

SQUID AS TROPHIC BRIDGES FOR PARASITE FLOW WITHIN MARINE ECOSYSTEMS: THE CASE OF *ANISAKIS SIMPLEX* (NEMATODA: ANISAKIDAE), OR WHEN THE WRONG WAY CAN BE RIGHT

E. ABOLLO*, C. GESTAL*, A. LÓPEZ†, A. F. GONZÁLEZ‡, A. GUERRA‡ and S. PASCUAL*

Long-term (1991–1997) information on parasitic infection by anisakid nematodes in cephalopods and top predators (marine mammals) of the south-eastern area of the North Atlantic underlines the important role of small cetaceans as final hosts for *A. simplex*. The ommastrephid squid *Illex coindetii*, *Todaropsis eblanae* and *Todarodes sagittatus* are the most important cephalopod paratenic hosts in the life cycle of the parasite. Information on parasite flow and parasite-caused diseases could be of use for stock assessment purposes. Moreover, it largely agrees with what is known about interactions between prey (squid) and predator (cetacean) in the same area. Parasitic castration and stomach wall ulceration were the most important parasite-caused effects recorded in infected cephalopods and cetaceans respectively.

Anisakid nematodes associated with aquatic organisms and piscivorous birds are an important component of marine ecosystems (Berland and Fagerholm 1994). Transmission of *Anisakis simplex* (Rudolphi, 1809) takes place in water and involves aquatic invertebrates and fish as intermediate or paratenic hosts (hosts that permit the maintenance, but not the development, of a larval parasite), and marine mammals as final hosts (Anderson 1992). According to Davey (1971), *A. simplex* is a cosmopolitan species, occurring worldwide in 23 cetaceans and 11 pinnipeds. Additionally, three other cetaceans and another pinniped were listed as hosts of *A. simplex* by Dailey and Brownell (1972). *A. simplex* is most frequently recorded in marine mammals in colder temperate and polar waters (Davey 1971).

Cephalopods also play an important role in the trophic web of marine ecosystems (Clarke 1980, 1996, Rodhouse *et al.* 1987, 1992, Lipiński *et al.* 1992, Rodhouse and Nigmatullin 1996, Guerra *et al.* 1993). Trophic relationships between species involved in predator-prey interactions significantly influences community structure and population dynamics. One way ecological relationships have been identified is through examination of parasites (Pascual and Hochberg 1996). Cephalopods serve as primary host for protozoan, dicyemid and many crustacean parasites. More commonly, they function as paratenic and/or reservoir hosts for metazoan parasites, so playing a vital role in the transfer of parasites through the foodweb to the final hosts, top predators

(Hochberg 1990). Canadian, Russian and Spanish workers have demonstrated the importance of quantitative studies in establishing predator-prey patterns of biological importance (Brown and Threlfall 1968, Gaevskaya and Nigmatullin 1976, Pascual *et al.* 1995a, b).

Understanding the ecological balance and trophic relationships of large predators, the “end users” of the marine environment, is important because they reflect and respond to long- and medium-term physical, chemical and biological changes in the sea. Studies of parasite transfer and parasite-caused diseases through the foodwebs have obvious meaning for most top level predators, especially for marine mammals (many considered protected species), man (as a result of food-borne anthroozoonoses) and wildlife resource managers in general.

Zelmer (1997) pointed out that, although a paratenic host is not always required, it is often critical in allowing the parasite to complete its life cycle by bridging an ecological gap. The word “paratenic” is derived from two Greek words, “παρά” (para) meaning “wrong way” and “τείνειν” (teinein) meaning “to make one’s way towards”. A paratenic host is therefore a host towards which a parasite makes its way wrongly. However, parateny is useful for the parasite and has impact in a number of important ways in epidemiology and pathogeny of parasitic diseases. Through paratenic transfer a parasite is able to survive in the absence of the normal host. In neritic foodwebs, a paratenic host squid may permit a parasite to infect a host that

* Laboratorio de Parasitología, Facultad de Ciencias del Mar. Universidad de Vigo, Apartado 874, 36200 Vigo, Spain (Corresponding author's email: spascual@setei.uvigo.es)

† C.E.M.M.A., Anxeriz 19 5°D, Milladoiro, 15895 Ames, A. Coruña, Spain

‡ Instituto de Investigaciones Marinas (C.S.I.C.), Eduardo Cabello 6, 36208 Vigo, Spain

could not be infected in any other way. Further, 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.

The aim of this paper is to re-emphasize the importance of cephalopods as hosts for parasites that may travel up the food chain to top predators such as marine mammals and man. In addition to their role as hosts and transmitters of parasites, cephalopods are affected by infestation. There is therefore a need to clarify the interactions between feeding relationships, parasitic diseases and transmission pathways. To this end, gross lesions and the histopathology of parasitic diseases in both cephalopods and their predators were studied.

MATERIAL AND METHODS

Three macroparasites were common to both elements of the predator-prey system, the nematodes *Anisakis simplex* and *A. physeteris* (Baylis, 1923) and cestodes belonging to the genus *Phyllobothrium* (Linton, 1922). In order to simplify the investigation, secondary parasites (those with prevalence – see below – of $10\% \geq P > 1\%$), i.e. larval forms L_3 , L_4 and adults of *A. physeteris*, and those not identified to species level, i.e. plerocercoids of *Phyllobothrium*, were excluded from the analysis. For this study, therefore, a single pathway of parasite development was chosen: the microgeographic predator-prey interaction between squid and cetaceans involving *A. simplex*.

Data were collected from 1991 to 1997 off the coast of Galicia (N.W. Spain) between 42 and 45°N and between 7 and 9°W. A total of 1 790 cephalopods (10 species), 80 stranded cetaceans (8 species) and 56 seabirds (6 species) were sampled and dissected carefully according to standard protocols (Borgsteede 1991, Pascual 1996).

Nematodes were preserved in 70% ethanol, then prepared for light microscopy (LM) and scanning electron microscopy (SEM) for identification. They were identified by examining the anatomy of the oesophago-intestinal region, the lip morphology, the opening of the excretory pore and the tail morphology. The number and distribution patterns of caudal papillae and papillae-like structures in adult male ascaridoid nematodes were elucidated according to the method documented by Fagerholm (1991). Parasitized tissues were fixed in 10% formaldehyde for 24 h, then embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin (H&E) and Wheatley trichrome (WT), following standard methods (Culling *et al.* 1985).

Table 1: Host-parasite distribution data

Taxon	Sample size	Prevalence <i>P</i> (%)	Numerical importance
Cephalopoda			
Octopodidae	217	1.84	0.04
<i>Octopus vulgaris</i>	150	2	0.02
<i>Eledone cirrhosa</i>	67	1.50	0.02
Sepiidae	198	2.52	0.04
<i>Sepia officinalis</i>	150	3.50	0.04
<i>Sepia elegans</i>	13	0.00	0.00
<i>Sepia orbignyana</i>	35	0.00	0.00
Loliginidae	110	7.27	0.19
<i>Loligo vulgaris</i>	50	16	0.19
<i>Alloteuthis subulata</i>	60	0.00	0.00
Ommastrephidae	1 265	15.81	0.73
<i>Todaropsis eblanae</i>	600	18.60	0.21
<i>Illex coindetii</i>	600	11	0.13
<i>Todarodes sagittatus</i>	65	33.80	0.39
Cetacea			
Delphinidae	74	59.45	0.58
<i>Delphinus delphis</i>	50	64	0.16
<i>Stenella coeruleoalba</i>	8	37.50	0.09
<i>Tursiops truncatus</i>	10	60	0.15
<i>Grampus griseus</i>	2	0.00	0.00
<i>Globicephala melas</i>	4	75	0.18
Kogiidae	1	100	0.24
<i>Kogia breviceps</i>	1	100	0.24
Phocoenidae	4	75	0.18
<i>Phocoena phocoena</i>	4	75	0.18
Balaenopteridae	1	0.00	0.00
<i>Megaptera novaeangliae</i>	1	0.00	0.00
Seabirds			
Phalacrocoracidae	31	0.00	0.00
<i>Phalacrocorax aristotelis</i>	30	0.00	0.00
<i>Phalacrocorax carbo</i>	1	0.00	0.00
Laridae	15	0.00	0.00
<i>Larus cachinnans</i>	15	0.00	0.00
Alciidae	4	0.00	0.00
<i>Alca torda</i>	4	0.00	0.00
<i>Uria aalge</i>	4	0.00	0.00
<i>Fratercula arctica</i>	2	0.00	0.00

Parasite occurrence was expressed in terms of prevalence ($P = \% \text{ infected}$), following Bush *et al.* (1997). Numerical importance (NI) was calculated as the proportion of parasitized individual hosts of the i^{th} species.

RESULTS

Parasites

Larval (L_3 , L_4) and adult forms of *Anisakis simplex* were found within 7 and 6 species of cephalopods and cetaceans respectively (Table 1). Diagnostic characters and other morphological features of systematic interest are illustrated in Figures 1 and 2. There was no *Anisakis*

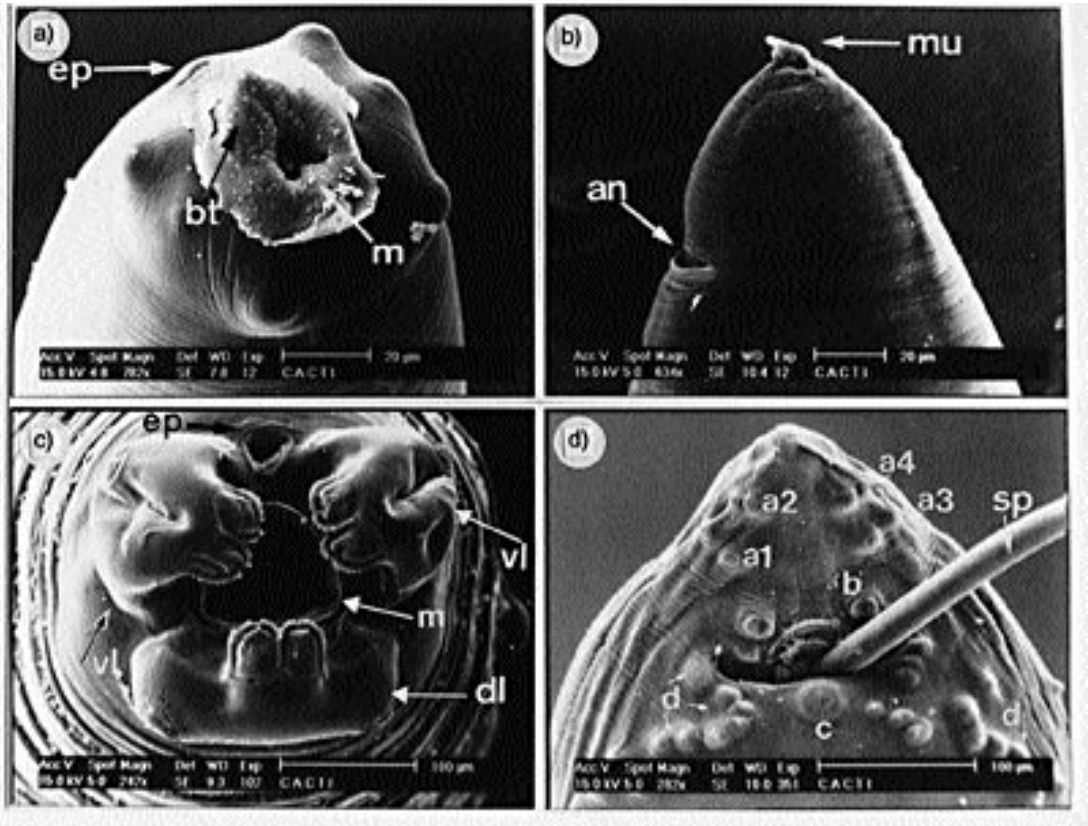


Fig. 1: *Anisakis simplex*. a, b) Third stage larva (L_3); (a) anterior end showing the excretory pore (ep), boring tooth (bt) and mouth (m); (b) tail end showing the anus (an) and mucron (mu). c, d), Adult; (c) anterior end showing the excretory pore (ep), mouth (m), dorsal lip (dl) and ventrolateral lips (vl); (d) tail end showing the spicule (sp) and the number and distribution pattern of papillae in adult males, distal papillae pairs (a1–a4), united paraoccal papillae (b), single preoccal median papilla (c) and subventral proximal papillae clusters (d)

infection of the seabirds examined. Ommastrephids were by far the most important cephalopod hosts of anisakids ($P = 63.4\%$, $NI = 67$), and delphinids had the highest levels of infection.

Gross lesions and histopathology

There was no gross pathology associated with parasitic infection in the ommastrephids examined. Worms were located macroscopically encysted in the sheath of connective tissue surrounding the mantle muscle, gonads, nidamental glands and the external and internal walls of the stomach (Fig. 3). Histologically, early infection consisted of necrotic tissue displaying a light inflammatory reaction; this stage was followed by haemocyte infiltration of the site. In more advanced cases of infection, most larvae were coiled inside a

concentrically constructed connective tissue capsule (fibroblast-like cells). Most infected organs examined displayed evidence of mechanical compression and displacement of host tissue elements at sites close to or in direct contact with the parasite larvae, with varying degrees of cellular infiltration. The spaces surrounding worms were usually coated with tissue fragments, cell debris and extensive secretions of mucus. Parasitic castration was observed in mature squid. Anisakid nematodes caused the partial destruction and alteration of gonad tissue and a partial inhibition of gamete formation in hosts.

In cetacean carcasses, anisakid nematodes were found free within the stomach and/or attached to the stomach wall. Gastric ulcers were noted in 15% of the animals examined, with clusters of anisakids embedded in the ulcers. Histologically, ulcerations were located in the mucosa and submucosa of the

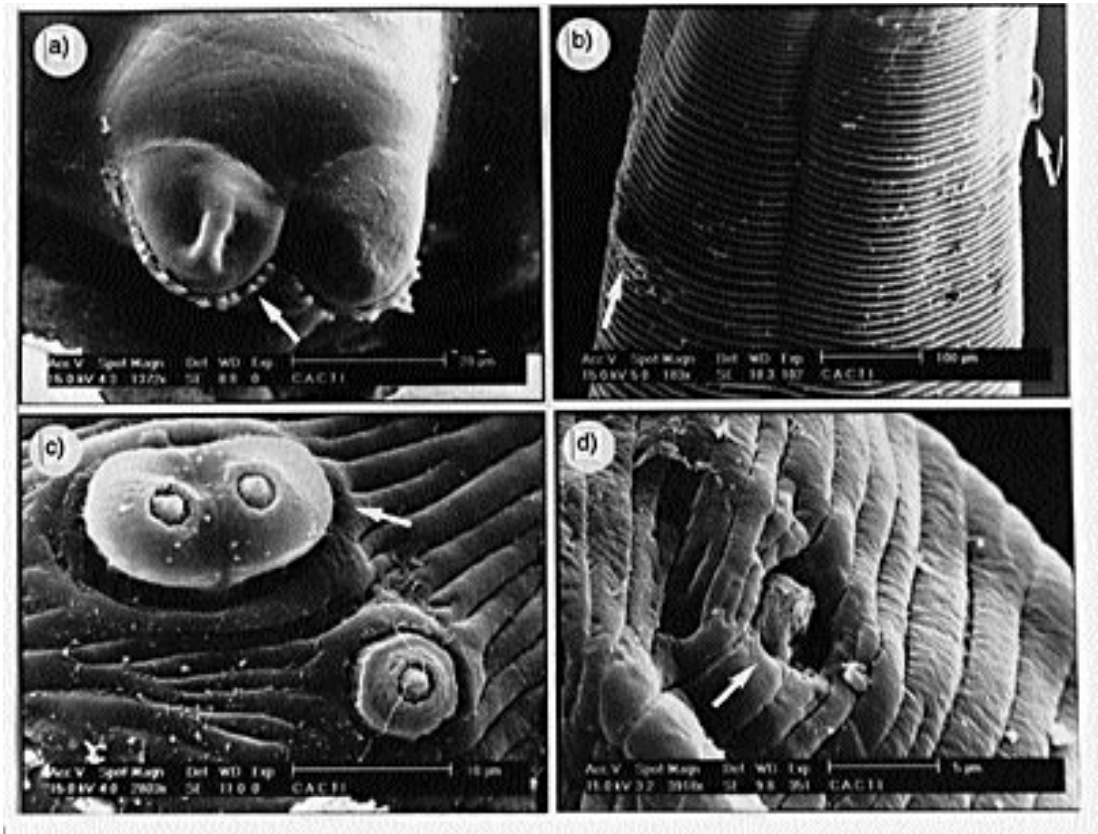


Fig. 2: Additional taxonomic characters observed in adult *Anisakis simplex*. (a) Lip with dentigerous ridges (arrow), (b) a pair of derids (arrows), (c) united paracloacal papillae pair forming double papillae (arrow), (d) phasmid (arrow)

stomach wall, but not in the muscularis (Fig. 4). The anterior extremities of the nematodes were situated deep in the submucosa and were encapsulated in an amorphous eosinophilic substance. The anterior extremities of nematodes and the vacated hyaline caps were surrounded by fibrin, necrotic host tissues and a varying degree of cellular infiltration, which included lymphocytes, macrophages and, mainly, eosinophils. *A. simplex* could be identified in histological sections by the presence of distinctive Y-shaped lateral chords and the absence of lateral alae extending from the cuticle.

DISCUSSION

Pathology

There is little information concerning cephalopod diseases or host defence responses against pathogens.

To date, most reports of macroparasitic infections in cephalopods are descriptive or taxonomic, and the parasite-induced pathology is rarely reported (see review by Hochberg 1990). In vertebrate hosts, anisakid infections often elicit a fibrin concentric encapsulation of the migrating larvae in host tissues (Lackie 1980), as was found in the squid paratenic host examined in this study. Elarifi (1982) suggested that the formation of fibrotic capsules around nematode larvae might prevent further migration and, as a result, the destruction of other host tissues (Pascual *et al.* 1995a). Cephalopod haemocytes have phagocytic capabilities and function primarily in the encapsulation of large parasitic organisms and in the repair of tissue damage resulting from mechanical or biological trauma (Ford 1992). Parasitic castration, as defined by Noble and Noble (1971, as cited by Hurd 1990) and Malek and Cheng (1974), was the most important pathological observation in squid. However, true castration, defined by Baudouin (1974) as an irreversible destruction of the reproductive tract, was not observed. Therefore,

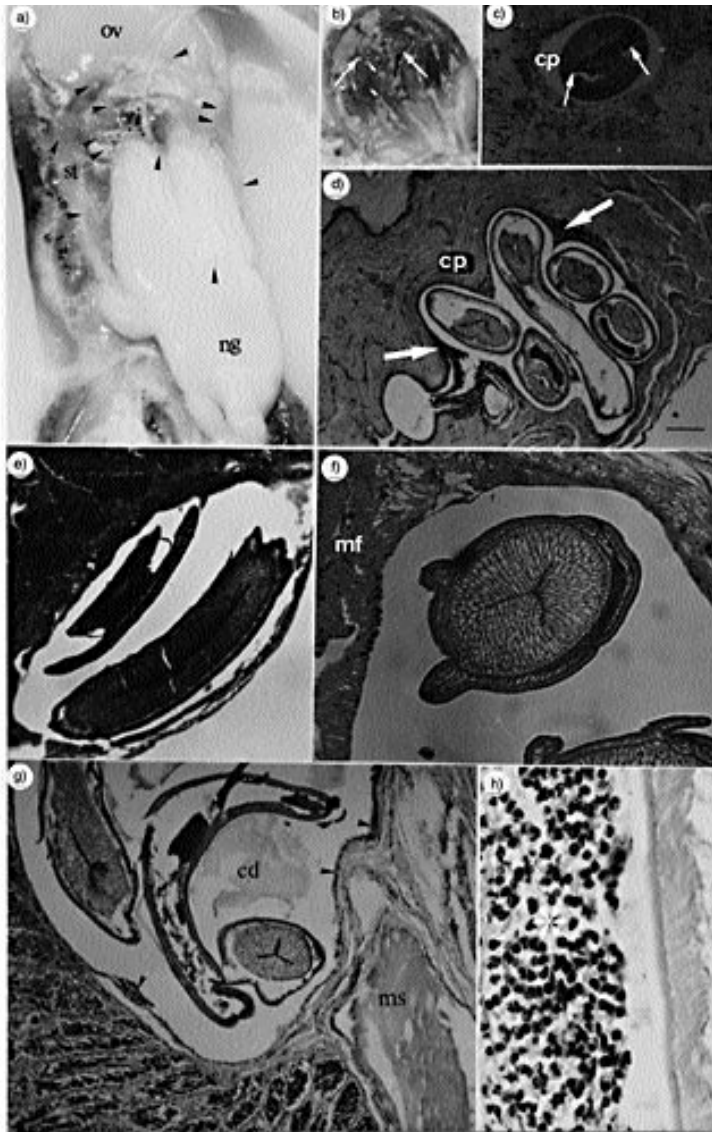


Fig. 3: Gross and micropathology caused by larval stage *Anisakis simplex* in ommastrephid squid. (a) Macrophotograph showing the presence of worm larvae (arrowheads) coiled and encysted in the stomach (st), nidamental gland (ng) and ovary (ov) of *Todaropsis eblanae*. (b) Anisakid larvae (white arrows) coiled and encysted within the stomach wall of *T. eblanae*. (c) Histological section (H&E, 10 \times) of the stomach showing an early infection in *Illex coindetii* (cp, encapsulated larvae; the white arrows show Y-shaped lateral chords). (d) Histological section (H&E, 4 \times) of the stomach of *T. eblanae* showing a later infection where the larva was coiled inside a concentrically arranged connective tissue capsule (cp) with varying degrees of cellular infiltration (white arrows). (e) Section (WT, 10 \times) showing a larva encysted in a connective tissue sheath in the nidamental gland of *T. eblanae*. (f) Section (H&E, 10 \times) showing the larva encysted in the muscle fibres of the mantle (mf). (g) Histological section (H&E, 4 \times) of connective-muscular tissue of the testis of *T. eblanae* showing mechanical compression and displacement of host tissue elements (cd, cell debris; ms, mucus secretion; arrowheads show cellular infiltration). (h) Histological section (H&E, 40 \times) showing heavy haemocyte infiltration (*) in response to the presence of larval worms

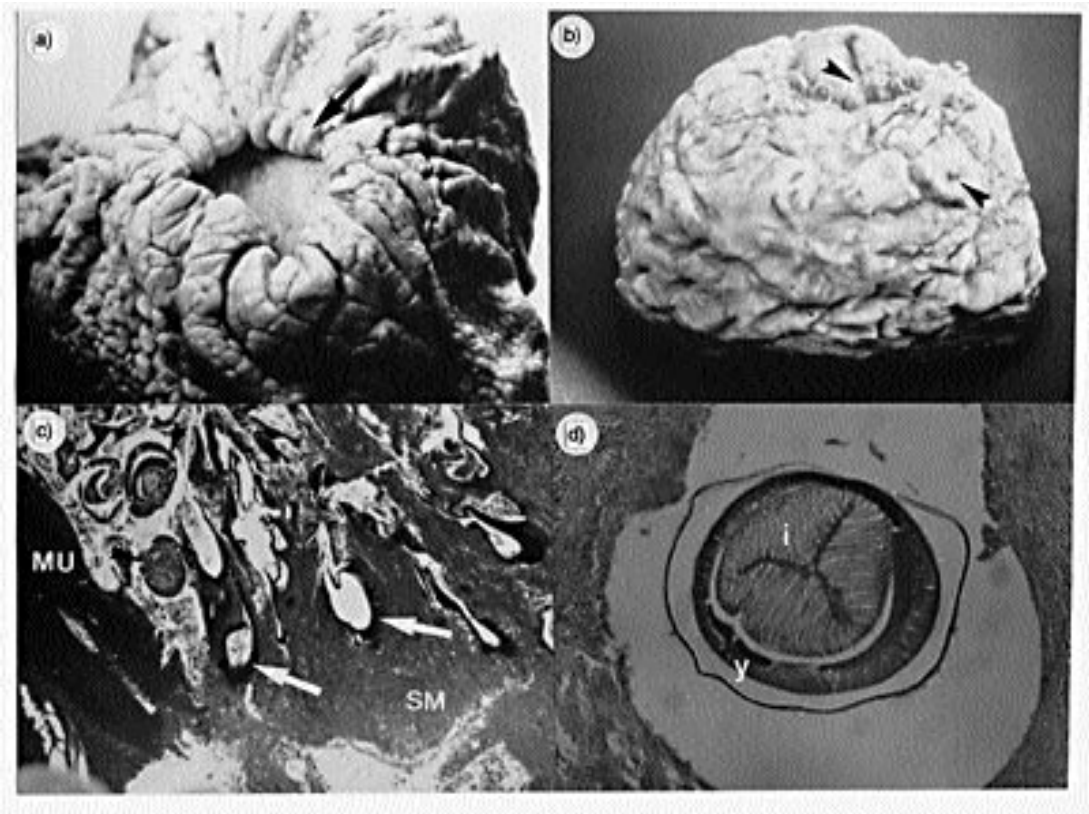


Fig. 4: Gross and micropathology caused by *Anisakis simplex* in the stomach wall of cetaceans stranded in NW Spain; (a) stomach wall ulceration (arrow) of *Delphinus delphis*; (b) cluster of *A. simplex* partially embedded in the inner wall of the stomach of *D. delphis*; (c) transverse section (WT, 4 \times) of the stomach of *Phocoena phocoena*, showing vacuolated hyaline caps (white arrows) in the submucosa (SM) and anisakine parasites (black arrowheads) in the mucosa; (d) tangential section (H&E, 10 \times) of the submucosa of the stomach of *D. delphis*, showing Y-shaped lateral chords (y) and intestine (i) of *Anisakis simplex* larvae

castration seems to be of limited value in discussing the impact of parasites on invertebrate host life histories and population dynamics (Hurd 1990).

The first record of pathology in marine mammals caused by *Anisakis* was documented by Murie (1868). Subsequently, a number of authors have described *Anisakis*-induced ulceration of the stomachs of pinnipeds and cetaceans. Although some investigators (Young and Lowe 1969, Cattán *et al.* 1976) conclude that anisakine-associated gastric lesions in marine mammals are caused by the nematodes themselves, others (Schroeder and Wegforth 1935, Griner 1974, Sweeney 1974) speculate that the anisakines merely invade and aggravate pre-existing ulcers. McClelland (1980) noted that gastric lesions in experimentally infected seals resulted from a host response to pene-

tration of mucosa and submucosa by *Phocanema decipiens* (Krabbe, 1878).

The severity of gastric lesions in marine mammals is presumably attributable to the mechanical irritation associated with large numbers of anisakids penetrating the stomach wall at a single point. Regardless of the factors which cause it, clustering of the anisakines penetrating the mucosa and submucosa of the stomach wall of cetaceans is clearly a form of behaviour which can be detrimental to the host either by causing or aggravating lesions. Clustering and associated pathology seem to be indicative of a breakdown in the mutual tolerance of host and parasite (McClelland 1980).

Larval anisakid nematodes are known to cause public health problems, e.g. anisakiasis (Sakanari

and McKerrow 1989). Considerable information is available in the literature on cases of human infections with larval anisakids and other nematode parasites following the ingestion of raw or undercooked squid and other improperly cooked infected seafood (Nagasawa 1993). In Spain, there have been 12 confirmed cases of anisakiasis (Arenal Vera *et al.* 1991, López-Vélez *et al.* 1992, Valero *et al.* 1992, Martín Cavanna *et al.* 1994, Martín del Olmo *et al.* 1996, Acebes Rey *et al.* 1996, Rodríguez Santiago *et al.* 1996). However, the real prevalence and incidence of anisakiasis is presumably much greater, as was noted in a study of seroprevalence against crude larval antigens of *Anisakis simplex* in a Spanish random population (García-Palacios *et al.* 1996). Because of the vagueness of the symptoms, anisakiasis has been often misdiagnosed as appendicitis, cholecystitis, diverticulitis, tuberculous peritonitis, cancer of the stomach or pancreas, or Crohn's disease (Sakanari and McKerrow 1989). In addition, recent studies have identified *A. simplex* as a new sea-food allergen in Spain (Audicana *et al.* 1995a, b, Montoro *et al.* 1997, Del Pozo *et al.* 1997). Larval anisakid parasites have been implicated in four cases of recidivorous acute urticaria/angioedema in patients who ingested undercooked infected parasitized squid in seafood dishes (Ardusso *et al.* 1996).

Squid as trophic channels for parasites

While knowledge of the trophic ecology of nektonic cephalopods is relatively extensive, their role in the ecology of parasite life cycles and in the transfer of parasites and parasite-caused diseases is incomplete. In the past 40 years, nearly 100 papers have addressed the trophic status of cephalopods in foodwebs of the North-East Atlantic. However, only some 12 publications have dealt with the role of squid as hosts for macroparasites in that geographic area (Hochberg 1990, Pascual *et al.* 1995b, c, 1996a, b). For the coast of North-Western Spain, González *et al.* (1994) analysed the diet of marine mammals, with special reference to Cephalopoda. Ommastrephids constituted 11.9% of the diet of small cetaceans. The results indicate that, whereas *Delphinus delphis*, *Tursiops truncatus* and *Phocoena phocoena* are primarily fish-eaters, *Grampus griseus* and *Globicephala melas* consumed only cephalopods.

Recently, Pascual *et al.* (1996a) analysing the parasite fauna of commercially exploited cephalopod populations off NW Spain, noted that ommastrephids were by far the dominant cephalopod hosts of *Anisakis simplex* B larvae, which complete their life cycle in the common dolphin *D. delphis*, the striped dolphin

Stenella coeruleoalba, the bottlenosed dolphin *T. truncatus*, the long-finned pilot whale *G. melas*, the harbour porpoise *P. phocoena* and the pygmy sperm whale *Kogia breviceps*. Olaso (1990) and Raserio *et al.* (1996) also found important predatory relationships between sympatric teleost-cephalopod nekto-benthic populations on the continental slope and adjacent continental shelf (100-500 m deep) off the coast of Galicia. Off the north-west coast of Spain, most fish species harbour large nematode populations of *A. simplex*, in particular the blue whiting *Micromesistius poutassou* and the horse mackerel *Trachurus trachurus* (Sanmartín Durán *et al.* 1989, Abaunza *et al.* 1995). This suggests considerable trophic exchange of nematode parasites between different species of paratenic hosts, most likely because of overlapping food resources. The final host populations in the parasite's life cycle are mainly piscivorous cetaceans that feed on blue whiting and horse mackerel (González *et al.* 1994). The degree to which parasites are exchanged between teleost fish and ommastrephids is reinforced by the stenoxenous condition (i.e. low host specificity) of the parasite species (Nagasawa 1990).

In the literature, there are surprisingly few records of *Anisakis* in piscivorous seabirds in view of the parasite's abundance in marine fish and squid (Smith and Wooten 1978). Regardless of the small sample size, the current work confirms that scarcity of *Anisakis* in these hosts and also that the paucity of the records does not reflect a lack of parasitological studies on seabirds, as suggested by other authors (Smith and Wooten 1978).

Despite adult forms of *A. simplex* having been reported in the odontocete *G. griseus* and in the mysticete *Megaptera novaeangliae* (Kikuchi *et al.* 1967, Davey 1971), the absence of parasites in the current material may well be explained by the small number of individuals examined (only two *G. griseus* and one *M. novaeangliae*).

Various authors (e.g. Sakanari and McKerrow 1989, Nagasawa 1990) have discussed the life cycle of *Anisakis* and the role of various hosts in detail, and concluded that euphausiids (which become infected by feeding on hatched second-stage larvae) serve as the primary intermediate hosts. However, fish and squid must be regarded as paratenic hosts. Smith (1983) reviewed the life history and ecology of *A. simplex* and discussed the status of teleosts and squid as hosts of its third-stage larvae. He concluded that, regardless of the role of fish and squid hosts, they are "obligatory from the ecological point of view in order for a life-cycle to be completed". Such consideration may apply to the interannual superabundance of *A. simplex* L₃ in a variety of squid

species in the south-eastern North Atlantic (unpublished data), despite the semelparous condition of the squid and the influence of oceanographic factors on their recruitment success (Coelho 1985, Rasero 1994).

It is hoped that this paper will serve to clarify the importance of squid as hosts for parasites and as essential bridges for the transmission of parasite-induced diseases through the food chain to the end-users of the marine environment. It is also hoped that this paper will stimulate more focus on the use of parasites as tags for trophic interactions.

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