid-1960s. Food consumption brings porpoises into contact with two important threats: persistent organic contaminants and fishing nets, both of which have potentially serious impacts (Santos *et al.*, 2004).

In the Kattegat and Skagerrak stomach contents of juvenile and adult harbour porpoises contained mostly Atlantic herring (*Clupea harengus*) while Atlantic hagfish (*Myxine glutinosa*) was also important for adults (Boerjesson *et al.*, 2003). In another study on animals stranded and by-caught in Denmark, cod (Gadidae), viviparous blenny (Zoarcidae) and whiting (Gadidae) made up most of the stomach contents while in the Netherlands whiting was the main prey, making up around 34 % of the total reconstructed prey weight (Santos *et al.*, 2005).

1.5 *Lagenorhynchus acutus* (Gray, 1828)

1.5.1 Distinctive characteristics

Atlantic white-sided dolphins are robust and , with a maximum girth of up to 60% of total length. The tail stock is laterally compressed into vertical keels and the beak is short (Jefferson *et al.,* 2008). These dolphins are impressively patterned and more colourful than most dolphins. Below the black or very dark grey back and dorsal fin a narrow, bright white patch on the side extends back from below the dorsal fin, overlaying a yellow blaze above a thin dark stripe running towards the flukes (Fig. 12). The belly and lower jaw are white, and the sides of the body are light grey. A black eye ring extends in a thin line to the upper jaw and a very thin stripe extends backward from the eye ring to the external ear. A faint grey stripe may connect the leading edge of the flipper with the rear margin of the lower jaw.

Male Atlantic white sided dolphins reach 270 cm and 230 kg, whereas adult females are about 20 cm shorter and 50 kg lighter (Cipriano 2002).

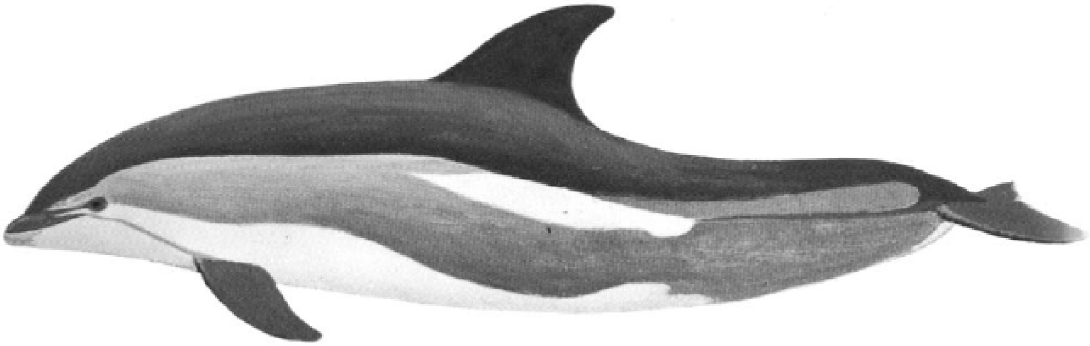


Fig. 12. *Lagenorhynchus acutus (*After Encyclopedia of marine mammals (Perrin WF, Würsig B, Thewissen JGM))

1.5.2 Geographic distribution

L. acutus is a deepwater species which ranges across the North Atlantic, from Cape Cod in the western North Atlantic to southern Greenland, across the Barents Sea to Svalbard and from there south to the North and Irish Seas as far south as Brittany (France) (Fig. 13) (Reeves *et al.*, 1999; Cipriano 2009). The species rarely enters the Baltic sea (Kinze *et al.*, 1997). It has been seen as far south as Strait of Gibraltar (Hammond *et al.*, 2008).

Fig. 13. Distribution of *Lagenorhynchus acutus*: cool, temperate and subarctic waters of the northern North Atlantic (Hammond *et al.*, 2008; © IUCN)

1.5.3 Diet

Atlantic white-sided dolphins feed on small schooling fish and squid. These include herring (*Clupea harengus*), small mackerel (*Scomber scombrus*), silvery pout (*Gadiculus argenteus*), blue whiting (*Micromesistius poutassou*), American sand lance (*Ammodytes americanus*), smelt (*Osmerus mordax*), silver hake (*Merluccius bilinearis*) and short-finned squid (*Illexiile cebrosus*) (Jefferson *et al.*, 1993; Reeves *et al.*, 1999). In the North Sea, oceanic cephalopods seem to be their main diet (Das *et al.*, 2001). Different prey species may predominate at different times of year, representing seasonal movements of prey, or in different areas, indicating prey and habitat variability in the environment (Cipriano 2002). For instance off the coast of New England, pelagic Atlantic herring (*Clupea harengus*) was the most important prey in summer but was rare in winter. (Craddock *et al.*, 2009). Atlantic white-sided dolphins apparently co-operate in their efforts to contain and attack schools of fish, a behaviour which is similar to that described for dusky dolphins off Argentina (Reeves *et al.*, 1999).

1.6 *Lagenorhynchus albirostris* (Gray, 1846)

1.6.1 Distinctive characteristics

The white-beaked dolphin has a robust appearance. The dorsal fin is in the middle of the back, erect and strongly curved. Adults grow between to 2.4 and 3.1 m long and may weigh between 180 and 350 kg. Males usually grow larger than females. The coloration is typically black on the back, with a white saddle behind the dorsal fin and whitish bands on the flanks that vary in intensity from a shining white to ashy grey (Fig. 14). Belly and beak are normally white, but the beak may be ashy grey or even darker; it may appear that a white beak is missing. The beak is only 5-8 cm long (Kinze 2009).

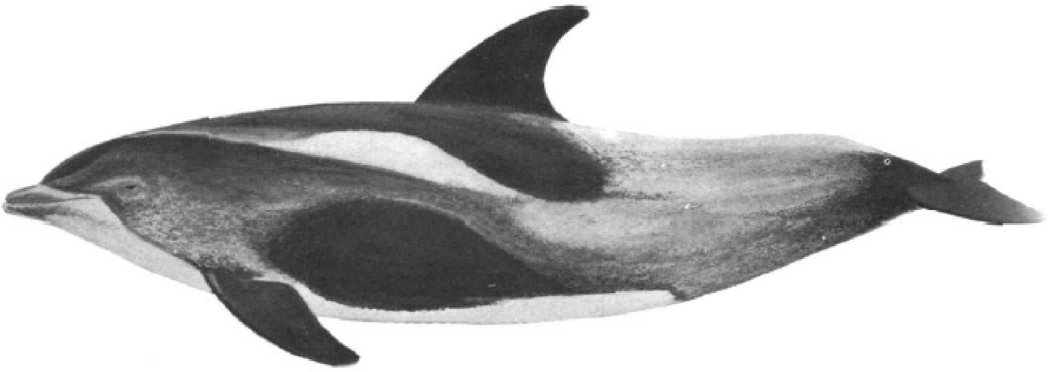


Fig. 14. *Lagenorhynchus albirostris* (After Encyclopedia of marine mammals (Perrin WF, Würsig B, Thewissen JGM))

1.6.2 Geographic distribution

This is the most northerly member of the genus *Lagenorhynchus* and has a wide distribution. The species is found in the immediate offshore waters of the North Atlantic, off the American coast from Cape Chidley, Labrador, to Cape Cod, Massachusetts; the Southwest coast of Greenland north to Godthab; off the European coast from Nordkapp in Norway south through the North Sea to the British Isles, Belgium, the Netherlands and Denmark (Fig.15).

L. albirostris is vagrant to France, the north coast of Spain, the Strait of Gibraltar, and the Mediterranean Sea (Rice 1998). It is only seen occasionally in inner Danish waters (Reeves *et al.*, 1999) and the Baltic proper (Kinze 2002).

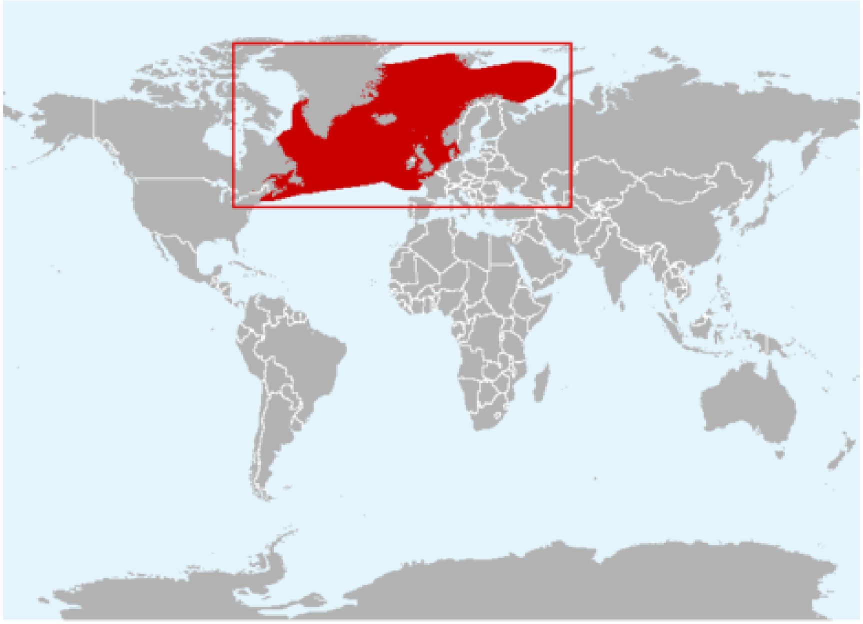


Fig. 15. Distribution of *Lagenorhynchus albirostris*: cool temperate and subarctic waters of the North Atlantic (Hammond *et al.*, 2008; © IUCN)

1.6.3 Diet

In all areas where stomach contents have been examined, clupeids (e.g. herring), gadids, e.g. Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), poor-cod (*Trisopterus minutus, T. luscus*), whiting (*Merlangius merlangus*), capelin (*Mallotus villosus*) and hake (*Merluccius merluccius*) have been found to be the principal prey of white-beaked dolphins. Others consumed include Scomber, Pleuronectes, Limanda, Eleginus and Hyperoplus as well as squid, octopus and benthic crustaceans (Reeves *et al.*, 1999).

2 AIMS OF THE STUDY

Knowledge about parasitism in cetaceans around Scotland is scarce. Studies of parasite assemblages produce valuable information about various aspects of marine mammals. Thus, in the present study special attention was paid to describe parasite distribution recovered by stomach contents of three species of cetaceans in Scottish waters.

We also characterized their diet in order to assess potential helminth transmission routes. Diet composition will influence cetacean parasite fauna, since feeding is the normal route by which many parasites reach marine mammals and as fish or cephalopod species, occurring in temperate and arctic waters, play different roles as intermediate hosts, (Klimpel *et al.,* 2004; Lehnert *et al.,* 2010) implies changes in the parasite frequency distribution among the host species.

Therefore the aims of this study are :

1. Examine nematode fauna morphology and distribution in the three species of odontocetes, originating from different geographical locations, along Scottish coastline;

2. Investigate the suspected relationship between the abundance of the four different parasite genera and the diet composition of their host.

3 MATERIAL AND METHODS

3.1 Parasitological analysis

3.1.1 Sampling

Scottish Agriculture College Veterinary Services Division, under a contract with the UK Department of Environment, Transport and the Regions, has coordinated and investigated marine mammals stranding in Scotland since 1 January 1992 (Santos *et al.*, 2001). In the present study stomach contents of 42 harbour porpoises (*Phocoena phocoena)*, 6 Atlantic white-sided dolphins (*Lagenorhynchus acutus*) and 8 white-beaked dolphins (*Lagenorhynchus albirostris*) stranded, due to various causes, all around the Scottish coast were collected between 2004 and 2014 (Fig. 16).

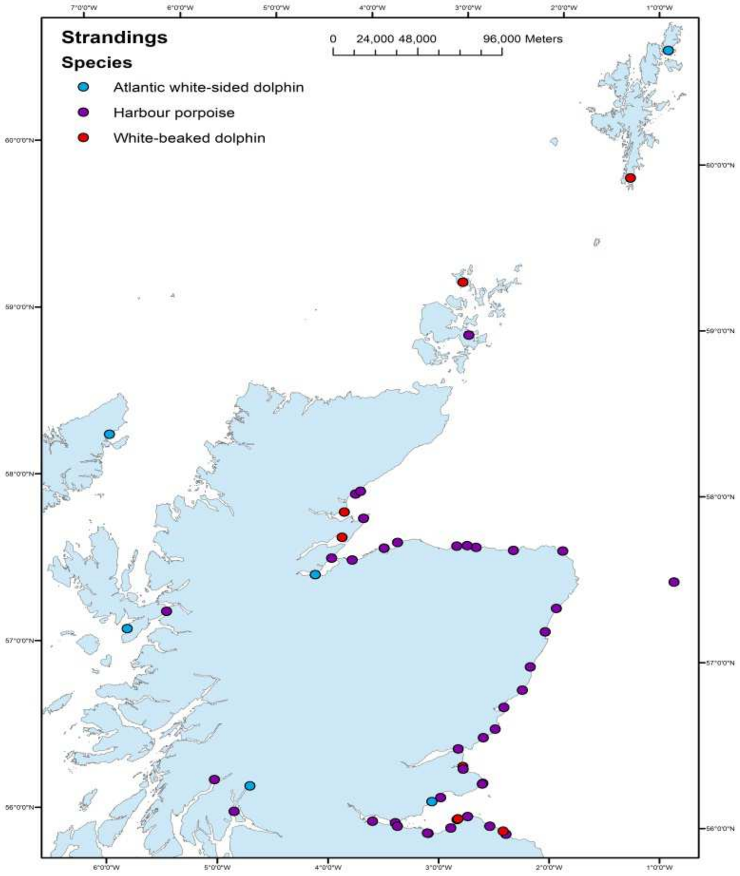


Fig. 16. Map of the study area, showing locations of strandings of 42 harbour porpoises, 6 Atlantic white-sided dolphins and 8 white-beaked dolphins from which stomach contents were obtained, between 2004-2014.

Data were collected off the coast of Scotland between 55 and 60 °N and between 1 and 5° W. Stranding locations were mapped using ArcGIS.

In Table 1 are showed the stranding locations of the three species, object of the present study, with the exception of the locations of two individuals of harbour porpoises and white-beaked dolphin, which are unknown.

Table 1. Details of stranding locations of the 42 harbour porpoises, 6 Atlantic white-sided dolphins and 8 white-beaked dolphins object of study

M reference SAC	Species	Date found	Longitude	Latitude	Region
M 008/04	*P. Phocoena*	22/01/2004	58.9616928	-2.88146186	Orkney
M 027/04	*P. Phocoena*	06/02/2004	57.2243805	-5.63613415	Highland
M 048/04	*P. Phocoena*	09/03/2004	55.9890289	-2.89921165	Lothian
M 101/04	*P.Phocoena*	14/05/2004	57.5858612	-3.91680408	Highland
M 127/04	*P.Phocoena*	23/06/2004	55.5914955	-5.15169621	Strathclyde
M 206/04	*P. Phocoena*	10/10/2004	57.6886024	-2.92917061	Grampian
M 055/05	*P.Phocoena*	12/03/2005	57.9838486	-3.91771436	Highland
M 123/05	*L. albirostris*	28/05/2005	56.3607559	-2.80597711	Fife
M 130/05	*P. Phocoena*	04/06/2005	57.4863892	-0.86239058	Grampian
M 203/05	*L. acutus*	07/08/2005	58.2664604	-6.32428741	Western Isles
M 303/05	*P. Phocoena*	29/11/2005	57.6835403	-2.7445569	Grampian
M305/05	*L. acutus*	02/12/2005	59.2790794	-2.95808935	Orkney
M 306 /05	*P. Phocoena*	06/12/2005	56.0406342	2.84423184	Lothian
M 037/06	*P. Phocoena*	19/02/2006	57.8387184	-3.82913613	Highland
M 038/06	*P. Phocoena*	20/02/2006	56.9687271	-2.2055583	Grampian
M 053/06	*P. Phocoena*	15/03/2006	56.8284378	-2.27524281	Grampian
M 090/06	*P. Phocoena*	06/04/2006	57.670433	-1.91949713	Grampian
M 094/06	*P. Phocoena*	11/04/2006	55.9584312	-2.39558363	Lothian
M 117/06	*P. Phocoena*	01/05/2006	56.7219505	-2.44277453	Tayside
M 126/06	*P.Phocoena*	07/05/2006	56.3455009	-2.80242419	Tayside
M 139/06	*P.Phocoena*	17/05/2006	57.5926094	-4.11292458	Highland
M 025/07	*P. Phocoena*	04/02/2007	56.0112648	-3.40498495	Fife
M 057/07	*L. acutus*	08/03/2007	56.1448288	-3.07990193	Fife
M 171 /07	*L. acutus*	26/08/2007	57.1066208	-5.98975945	Highland
M 222/07	*L. acutus*	19/11/2007	57.4894142	-4.2604785	Highland
M 049/08	*P. Phocoena*	05/03/2008	55.9532318	-3.10337925	Lothian
M 119/08	*P. Phocoena*	25/05/2008	56.2280922	-5.0763135	Strathclyde
M 147/08	*P. Phocoena*	08/07/2008	57.6928825	-2.83029628	Grampian
M 001 /09	*P. Phocoena*	05/01/2009	57.1817741	-2.07274103	Grampian
M 013 /09	*L. albirostris*	24/02/2009	55.9763107	-2.42140388	Lothian
M 028/09	*P. Phocoena*	17/03/2009	56.0601234	-2.74830747	Lothian
M 035/09	*P. Phocoena*	21/03/2009	55.990818	-3.38499808	Lothian
M 044/09	*P. Phocoena*	27/04/2009	56.4664497	-2.8536613	Lothian

M 054/09	*P. Phocoena*	30/04/2009	56.1706543	-3.00008988	Tayside
M162/09A	*L. acutus*	03/11/2009	60.6905746	-0.90675461	Fife
M 192/09	*L. albirostris*	31/12/2009	57.8727112	-4.01635551	Shetland
M 006/10	*P. Phocoena*	11/01/2010	55.9549675	-3.10983396	Highland
M 009/10	*P. Phocoena*	12/01/2010	56.262104	-2.61825848	Lothian
M 010/10	*L. albirostris*	13/01/2010	59.9218636	-1.29345989	Fife
M 036/10	*P. Phocoena*	20/02/2010	57.670433	-1.91949713	Shetland
M 114/10	*L. albirostris*	19/06/2010	56.045177	-2.83483600	Grampian
M 286/10	*P. Phocoena*	21/11/2010	56.0045891	-2.543573	Lothian
M 301/10	*P. Phocoena*	09/12/2010	57.6636887	-3.6191587	Lothian
M 308/10	*P. Phocoena*	15/12/2010	56.0432243	-4.8768796	Grampian
M 051/11	*P. Phocoena*	07/03/2011	56.2584877	-2.6230323	Strathclyde
M 321/11	*P. Phocoena*	15/11/2011	57.3237152	-1.9717294	Fife
M 005/12	*L. acutus*	05/01/2012	56.2007637	-4.7483906	Grampian
M 034 /12	*P. Phocoena*	02/02/2012	56.5369873	-2.6259841	Strathclyde
M 036 /12	*L. albirostris*	04/02/2012	57.7198486	-4.0246295	Tayside
M 037/12	*P. Phocoena*	05/02/2012	56.5904922	-2.5177693	Highland
M 062/12B	*P. Phocoena*	28/02/2012	56.0167961	-3.61373878	Tayside
M 107/12	*P. Phocoena*	13/04/2012	57.7002487	-3.4949324	Central
M 388/12	*P. Phocoena*	28/11/2012	57.669857	-2.3888816	Grampian
M 413/12	*P. Phocoena*	26/12/2012	58.0016136	-3.8712888	Highland
M 040/14	*P. Phocoena*	17/02/2014	NA	NA	NA
M 070/14	*L. albirostris*	22/03/2014	NA	NA	NA

Use of stranded animals for dietary analysis has evident drawbacks, e.g., concerning the representativeness of the sample. For a large-scale survey, at the same time the use of stranded animals has several advantages over taking biopsies from living animals in the wild. Sampling from dead animals is less expensive, raises no ethical issues, and provides access to all tissues, not simply blubber, as well as a wealth of ancillary information on size, age, reproductive status, condition and pathology (Pierce *et al.*, 2008).

Animals sampled, ranged in decomposition state from extremely fresh (point 2a on the ECS scale) to moderately decomposed (point 3). Animals were necropsied following the ECS protocol of Kuiken & García Hartmann (1993). Basic data collected from each animal included stranding location (latitude and longitude), date (month and year fraction), species, sex, total length. Body length ranged from 99 to 258 cm (mean ± SD: 156.3 ± 52.5).

Following post-mortem examination, the complete digestive tract was removed and all stomach compartments (i.e. fore stomach, main stomach, pyloric stomach) were examined separately and the contents removed.

Total recovery of parasites was attempted by the vets at Culloden Veterinary Clinic in Inverness. Later on the samples were sent to Oceanlab in Newburgh where we separated parasites from food items using a series of sieves of different mesh size. Nematodes are very sensitive and perishable and to keep them in good condition, they were collected in a small vial or tube and preserved in 70 % ethanol for reason of safety, although formalin would have been more suitable for preventing samples from deteriorating until the identification. In this way nematodes maintained their structure, though they became discoloured after some time. The vials have been labelled with a code/number which was recorded on an Excel sheet with all the details of the sample. After collection, samples were packed in strong cardboard boxes and placed in a shaded area where conditions were cool.

3.1.2 Sample analysis

For diagnostic morphological studies, the nematodes were cleared in lactophenol under fume hood, due to its dangerous effect on human health. To look at nematodes closely for identification, nematodes has been picked from the sample using a tweezers and placed with the help of a mounted needle on a glass block or a Petri dish with two or three drops of formalin or ethanol to keep the nematode from drying out. Finally, with some training, it was possible to tentatively identify the worm(s) on the glass bock, under a compound stereomicroscope by examining the following morphological characters: anatomy of the oesophago-intestinal region, lip morphology, opening of the excretory pore, tail morphology (see Fig. 17); except one individual of harbour porpoise and one of white-beaked dolphin, for which the identification was unpratical cause of the high state of parasite .

Parasite loads in the stomach, in terms of parasite numbers and total dry weight, were estimated for all the three species of odontocetes. Because of the high volume of nematodes present in some samples, it was not practical to count the total number of worms. Thus, the number of anisakid nematodes from each host was estimated from subsamples of the previously weighed worm sample previously weighted. Using a digital scale and a tally counter, the weight (w) and the number of parasites present in each of four subsamples (n) were assessed.

The choice of evaluating four sub-samples rather than a big one has been made with the aim to obtain representative values.

The total number of parasites (N) from each sample was estimated by applying the following equation to each subsample and then taking an average:

$$N = (W/w)*n$$

Where:
W = weight of the parasites in the sample
w = weight of the parasites in each subsample
n = number of parasites in each subsample

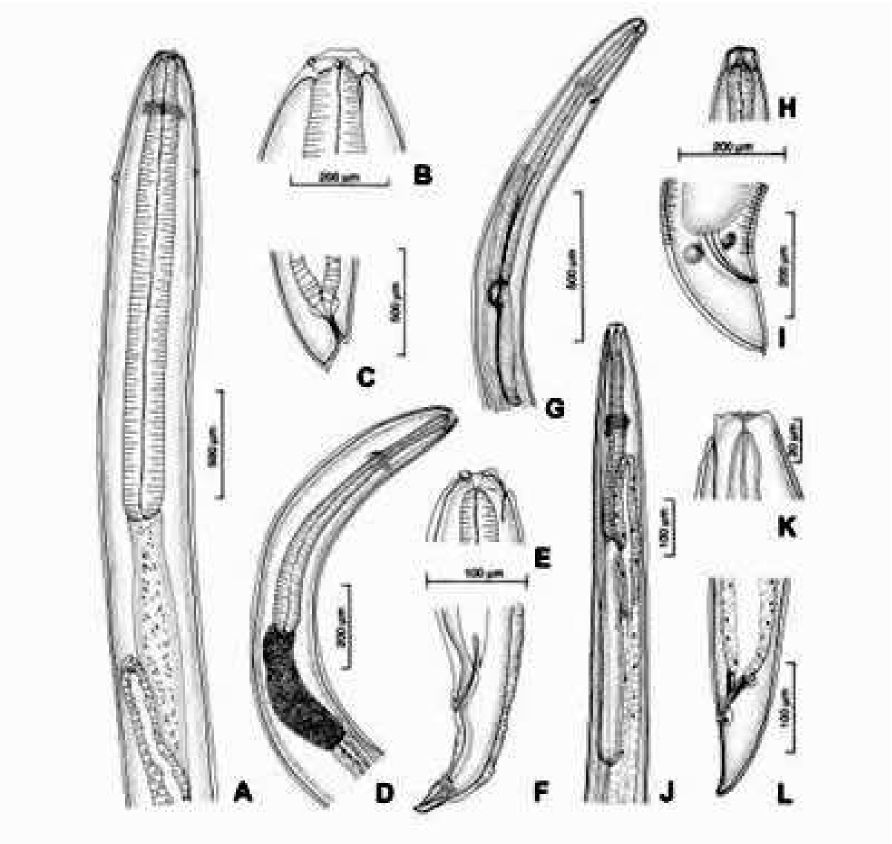


Fig. 17. Anatomy of the oesophago-intestinal region, lip morphology, opening of the excretory pore, tail morphology of **A-C** *Pseudoterranova* spp; **D-F** *Anisakis* spp.; **G-I** *Hysterothylacium aduncum*; **J-L** *Contracaeum* spp.(From Anderson, 2000).

An high variability in sample sizes occurred and some samples included more than 10000 of individuals, while in someone less than 10 individuals were found. This variation in worm numbers per stomach could be real because of inherent natural variability. But at the same time it is not impossible that the use of the parasites number as variable is not free of potential sampling biases that can occur any time samples are not random samples and some individuals are more likely than others to be chosen. A preliminary assessment of the distribution of each parasite genera, among the three stranded hosts, was expressed in terms of occurrence.

About abundance and intensity (of infection), as defined by Bush *et al.,* (1997), they weren't calculated for each host species as, since due to the wide variation in samples size, it wasn't practical assess the number of individuals of a particular parasites species in every single infected host.

3.1.3 Modelling approach

All statistical analyses were carried out using Brodgar 2.7.4 software (www.brodgar.com, Highland Statistics Ltd.).

The Atlantic white-sided dolphin, white-beaked dolphin and harbour porpoise data were analysed separately. The principal focus of these analyses was to examine the relationships between the variation in the burden of separate parasite genera and the set of potential explanatory variables. Six response variables (number and blotted weight of all parasites, presence/absence of *Anisakis,* presence/absence of *Pseudoterranova,* presence/absence of *Hysterothylacium,* presence/absence of *Contracaecum*) were used to predict parasite burden. The explanatory variables were seven, of which five were continuous (longitude, latitude, body length, month, year fraction) and two were nominal (sex, species code) (Table 2).

Table 2. List of avaiable explanatory variables

Explanatory variables	Remarks
Body length	Continuous variable . Medium body length is 156.3 cm
Month	Continuous variable*
Year fraction	Continuous variable with values between 0 (1 January) and 1(31 December)
Longitude	Continuous variable
Latitude	Continuous variable
Sex	Nominal variable with values 0 (female) and 1(male)
Species code	Nominal variable with values 1 (harbour porpoises), 2 harbour porpoises and 3 (Atlantic white-sided dolphins)

* the variable month is nominal, but in the present study we treated it as continuous

An initial graphical data exploration was conducted to ascertain the spread and distribution of the data, to identify outliers and to examine the relationships between variables (Zuur *et al.,* 2010). Boxplots were generated for both the explanatory and response variables to identify any extreme values and to determinate whether the data required transformation. The boxplots indicate a few extreme values, for example, number of parasites (Fig. 18) and length (Fig. 19), although these values were not errors.

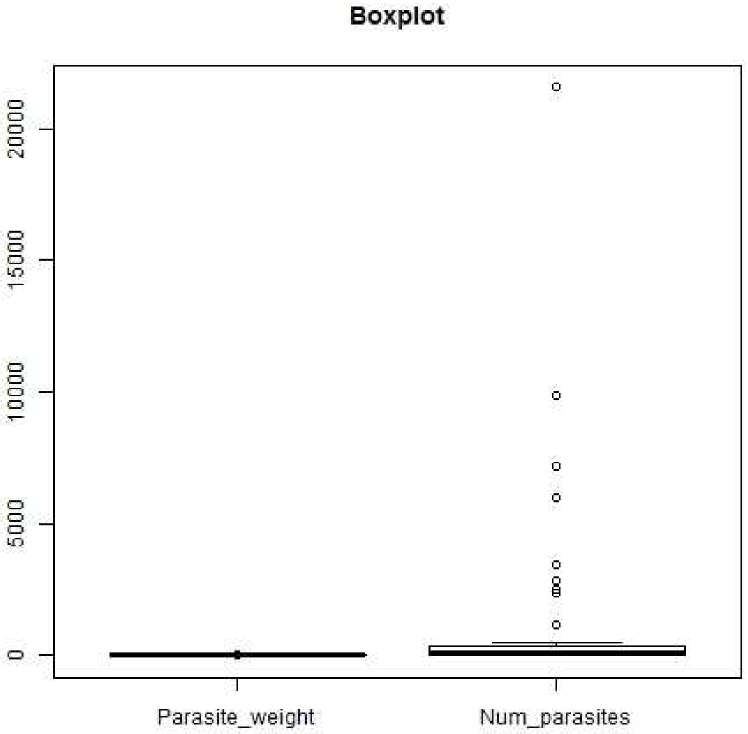


Fig 18. Boxplot of blotted weight and number of parasites from the stomach of stranded hosts

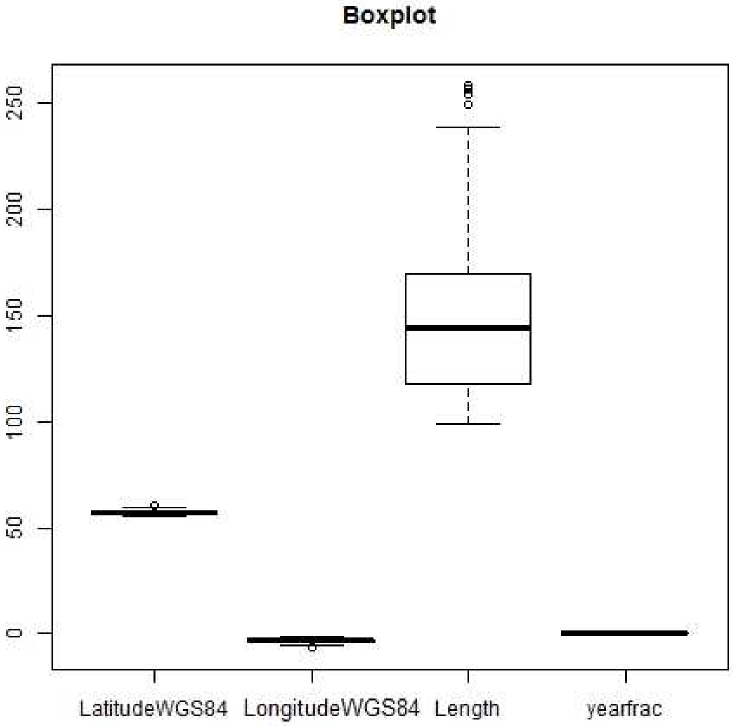


Fig 19. Boxplots of four (Latitude, Longitude, Length and Year fraction) of the six explanatory variables

The data exploration applied on the two response variables indicates that a transformation on the number of parasites is required and that a log (Y+1) transformation was needed to improve the linear relationship between the variables.

A pairplot (Fig. 20) for the continuous explanatory variables gives no immediate indications of collinearity between the explanatory variables.

The first row shows the relationship between the parasite number and each of the continuous explanatory variables. The scatter of points indicates that the parasite number and length are correlated and that it is correlated to longitude as well. The cross-correlation between the number of parasite and length is 0.49.

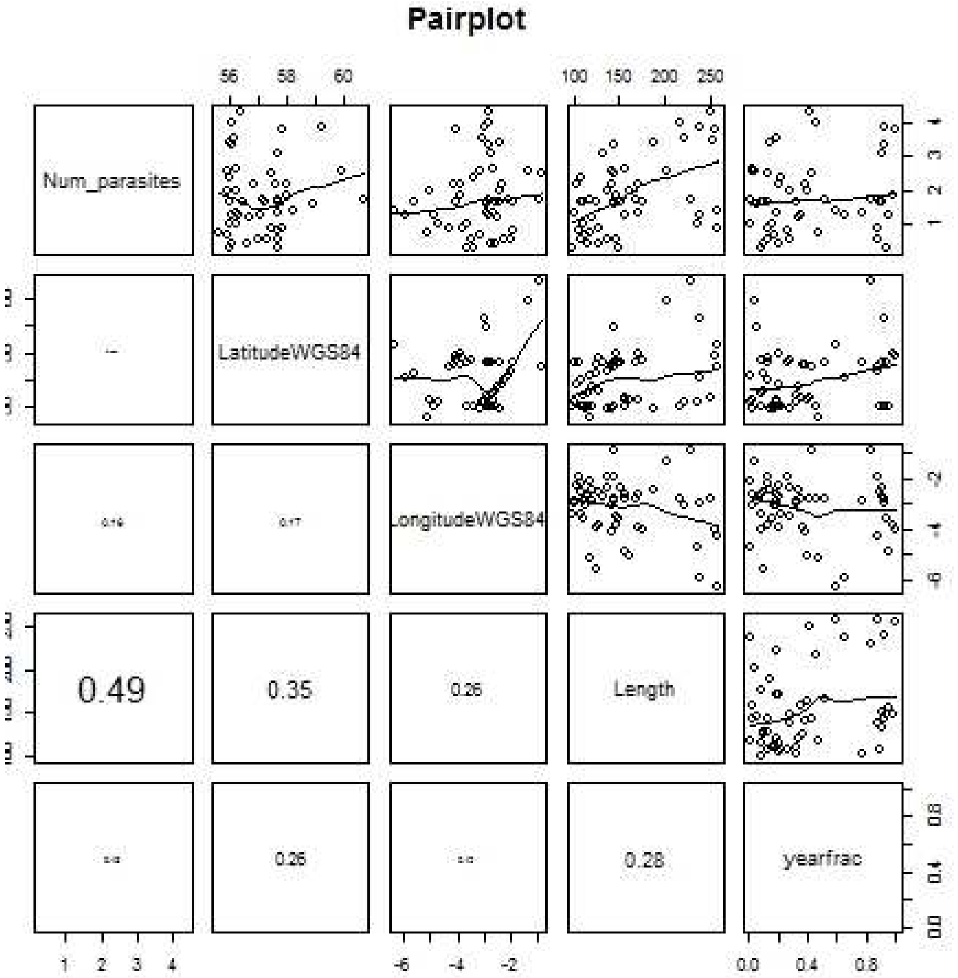


Fig 20. Pairplot between parasites number and four explanatory variables ((Latitude, Longitude , Length and Year fraction). The graphs above the diagonal are scatterplots, and numbers below the diagonal represent (absolute) correlations between variables. (Zuur *et al.*, 2007)

For models in which parasites number among host species stomachs was the response variable, a Negative Binomial distribution was used.

For models in which the parasite blotted weight was the response variable, a Gaussian distribution was applied. For models in which the presence of each parasites genera was the response variable, a Binomial distribution with log-link function was employed.

The extremely high number of zeros tells us that we should not apply an ordinary Poisson or negative binomial GLM as these would produce biased parameter estimates and standard errors (Cameron & Trivedi 1998; Zuur *et al.*, 2009a).

We recognize that these zeros are not real, since the zero doesn’t represent the real absence of worms in the stranded hosts but their presence is due to sampling errors during the necropsy. Thus Negative Binomial distribution with a very low theta value, instead of zero-inflated, was considered more appropriate to use. To answer specific questions about relationships between variables we used generalised additive models (GAMs) (Hastie & Tibshirani 1990; Zuur *et al.*, 2007, 2009). GAM is basically a smoothing equivalent of generalised linear modelling (GLM) (see McCullagh & Nelder 1989; Hastie & Tibshirani, 1990).

For each nominal explanatory variable used in the models a smoother was employed and for the smoothers fitted were fixed value of 4 degree of freedom. Optimal models were determined using combined ‘forwards’ and ‘backwards’ stepwise selection, being those with the lowest Akaike information criterion (AIC) value (Akaike 1973) and in which all explanatory variables retained had significant effects ($p < 0.05$).

The selection process also took into account explanatory variable significance (i.e. normally adding the variable with lowest *p*-value or removing the variable with the highest *p*-value) and deviance explained (favouring high values).

Using the Brodgar tabsheet ‘Specialised corner’ was possible to fit the full model, and a nested model and to compare the models with an *F*-test. Generally, the best model is that with the lowest value for the Akaike Information Criterion (AIC), in which all remaining explanatory variables had significant effects (Pierce *et al.*, 2008) but the *F* test can be used when AIC values of different models are quite similar.

3.2 Diet data analysis

Prey remains recovered from stomach contents consisted principally of otoliths and bones of fish and beaks (mandibles) of cephalopods, which were identified using reference material and published guides (Clarke 1986; Härkönen 1986; Watt *et al.*, 1997). The majority of identifications for fish were based on otoliths.

Not all otholiths, apart from cod (*Gadus morhua*) and whiting (*Merlangius merlangus)*, were identifiable to species level and some composite categories were therefore used.

For statistical analysis of numerical importance, herring and sprat were grouped as Clupeidae since they could not always be distinguished from each other. Haddock (*Melanogrammus aeglefinus*), saithe (*Pollachius virens*), and pollack (*P. pollachius*) have very similar otoliths and were therefore grouped for statistical analysis as well.

Three higher level taxa, namely gadids (Gadidae), sand eels (Ammodytidae) and gobies (Gobiidae), were employed for statistical analysis as well, while for cephalopods the genus *Alloteuthis* was identified. Otoliths are subject to digestive erosion but cephalopod beaks are relatively robust to mammalian digestive processes (Tollit *et al.*, 1997). Therefore of 42 harbour porpoises, 6 Atlantic white-sided dolphins and 8 white-beaked dolphins stomach contents just 34 harbour porpoises, none white-beaked dolphins and 1 Atlantic white-sided dolphins were employed for statistical analyses on diet composition.

The majority of the statistical analysis has concerned variation in numbers of parasites across prey species. To detect statistically significant differences in number of parasite during univariate analyses, we ran some models. We first investigated the relationship between the number of parasites , as response variable, and all the prey taxa detected from the stomach. The explanatory variables, all nominal, were: presence of *Alloteuthis*, presence of haddock, saithe and pollack together, presence of Gadidae, presence of Gobiidae, presence of Clupeidae, presence of cod, presence/absence of whiting. For the response variable a Negative binomial distribution with logit link function was applied and for the explanatory variables four degrees of freedom were fixed. In the other models run, the response variable was the parasites number and the explanatory variables were: latitude, longitude, length and year fraction of the three host species and the presence/absence of the prey *Alloteuthis* as nominal variable. To find the optimum models, as for the parasitological analysis, forward and backward selection was applied.

4 RESULTS

4.1 Parasite data

Four anisakid genera (*Anisakis, Pseudoterranova, Contracaecum* and *Hysterothylacium*) were recovered from the hosts' stomachs. All the three host species harboured the four parasite species complexes, with the exception of one female of *Lagenorhynchus acutus* infected with at least 2 species of helminths (*H. aduncum* and *Contracaecum*). In all the three hosts, the most frequently occurring taxon was *Pseudoterranova*, with 50% occurence in both harbour porpoise and white-beaked dolphin and 83.33% in Atlantic white-sided dolphin. In the Atlantic white-sided dolphin, *Anisakis* was the most frequent (66.67%) and it was the second most frequent in harbour porpoise (45%) as well. The frequency for the remaining species *Contracaecum* was low (17.5%) in harbour porpoise, but was high (75 %) in white-beaked dolphin (Fig. 21).

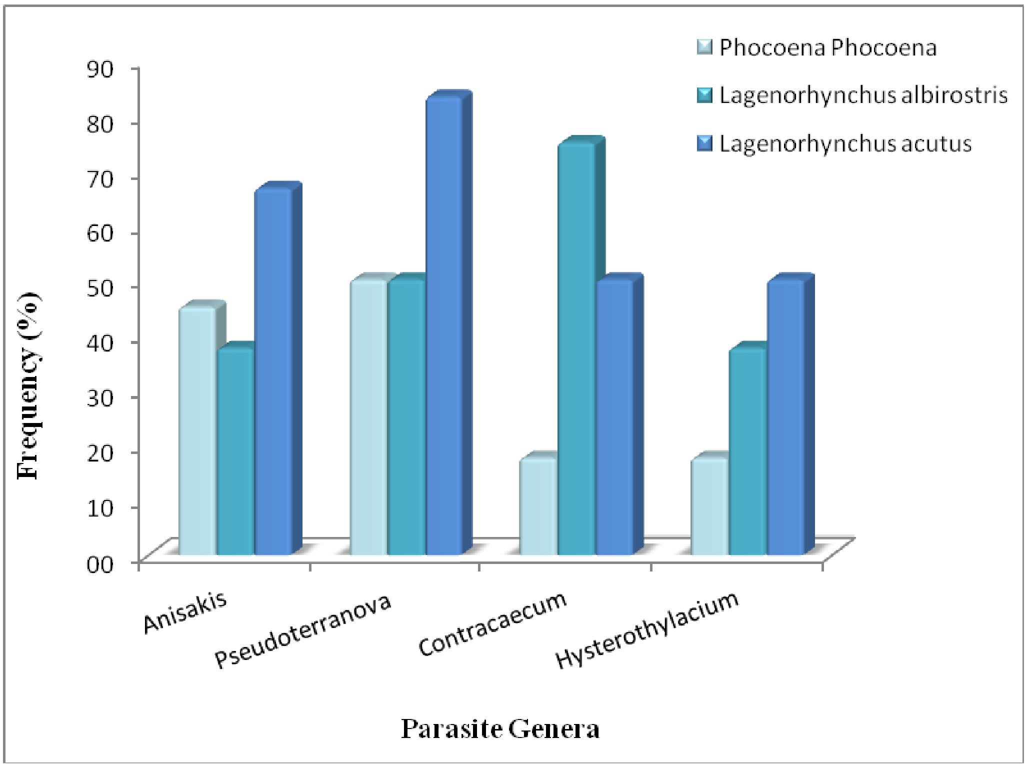


Fig. 21. Anisakid species frequency distribution among 3 cetacean species from Scotland: *Phocoena phocoena* , *Lagenorhynchus acutus,* and *L. albirostris*

The number of parasite species per host based on presence/absence data was tested in a generalized additive model (GAM) and there was a significant relationship between the presence of *Hysterothylacium aduncum* and the explanatory variable month. The smoothing curve for month is presented in Figure 22, and it shows a clear (but weak) seasonal pattern.

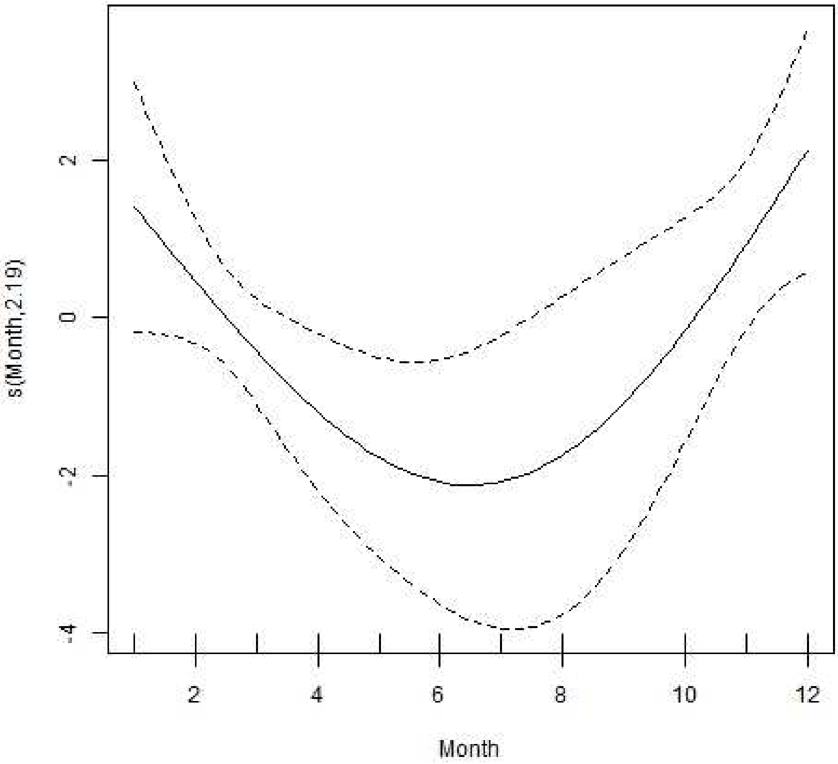


Fig. 22. Smoothing curve for month for the presence of *H. aduncum* data

This model was checked by adding, to the variable month, the nominal variable species code (as a factor). Comparing the full model with a nested model with the species code through the *F*- test, obtained from an ANOVA table (Table 3), no gain from including the additional factor was detected ($p = 0.364$). Therefore it is confirmed that there were no differences between species and the optimal GAM model is the first one:

$$Y1 \sim 1 + s(Month, k = 4)$$

Table 3. ANOVA table for the both models

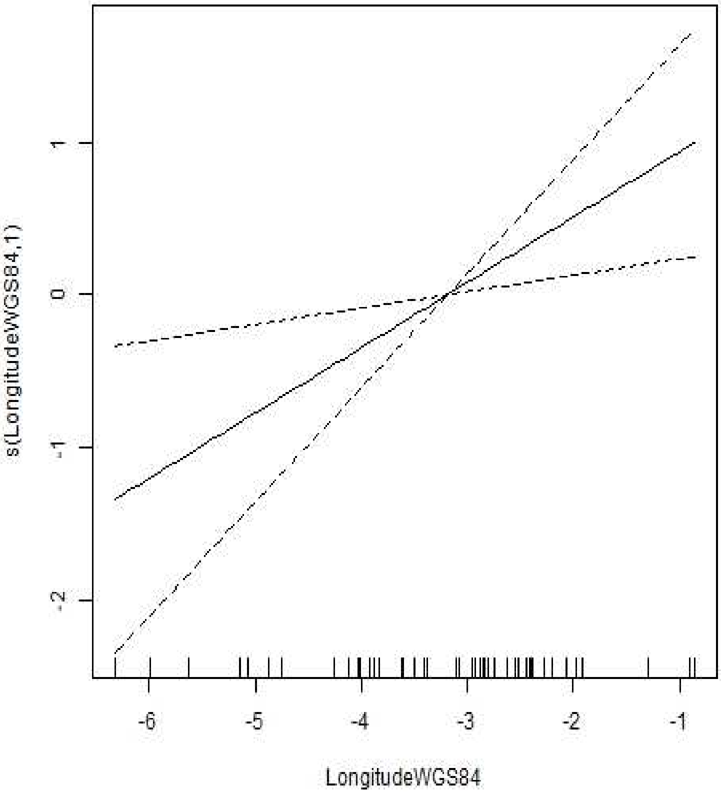
Model	Resid. df	Resid. Dev	df	Deviance	F	Pr(>F)
1	48.805	42.1				
2	45.702	38.8	3.1031	3.2999	1.0634	0.3645

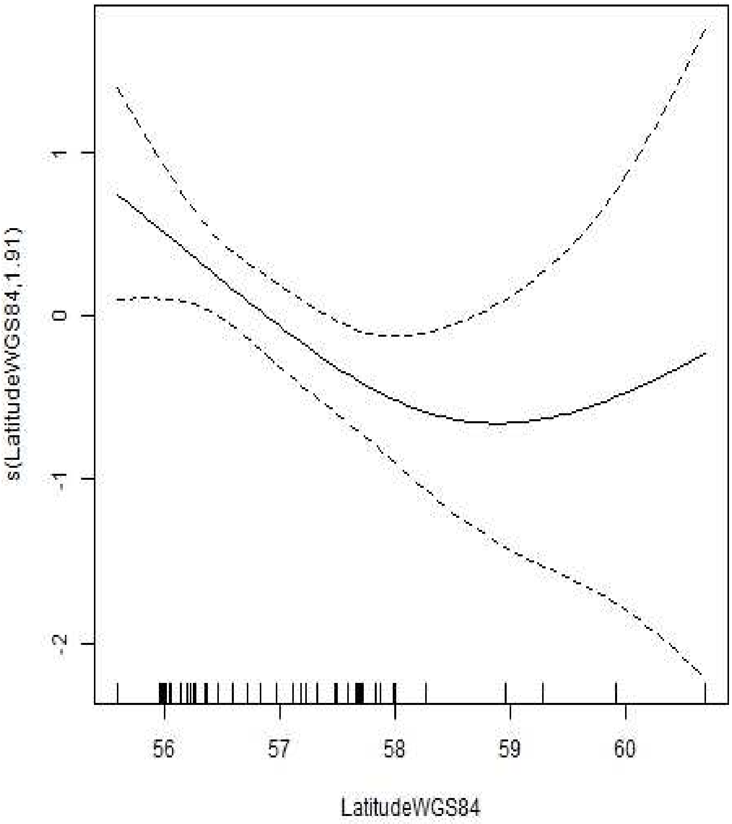
Geographical differences were observed in parasites number of all anisakid species pooled together ($p < 0.001$). It was highest in cetaceans from the East area and lowest in cetaceans from the north coast of Scotland. The parasite number also increased significantly with the length of the animal and during the year, but with a significant seasonal pattern only for *Phocoena phocoena*.

The GAM model for the parasite number is as follows:

Y1 ~ 1 + as.factor(spp_code) + s(LatitudeWGS84, k = 4) + s(LongitudeWGS84, k = 4) + s(Length, k = 4) + s(yearfrac, k = 4, by = as.factor(spp_code))

GAM smoothers curves for the effect of the explanatory variables on the parasites number are shown in Fig. 23.


s(LongitudeWGS84,1)
1
0
-1
-2
-6
-5
-4
-3
-2
-1
LongitudeWGS84


s(LatitudeWGS84,1.91)
1
0
-1
-2
56
57
58
59
60
LatitudeWGS84

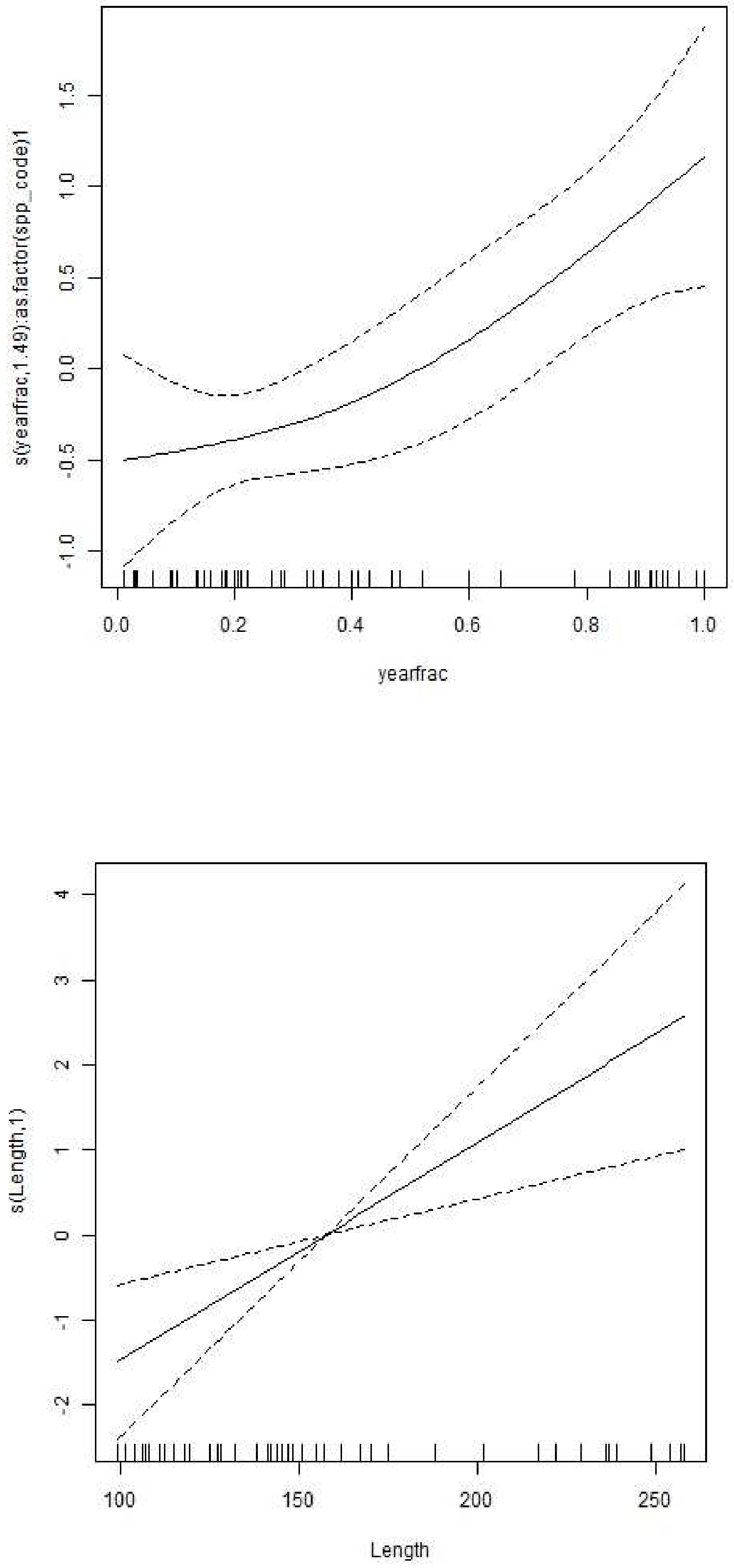


Fig. 23. GAM smoothing curves fitted to effects of explanatory variables (Longitude, Latitude, Year fraction and Length) on the parasites number. Smoothers are shown only for continuous variables with significant effects. Dotted lines are the approximate 95% confidence limits.

Similarly, the parasite burden of the all anisakid genera, pooled together, was related to the year fraction of stranding and significant differences were found between the species, but no significant difference was found between the sexes. The effect of year fraction was statistically significant only or the individuals of *Lagenorhynchus albirostris*, as shown in Fig. 24.

The best GAM model for the parasite burden is as follows:

Y1 ~ 1 + as.factor(spp_code) + s(yearfrac, k = 4, by = as.factor(spp_code))

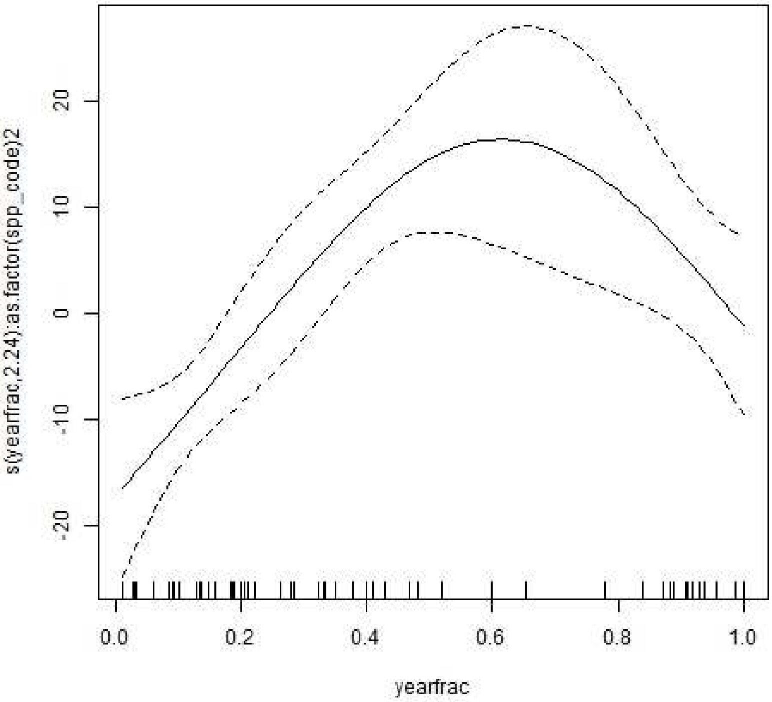


Fig 24. Smoothing curves fitted to effect of the year fraction of stranding on the parasites burden in the GAM model with Gaussian distribution.

Final models for the presence of *H.aduncum* and the parasite number and burden are also summarized in Table 4. All these models tended to have a reasonably high value for % deviance explained, ranging from 25.1 % to 68.8%, hence models can be considered satisfactory.

Table 4. Summary of generalised additive models for the presence of *H.aduncum* and the parasites number and burden in all cetacean species. For each model , all significant explanatory variables are listed with their associated probability (*p*) value, percentage of deviance explained (DE), Akaike Information Criterion (AIC) value and degrees of freedom in parentheses for smoothers (s). Where there was no significant effect, the relevant cell in the table is left blank .The explanatory variables were: month, longitude, latitude, length, year fraction and species code (where spp_code 1 is related to *Phocoena phocoena*, spp_code 2 to *Lagenorhynchus albirostris* and spp_code 3 to *L.acutus*).

	Explanatory variables					**DE**	**AIC**
	Month	**Long.**	**Lat.**	**Length**	**Year fraction**		
Parasite number		(1.0) 0.0075	(1.91) 0.0265	(1.0) 0.0009	factor(spp_code)1 (1.49) 0.0006	68.8	627.63
Parasite burden					factor(spp_code)2 (2.24) 6.38e-05	59.7	340.86
Presence of *H. aduncum*	(2.19) 0.0184					25.1	48.49

4.2 Diet data

Generalised additive models (GAMs) using a negative binomial distribution and logit link function were used to test the link between parasite number with the presence of prey groups.

The GAM models showed no significant relationship between parasite number and the presence of particular prey types: whiting (*Merlangius merlangus)* (*p* = 0.1330), Gobiidae (*p* = 0.5307), Clupeidae, Gadidae (*p* = 0.8921), Sand eels (*p* = 0.0636).

The model we ran was in the following form:

Y1 ~ 1 + as.factor(clupidae) + as.factor(cod) + as.factor(gadidae) + as.factor(gobiidae)
+ as.factor(had_sai_poll) + as.factor(sandeel) + as.factor(whiting) +
as.factor(alloteuthis)

This model was checked by removing the non-significant explanatory variables with the exception of the presence of Clupeidae, the presence of Had_sai_poll , the presence of cod and the presence of *Alloteuthis*. In the following model no significant interaction between the presence of Had_sai_poll (*p* = 0.156) and the presence of cod (*p* = 0.217) with parasite number was detected.

Therefore another model in the following form was run:

Y1 ~ 1 + as.factor(clupidae) + as.factor(alloteuthis)

which showed a significant interaction between parasite number and the presence of *Alloteuthis* (*p* = 3.15e-07) and the presence of Clupeidae (*p* = 9.98e-05).

Running these explanatory variable, separately, in two different models with the common response variable parasite number, it was detected that the strongest relationship was with the squid genus *Alloteuthis*. The model for this species had the highest value of deviance explained (36.3%) and the lowest AIC value (375.79) (Tab. 5).

Therefore the optimal single prey species GAM model is in the form:

Y1 ~ 1 + as.factor(*Alloteuthis*)

Table 5. Summary of generalised additive models for the presence of main prey groups and the parasites in 34 harbour porpoises and 1 Atlantic white-sided dolphin. For each of the two models, all significant explanatory variables are listed with their associated probability (*p*) value, percentage of deviance explained (DE) and Akaike Information Criterion (AIC).

Prey group	***p*-value**	**DE**	**AIC**
Alloteuthis	1.06e-07	36.3 %	375.79
Clupeidae	2.72e-07	11.7 %	416.56

Since the effect of the presence of *Alloteuthis* on parasite number was significant, we ran additional models to find which other factors influenced this relationship. The analysis was repeated, the presence prey data were dropped from the model with the exception of the genus *Alloteuthis* and another two explanatory variables longitude and year fraction were added. However, this GAM model showed no significant relationships ($p > 0.05$):

Y1 ~ 1 + as.factor(alloteuthis) + s(LongitudeWGS84, k = 4) + s(yearfrac, k = 4)

5 DISCUSSION

5.1 Geographical and seasonal variation in parasite distribution

The present work is the first quantitative study of the nematode fauna in stomach contents of cetaceans in Scottish waters. Here we used a 10-year database of dolphins and porpoises stranded around the coast of Scotland to explore the anisakid parasite infections in this wild population of marine mammals. Samples were examined from 42 harbour porpoises (*Phocoena phocoena),* 6 Atlantic white-sided dolphins (*Lagenorhynchus acutus*) and 8 white-beaked dolphins (*Lagenorhynchus albirostris*). There are undoubtedly some potential biases in such a study on parasites of cetaceans because stranded animals are the only source of samples, and their parasite faunas may not be representative since small sample size it is unlikely that many patterns in the data could be detected. Given the relatively small sample size, it is difficult to disentangle the effects of the various possible factors influencing parasite presence and abundance. Nevertheless, interesting preliminary inferences can be made.

General patterns and trends

In the present study infection data for *Anisakis* indicates high prevalence, both in harbour porpoise and in white-sided dolphin, probably reflecting its incidence in intermediate hosts due to its low host specificity (Smith & Wootten 1978). Similar levels of infection, in the same host species, have been reported for the anisakid *Pseudoterranova*, while the white-beaked dolphin showed a high prevalence of *Contracaecum*. Univariate analysis indicated that latitude, longitude, animal length, and time of year of stranding, all contributed significantly to variability in parasite number.

Seasonal variation

A significant relationship was found between the presence of the genus *Hysterothylacium* and the month of host stranding. A decrease in parasite abundance was observed in the period between April and August, therefore differences in infestation rate may be a seasonal effect related to prey availability, since feeding represents the main parasite transmission route. Our results generally agree with the extensive survey by Scott and Fisher (1958a) which suggested that the harbour porpoise in the inshore waters off Eastern Canada during the summer carry relatively few adults of *Anisakis*. Data given by Brattey and Stenson (1995) suggested that the harbour porpoise occupying inshore waters of southeastern Newfoundland, during the summer months, is not a major source of larval *A. simplex* for local fish stocks as well.

The parasite burden of the white-beaked dolphin showed an increase in April and October, with a peak abundance in August. Weir et al. (2007) reported a marked seasonality of white-beaked dolphin distribution, with a clear peak in relative abundance during August in Aberdeenshire waters. The summer increase in occurrence of this species is contrasted with the marked winter/spring peak relative abundance of bottlenose dolphins in Aberdeenshire waters (Stockin *et al.* 2006). Such seasonal shifts in distribution may help to explain the change in anisakid infection levels. Therefore, there are a number of potential explanations for the seasonal effects on parasite burden in this species, including migrations for feeding requirements and inter-specific competition with other cetacean species.

Geographical patterns

Our findings reveal there is a local geographical pattern in helminth communities in the stomachs of the 56 stranded hosts. Parasite prevalence seems to be somewhat higher in the eastern areas, and lower in the northern areas than in the southern areas. These differences in parasite numbers between porpoises and dolphins, from different regions, might reflect a direct effect related to more highly infected prey acting as intermediate host in eastern and southern areas.

Size effects

In addition, increasing host length, related with age, appears to be associated with increasing nematode number, probably because the oldest during their life have eaten more infected intermediate hosts without acquiring immunity. In agreement with this hypothesis is the observation, made by Bull and Jepson (2006), that the stomachs from heavily infected porpoises were generally found to be emptier than those from parasite-free porpoises because likely a high parasite burden might cause ill health and leads animals to stop feeding.

5.2 Parasite number variation related to diet composition

Cause of Death and Diet: A possible source of bias

Other studies have shown dietary differences between by-caught and stranded porpoises *(e.g.,* Lick 1991, Aarefjord *et al,*. 1995, Benke *et al.,* 1998).

Rogan & Berrow (1996) found that, in Ireland, bycaught porpoises had eaten less clupeids and whiting than had stranded porpoises.

The marine mammal diet has been shown to vary according to the age of the individuals and the abundance of prey species, or as a function of season or geographic location as well (Evans 1987, Lick 1991, Pierce *et al.* 1991b, Aarefjord *et al.* 1995, Tollit *et al.* 1997, 1998).

Various sources of potential bias are associated with reconstruction of diet from stomach contents (Härkönen 1986, Pierce and Boyle 1991). The main characteristic of stranded materials, compared to material obtained from freshly by-caught animals, is that the stomach content is often reported to be merely made up of digested material, in fact differential digestion rates of prey species may affect the assessment of the relative importance of prey species (Pierce and Boyle 1991).

Squid as trophic channel for parasites

Our results suggest a significant relationship between parasite number and the presence of the cephalopod taxa *Alloteuthis*, therefore this prey group is probably an important dietary source of nematode for the hosts species in our study area.

However, it is important to acknowledge that the estimate of the parasite effect on diet composition is affected by biases because of the small number of individuals for which we had diet samples (34 harbour porpoises, no white-beaked dolphin and just 1 Atlantic white-sided dolphin on 42 harbour porpoises, 6 Atlantic white-sided dolphins and 8 white-beaked dolphins of which we had parasite samples). Hence, it is unlikely that many patterns in data could be detected but it was still interesting to look at an hypothetical main parasite route transmission.

This link between the presence of *Alloteuthis* in the diet and the parasite number could be explained by a higher level of parasite infection in squid from this area, probably due to the quantitative distribution of infective forms in planktonic animals and fish (the squid prey), the abundance of the final host (the squid predators) , the degree of interchange between all of them via food and the age or size maturity of squid.

Accordingly, the number of parasites has been observed to increase with increasing host size. As the size-maturity and age of squid are interrelated (Gonzalez et al. 1992), a similar trend could be expected between helminth infection and squid age.

Furthermore, long-finned squids are neritic, demersal species associated with coastal waters.

In neritic foodwebs, a paratenic host squid can be infected several times, so permitting the infective stages of the parasite to be concentrated. Consumption of the squid by a suitable host then results in a heavy infection (Abollo *et al*. 1998).

According to the literature, at a local scale, the parasite fauna of pelagic fish species tends to be distinctive and comparatively poor compared to that of coastal hosts. This is due to the absence of intermediate hosts; thus, the likelihood of parasite exchange is decreased and infective stages are diluted in the pelagic environment (Hoberg & Adams 2000, Raga *et al.* 2009).

5.3 Future research

The present study permits only tentative conclusions about the population ecology of these four anisakids species because current evidence is based on observational data, often on small sample sizes, and data come only from the cetacean hosts. It is, however, only a preliminary study, thus more samples should be collected with detailed study into seasonal and spatial heterogeneity but in respect of the basic descriptive information about which parasites are found in the dolphins, and how many parasites .

Further empirical studies examining the host immunocompetence would enhance our understanding of the factors affecting the dynamics of the parasite community.

To fully understand the relationship between host species and parasite transmission routes, we need additional data on host diet and parasite lifecycles in the Northern North Sea.

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ACKNOWLEDGEMENTS

First I would like to thank Prof Graham Pierce of University of Aberdeen for all his help, support and guidance throughout the project.

Vorrei inoltre ringraziare la Prof.ssa Elena Fabbri per la sua professionalità, disponibilità ed assoluta umanità mostrata.

Un grazie a mio padre, che ha da sempre finanziato e creduto nel mio potenziale, che mi ha educata alla razionalità e con meno successo alla guerra contro l’entropia, che mi ha spronata a volte anche portandomi al limite, che ha sofferto l’attesa di questo giorno come se fosse il mio e che mi ha aiutata costantemente a superare l’attrito da primo distacco senza mai lasciarmi uscire fuori strada.

Un grazie a mia madre, che con tanta pazienza ha supportato e sopportato i miei progetti, i miei crolli e le mie battaglie senza mai smettere di credere nelle mie vittorie e che con il più sconfinato degli amori mi ha da sempre lasciato libera di scegliere e di prepararmi per la vita che ho sempre desiderato.

Un grazie a Paolo, compagno di vita e di studi, che ha apportato la diligenza, la concretezza, l’amore e la stabilità alla mia vita.

Un grazie a mio fratello Biagio, amico di sempre, che mi ha insegnato a credere fermamente nei miei obbiettivi.

Un grazie a tutti quelli che hanno sminuito o sottovalutato i miei sforzi e i miei traguardi, offrendomi la prospettiva di un futuro che ci avrebbe accomunati se anch’io insieme a loro avessi iniziato a smettere di avere il coraggio di sognare.

Infine un ringraziamento particolare va a me stessa, grazie unicamente alla quale nonostante le continue cadute ho trovato sempre la motivazione e la forza per rialzarmi, sempre pronta a rideterminare i miei obbiettivi confidando sempre nelle imprevedibili risposte dell’universo.

APPENDIX

Table 6. Details of the three host species used in the parasite analysis.

M reference SAC	Species	Longitude	Latitude	Lenght	Sex	Month	Year fraction	Parasite weight	Parasite number	P/A Anisakis	P/A Pseudoterranova	P/A Contracaecum	P/A Hysterothylacium
M 008/04	*P. Phocoena*	58.96	-2.88	145	M	1	0.06	0.14	37	0	1	1	1
M 027/04	*P. Phocoena*	57.22	-5.64	125	M	2	0.10	0.24	44	NA	NA	NA	NA
M 048/04	*P. Phocoena*	55.99	-2.90	121	F	3	0.19	1.75	NA	1	0	0	0
M 101/04	*P. Phocoena*	57.59	-3.92	126	F	5	0.37	NA	NA	0	1	0	0
M 127/04	*P. Phocoena*	55.59	-5.15	118	F	6	0.48	0.02	5	0	1	1	0
M 206/04	*P. Phocoena*	57.69	-2.93	101	M	10	0.78	1.17	21	1	0	1	0

M 055/05	*P. Phocoena*	57.98	-3.92	170	F	3	0.20	0.68	145	NA	NA	NA	NA
M 123/05	*L. albirostris*	56.36	-2.81	249	F	5	0.41	13.72	21558	NA	NA	NA	NA
M 130/05	*P. Phocoena*	57.49	-0.86	144	F	6	0.43	0.17	336	1	1	1	0
M 203/05	*L. acutus*	58.27	-6.32	258	M	8	0.60	1.14	26	1	1	0	0
M 303/05	*P. Phocoena*	57.68	-2.74	132	M	11	0.91	23.16	1152	0	0	1	0
M305/05	*L. acutus*	59.28	-2.96	239	M	12	0.92	17.8	7171	1	0	1	0
M 306 /05	*P. Phocoena*	56.04	-2.84	144	M	12	0.93	12.72	2343	1	1	0	1
M 037/06	*P. Phocoena*	57.84	-3.83	128	M	2	0.13	0.05	7	1	0	0	0
M 038/06	*P. Phocoena*	56.97	-2.21	108	M	2	0.14	0.01	3	0	0	1	0

M 053/06	*P. Phocoena*	56.83	-2.28	104	F	3	0.21	1.34	144	0	1	0	1
M 090/06	*P. Phocoena*	57.67	-1.92	106	M	4	0.26	0.03	6	0	0	1	0
M 094/06	*P. Phocoena*	55.96	-2.40	138	M	4	0.28	0.47	102	1	1	1	0
M 117/06	*P. Phocoena*	56.72	-2.44	119	M	5	0.33	0.12	15	0	1	1	0
M 126/06	*P. Phocoena*	56.35	-2.80	157	F	5	0.35	0.07	17	0	1	1	0
M 139/06	*P. Phocoena*	57.59	-4.11	141	M	5	0.38	2.91	147	0	0	1	0
M 025/07	*P. Phocoena*	56.01	-3.40	99	F	2	0.09	0.03	1	1	0	1	0
M 057/07	*L. acutus*	56.14	-3.08	222	F	3	0.19	15.99	3450	1	1	0	0
M 171 /07	*L. acutus*	57.11	-5.99	237	M	8	0.65	0.08	17	0	1	1	0

M 222/07	*L. acutus*	57.49	-4.26	257	M	11	0.88	0.3	7	0	1	1	1
M 049/08	*P. Phocoena*	55.95	-3.10	112	F	3	0.18	0.04	4	0	0	1	0
M 119/08	*P. Phocoena*	56.23	-5.08	162	F	5	0.40	0.86	105	1	0	0	0
M 147/08	*P. Phocoena*	57.69	-2.83	167	F	7	0.52	0.09	50	1	1	1	0
M 001 /09	*P. Phocoena*	57.18	-2.07	118	M	1	0.01	0.11	51	0	1	1	0
M 013 /09	*L. albirostris*	55.98	-2.42	188	F	2	0.15	10.78	2482	0	0	1	1
M 028/09	*P. Phocoena*	56.06	-2.75	115	M	3	0.21	0.03	2	1	1	0	0
M 035/09	*P. Phocoena*	55.99	-3.38	107	M	3	0.22	0.11	224	1	1	0	0
M 044/09	*P. Phocoena*	56.47	-2.85	111	M	4	0.32	2.06	220	1	1	1	0

M 054/09	*P. Phocoena*	56.17	-3.00	101	F	4	0.33	0.06	49	0	0	1	0
M162/09A	*L. acutus*	60.69	-0.91	229	M	11	0.84	0.24	55	1	1	1	0
M 192/09	*L. albirostris*	57.87	-4.02	254	F	12	1.00	13.45	5976	1	1	1	1
M 006/10	*P. Phocoena*	55.95	-3.11	142	M	1	0.03	1.7	367	0	0	1	0
M 009/10	*P. Phocoena*	56.26	-2.62	157	F	1	0.03	0.98	468	0	1	1	0
M 010/10	*L. albirostris*	59.92	-1.29	202	M	1	0.03	0.78	397	1	0	1	1
M 036/10	*P. Phocoena*	57.67	-1.92	144	F	2	0.14	0.41	45	1	1	1	0
M 114 /10	*L. albirostris*	56.05	-2.83	217	F	6	0.47	40.3	9851	1	1	1	0
M 286/10	*P. Phocoena*	56.00	-2.54	108	M	11	0.89	0.51	49	0	1	1	0

M 301/10	*P. Phocoena*	57.66	-3.62	148	M	12	0.94	0.03	1	0	0	1	1
M 308/10	*P. Phocoena*	56.04	-4.88	155	F	12	0.96	0.2	19	1	0	1	0
M 051/11	*P. Phocoena*	56.26	-2.62	170	F	3	0.18	0.18	23	0	1	0	0
M 321/11	*P. Phocoena*	57.32	-1.97	138	F	11	0.87	0.08	3	0	0	0	1
M 005/12	*L. acutus*	56.20	-4.75	236	M	1	0.01	0.09	9	1	0	0	0
M 034 /12	*P. Phocoena*	56.54	-2.63	116	M	2	0.09	21.82	4	1	0	0	0
M 036 /12	*L. albirostris*	57.72	-4.02	175	M	2	0.09	0.08	48	0	1	1	0
M 037/12	*P. Phocoena*	56.59	-2.52	127	NA	2	0.09	0.06	2	1	0	0	0
M 062/12B	*P. Phocoena*	56.02	-3.61	111	F	2	0.16	0.02	9	1	0	1	1

M 107/12	*P. Phocoena*	57.70	-3.49	147	M	4	0.28	0.04	3	0	1	0	0
M 388/12	*P. Phocoena*	57.67	-2.39	151	M	11	0.91	0.17	47	1	0	1	0
M 413/12	*P. Phocoena*	58.00	-3.87	147	NA	12	0.99	9	78	1	1	1	1
M 040/14	*P. Phocoena*	NA	NA	NA	M	2	0.13	0.435	313	1	1	1	1
M 070/14	*L. albirostris*	NA	NA	253	F	3	NA	6.95	2818	1	1	0	0