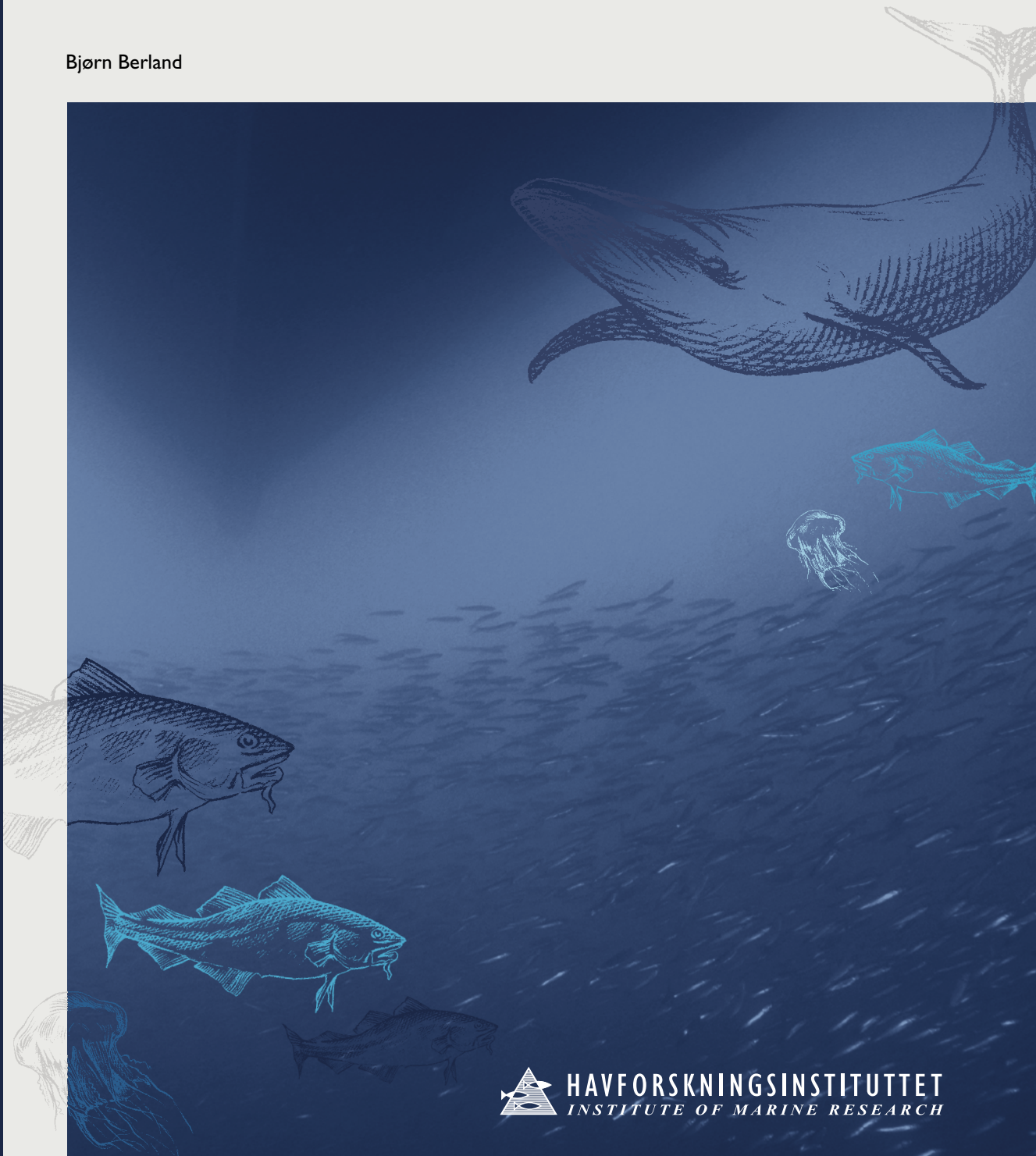


Musings on nematode parasites

Bjørn Berland



PROSJEKTRAPPORT



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De fleste vitenskaplige rapporter følger et standard oppsett: Tittel, introduksjon, materiale og metoder, resultat og diskusjon. Det gjør ikke denne rapporten. Forfatteren har arbeidet med parasitter generelt og nematoder spesielt, i mer enn 50 år, og presenterer her en del basiskunnskap om nematoder. Videre beskriver han hva vi vet og ikke vet om morfologi, biologi og livssykluser hos nematoder i marin fisk og fiskespisende sjøpattedyr og fugler. Den innkapslede nematodelarven i fisk som en møter på når en fisker, tilbereder fisk på kjøkkenet eller når familien spiser middag kan være mer harmløs enn vi tidligere har regnet med.

Summary (English):

Most scientific papers follow a standard template: Title, introduction, material and methods, result and discussion. The present work breaks this standard procedure. Having worked for more than fifty years on parasites in general, and nematodes in particular, I present to the educated public first some basic knowledge on the nematodes, and next describe what we do know, and what we do not know, about the morphology, biology and life cycles of nematodes in marine fish and fish-eating mammals and birds. The encapsulated nematode larvae in fishes encountered by anglers, when preparing fish in the kitchen, or by families at the dinner table, may be less harmless than formerly assumed.

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MUSINGS ON NEMATODE PARASITES

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Preamble

Most scientific papers follow a standard template: title, introduction, material and methods, results and discussion. The present work breaks this standard procedure. Having worked for more than fifty years on parasites in general, and nematodes in particular, I present to the educated public first some basic knowledge on the nematodes, and next describe what we do know, and what we do not know, about the morphology, biology and life cycles of nematodes in marine fish and fish-eating mammals and birds. The encapsulated nematode larvae in fishes encountered by anglers, when preparing fish in the kitchen, or by families at the dinner table, may be less harmless than formerly assumed.

Introduction

Symbiosis means living together. Traditionally the term has been used for the mutually beneficial relationship between two partners which are separate species, and as such it is still used in many textbooks. The term, coined by H.A. de Bary in 1879, is neutral; the relationship may be neutral for both, or positive for one and negative for the other, or positive for both. They may live in close proximity, or one may gain protection or left-overs from the other's food (**commensalism**). In **phoresy** the lesser partner, *phoront*, may get a free ride on the larger one. In **inquilinism** the lesser partner may seek shelter with or in the larger partner. The "positive and negative" symbiosis is **parasitism**. The relationship may be very intimate, the smaller partner may live on – as an **ectoparasite**– or as an **endoparasite**– in the other, who provides "board and lodging". The smaller partner is termed **parasite**, the larger one the **host**. The relationship is positive for the parasite, negative for the host. The parasite is completely dependent on the host, but the host can live quite happily without parasites.

The symbiosis that is mutually beneficial for both partners is called **mutualism**. Biology has many examples of positive cooperation between organisms: plant flowers attract insects, which are paid with nectar for picking up pollen and bring it to the next plant for pollination. Among free-living animals, birds pick ticks and insects off grazing cattle, and in the sea some fish and shrimp species are known to pick ectoparasites off fish – this "de-lousing" is known as **symbiotic cleaning**.

Parasites are regarded as having a negative influence on their hosts, and they may even kill them. Or is it possible that some parasites, at some stage in their life cycles, may be useful for the host, or do useful work? We make the definitions of the various types of symbiotic relationships, but as the animals cannot read they must find good solutions to survive on or in a host and to reproduce in time.

The many definitions of parasitism always stress that the host and the parasite are separate species. But if that requirement is waived, **viviparity**, with one or more embryos developing in the female oviduct or uterus seen in many vertebrates, man included, is a *temporary parasitism for the benefit of the species*. Controversial? Yes, but the organisms do not know our scientific definitions and they do what serves the species (see Brinkmann 1970).

General morphology

The phylum Nematoda – roundworms – is a very special one, comprising a large group of animals. Most are quite small, free-living in mud and soil in freshwater, in the sea and on land, and many are plant parasites. The latter suck juices from plant cells and roots and some are serious agricultural pests. But many are parasites in animals – in invertebrates and vertebrates: fishes, amphibians, reptiles, birds and mammals – in their digestive tracts, muscles or other tissues, including blood. Most have complicated life cycles, some using invertebrates as intermediate hosts for larval stages, which later develop to maturity in vertebrate final hosts. A few reach considerable body size, even several meters. Many are serious parasites of man, domestic animals, and wildlife. For an overview of the nematode parasites of vertebrates, see Anderson (2000).

There is a famous adage by N.A. Cobb, cited by Roberts & Janovy (1996) in their introduction to the nematodes: *If the entire earth, except the nematodes, were to disappear, the nematodes would form a “veil” making the outline of our globe visible.*

Nema means thread, thus indicating that they are long and slender. But the vernacular *roundworm* tells that their bodies are circular in cross section. In contrast to other parasitic helminths, such as the flatworms, comprising the monogeneans, trematodes and tapeworms, the nematodes have an outer cuticle that gives their body structural rigidity. The internal turgor keeps the body “pumped up”. The cuticle and underlying muscles form an outer tube, and a central digestive tract runs almost the entire length of the animal, with a mouth at the anterior and a rectum with anus near the tip of the tail. The mouth may be a narrow tube or a spacious buccal capsule, or the head may even bear lips. The mouth is followed by the muscular oesophagus, which has radiating muscle fibres and glandular tissue. As the body is turgid, the oesophageal muscles pump food or fluid into the intestine.

A typical cross section of the nematode body – *Ascaris* is a good textbook example – shows the outer cuticle with underlying hypodermal cells. In the dorsal and ventral midline runs a thickened chord with a longitudinal nerve in each chord, and a pair of lateral hypodermal thickenings is also present. Below the hypodermal cells are longitudinal muscle cells. Thus the body section is seen to be divided into four quadrants, each with a number of muscle cells (Fig. 1). *Ascaris* from pig is the textbook nematode, but it is too large to be placed on a slide for microscopy. The smaller nematodes from fish are better alternatives: they fit on a slide, alive, or fixed and made partly transparent in glycerol, all internal structures can be seen in the microscope.

In most animals the nerves send fine branches to the muscles, but in nematodes each muscle cell sends at least one “arm” to the nearest longitudinal nerve (Soleim & Berland 1981). An impulse in the dorsal nerve makes the left and right muscles in the body’s dorsal half contract, and an alternate similar impulse in the ventral nerve makes the ventral muscles contract. The dorsal and ventral muscles are stimulated alternately. The nematode body moves only in the dorso-ventral plane – they “nod” – but as nematodes kept alive in a Petri dish lie on their sides, they seem to move laterally.

Returning to the cross section, the digestive tract – oesophagus and intestine – is centrally located. Between the gut and the body wall muscles is a body cavity. This is a *pseudocoel*, because it is not lined with a thin epithelium, as in the true coelomate groups.

Reproduction

The reproductive organs are located in the pseudocoel. Nematodes have separate sexes – males and females. Most females have two *ovaries* in which the ova are formed. Each ovum is released into the oviduct where it becomes fertilized by waiting male spermatozoa. Upon fertilization an egg shell quickly forms around the egg, which is pushed down the coiled oviducts which widen and join to form a Y-shaped uterus. This leads via a single vagina to the vulva, which opens somewhere on her ventral surface. The oviducts and uteri become filled with *eggs*. Some lay undeveloped eggs, in others an embryo develops in each egg, and in some small larvae are present when the egg is laid. A larva in the egg may moult, thus the hatching larva may be surrounded by one or two shed cuticles (Køie *et al.*, 1995). In others the females are loaded with wriggling naked or sheathed larvae. They may circulate in the host’s blood as *microfilariae* to be sucked up by blood-feeding insects, or voided into water where they, as part of the life cycle, become ingested by small crustacean intermediate hosts.

The nematode male has only one testis; the produced sperm are conveyed in a sperm duct that via a ventral *ejaculatory duct* opens into the cloaca. In the dorsal wall of the cloaca most male nematodes have a pair of copulatory *spicules*, a few have only one or none. The male tail is curved ventrally – it is hook-shaped (Fig. 4D). Presumably he coils his tail around the female body during copulation, inserting one or both spicules into her vulva/vagina and transfers sperm, which in the larger species have a long way to travel up the female ducts to reach the ova produced by the ovaries.

As the nematode body is turgid, the copulatory process may not be as easy as it looks. The male must first locate the female genital opening, the vulva, for which purpose he has a number of sensory caudal papillae. The number and relative positions of these papillae are taxonomically important. Although the male tail is curved, he must really coil his tail firmly around the female to hold her in a solid grip, and as they both are turgid, he must use force to insert his spicules to open her vulva. This can be imagined as two plastic bottles trying to make bodily contact, one trying to coil itself around the other one. The male should flatten his caudal ventral side, and he should coil his tail to completely encircle the female body (Berland 1999). Can he do it? It seems that very few people have observed parasitic nematodes in copulation, but students of free-living nematodes must have made observations. The female’s body diameter seems to match the curved male tail.

It is almost axiomatic that nematodes have only longitudinal body-wall muscles. However, many line drawings of nematodes show the males to have ventral oblique lines, representing muscles, for a considerable distance in front of the cloaca (Fig.4D). By cutting transverse thin slices with a scalpel of a typical large male, and studying them in the microscope, it is easily seen that muscles in the two ventral quadrants run postero-ventrally towards the midline. When these *copulatory muscles* (Chitwood & Chitwood 1974 p. 51) contract, the male posterior becomes strongly coiled and, as the ventral midline is pulled “upwards”, the ventral body wall becomes flattened, thus ensuring intimate body contact. The two rows of caudal papillae now come to border the flattened ventral surface of the copulating male and the more centrally placed papillae in the immediate vicinity of the cloaca may sense the position of the vulva (Berland 1998c). Admittedly, this is qualified speculation, as few seem to have seen this in parasitic nematodes. A large sample of live nematodes killed in liquid nitrogen may yield a few pairs “caught in the act” to verify my hunches. In some nematodes the precloacal muscles may be arranged as a rosette, creating a sucker.

Many nematodes have tail “wings” – *caudal alae*. When a male, or its cut-off tail, is placed between slide and cover glass to permit a ventral view, the curved tail becomes straightened, and what appear to be alae become visible. Admittedly, some nematodes do have wide caudal alae, but SEM shots of male tails probably give a truer picture of assumed alae. At least some nematode males have a spoon-shaped tail.

The male strongylid nematodes have their tails modified to a *bursa copulatrix*, with the sensory papillae forming rays, similar to the rays keeping an umbrella open. In the spirurine nematodes, as their name indicates, the male tail is spirally coiled in cork-screw fashion. By comparison, the bodies, and shells, of gastropod molluscs are coiled either to the right (dextral) or left (sinistral). From what I have seen the posterior male body and tail in the spirurine nematodes are sinistral, but is this a taxon character?

Does it matter how the male clings to the female? In some nematodes the vagina runs anteriorly from the vulva, in others posteriorly. Are the spicules so flexible that it does not matter how he makes contact? The male ejaculatory duct becomes filled with spermatozoa, which have to be forced via the spicules into the female. Presumably muscles in the duct wall contract, and the tight coiling of his tail may exert so much pressure that the sperm are ejaculated?

The male’s cuticular spicules are formed in a pair of sacs in the cloaca’s dorsal wall. In many species, they are short and of equal size, in others one may be short, the other very long. The spicules commonly have delicate alae. It is easy to understand that a pair of short equal spicules may open the vulva, but they cannot reach far inside her. But in others with one short and one long spicule, the short one may open the vulva, or does it guide the long one? In some nematodes the spicules are very long. Do they extend so far into the female uteri/oviducts that the sperm are delivered “where they are needed”? Do the spicular alae form a closed tube, turning the spicule into a cannula or hypodermic needle? Which roles do spicules’ shape and length – the lock and key principle - play in avoiding mating with wrong species?

Textbooks tell us that practically all animals have very small tailed spermatozoa, like tiny tadpoles, that swim. But nematodes are exceptional in that their sperm are amoeboid and they do not swim but “creep” up the female ducts to reach the ova produced by the ovaries. They are large enough to be seen by microscopy as small grains in cleared specimens. In the larger species the spermatozoa have a long way to travel; do they creep slowly, or do the long spicules place them far up in the uteri or oviducts? Can the uteri and oviducts make reverse peristaltic contractions to bring the spermatozoa to their goal?

Ova are discharged from the solid ovaries into the oviducts, where spermatozoa fertilize them. Very quickly material in the egg cell forms the egg shell, which may be quite thick and ornamented. The oval eggs usually measure about 50 – 90 μm by 40 – 60 μm , but in species with large larvae, the eggs may be larger to provide sufficient “building material”.

The egg production may be very large. In their textbook, Roberts & Janovy (1996) state that a female *Ascaris lumbricoides* may contain 27 million eggs at a time, with 200,000 laid per day. This translates to more than 2 eggs per second!

It is general biological knowledge that the male and female gametes each contribute one set of haploid chromosomes. The resulting fertilized ovum, the zygote, has the full diploid chromosome complement. In almost all animals, during the following cell divisions – mitoses – each and every cell has the full chromosome genetic code, be they muscle or liver cells. But only the relevant part of the code is used in the various tissues and organs. This is the basis for the successful cloning of animals from somatic cells.

In the nematodes the fertilized egg – the zygote – also has the full genetic set-up. The zygote divides to give two cells, which during the next mitoses yield cells that become specialized, some being destined to become hypodermis, muscle, gut, etc. However, the nematodes differ from other organisms in that during each division, excepting cells set aside to become future gonads, some of the chromatin is deleted; the resulting cells do not have the full genetic “toolbox”, but only that part of it that is needed for the specialized cells to do their job. This is called chromatin diminution, discovered by Theodore Boveri in 1887 in the horse ascarid *Parascaris*, but has later been shown also in the pig ascarid *Ascaris suum*. The modern techniques of molecular biology may verify if this “throwing out” of chromatin is valid for nematodes in general.

The nematodes have strictly determinate cleavage and ontogeny and their development follows a set programme, which after a number of steps forms the complete specimen. The fate of each cell is determined. Nematodes thus have cell constancy, each species having a fixed number of somatic cells, but of course the gametes – ova and sperm – are not in this number. Nematodes cannot repair damage. The famous “model nematode”, *Caenorhabditis elegans*, has 959 somatic cells, plus a variable number of germ cells. If this applies to all nematodes, their bodies grow by increasing the volume of each cell. Some textbooks claim, that in large nematodes, cells in the intestine and hypodermis divide further, thus breaking the cell constancy rule.

Their life cycle is fixed; they have five stages with a total of four moults, or ecdyses, between them. In some a tiny 1st stage larva hatches from the egg, in others the 2nd or 3rd stage (Køie *et al.*, 1995). It is generally assumed that the 3rd stage is the infective one for parasites. In the final host the 3rd stage larva grows, and at the 3rd moult the 4th stage appears. After some body growth and the final 4th moult, the 5th stage is formed, but it still requires some time for it to grow, develop to maturity and copulate, becoming the sexually mature adult male or female.

Parasitic nematodes in marine fishes, mammals and fish-eating birds

Nematodes are common parasites in freshwater and marine fish, amphibians, reptiles, birds and mammals, including wildlife and our domestic farm animals. But in practice only fish caught by sport and holiday activities may let children and adults handle fish, and gutting them to see what they have eaten, and possibly notice their parasites, not only nematodes. As student I studied nematodes in marine fish in Norway, and have since searched fish for parasites on several continents. In the following account I shall deal with the common ascarid parasites in marine fish, and their life cycles. However, several other nematodes than these occur in the intestines of fish (Berland 1961a, 1970). All have interesting life cycles with larval stages in prey the hosts feed on.

In principle it is not allowed to import live fish. But ornamental fresh-water fish are imported to supply the many aquarium shops. These tropical fish may harbour several exotic parasites. A nematode, *Camallanus cotti*, originally from Japan, is now widely distributed worldwide. The female, attached to the rectum of the fish, sheds tiny live larvae that are eaten by copepods, which in due course re-infect fish. However, when no copepods are present, it was experimentally shown by Levsen (2002) that it is able to complete its life cycle.

The presence of nematodes in fish, known as “kveis” in Norway, has for centuries been well known to fishermen and coastal people. As a student in the 1950s, I described and identified the common nematodes in some marine fishes in Norway (Berland 1961a). In the North Atlantic, and other seas, marine fishes are often teeming with nematodes: adult worms in stomach and intestine, but more striking are the large numbers of nematode larvae encapsulated on and in their viscera and also in their somatic muscles. Their life cycles involve invertebrates, mainly small crustaceans, as first intermediate hosts. These transfer the tiny larvae to feeding fish, in which the larvae grow and develop to the infective 3rd stage, usually encapsulated on and in viscera and flesh. Small fish, and the viscera of gutted fish, with encapsulated larvae, are eaten by larger fish, thus their parasites are carried up in the food chain. The larvae become digested free, and they may bore through the gut wall of the new fish and become re-encapsulated in this new paratenic or transport host. This process can be repeated as large fish feed on smaller fish; thus old, large carnivorous fish may accumulate very large numbers of nematode larvae in their bodies.

In order to reach maturity the larvae must reach the stomach of their normal definitive hosts: carnivorous fish, aquatic birds (cormorants) and marine mammals – seals and whales, which are known to harbour many fairly large nematodes in their stomachs (Fig. 5). A fish may harbour several larval types, which when eaten by a bird or mammal all arrive in warm host stomach. In a whale the “correct” larvae will thrive, grow, moult, and reproduce, while the

“wrong” larvae do not thrive and are lost. Depending upon their taxonomic position, in their correct hosts they moult, grow and reproduce, while those ending in unsuitable hosts may be digested or lost.

Classification and life cycles

The nematodes dealt with here are ascarids, they belong to the family Anisakidae. The subfamily Anisakinae comprises the genera *Anisakis*, *Pseudoterranova*, *Contracaecum* and *Phocascaris*, all of which mature in the stomach of whales, seals or marine birds. A sister subfamily comprises the genus *Hysterothylacium*, with adults in the gut of fish.

The genus *Hysterothylacium*, formerly known as *Contracaecum* and *Thynnascaris*, are strict fish parasites. In the North Atlantic cod and many other fish are hosts to a large nematode, *H. aduncum*, both as adults and larvae (Berland 1961a, 1989, 1991) (Fig. 3). Other species in the genus *Hysterothylacium* and related genera are known from other fish hosts in all seas. Most parasitic nematodes are restricted to particular sections of the digestive tract, but *H. aduncum* occurs in the entire gut, and its cervical wings – alae – permit it to swim freely the length of the digestive tract. It is never attached to the gut mucosa.

H. aduncum and related species have three lips, its oesophagus has a posterior ventricular appendix and its intestine has an intestinal caecum (Fig. 3C), the opposed appendix and caecum explain the old name *Contracaecum* (which is a valid genus, see below). The excretory pore is at the level of the nerved ring. The conical “cactus tail” possibly may give them a “toe-grip” when “swimming” in the gut contents. In a dead fish it is quite common to see the stomach worms leave the host via mouth and gills, and those in the intestine wriggle out through the anus. Berland & Egidius (1980) reported tangled clusters of large mature *H. aduncum* specimens, forming plugs in the anterior intestines of farmed marine rainbow trout, as possible cause of mortality.

Most parasites, not only the nematodes, are host specific. A genus or species of a parasite is often restricted to one or a few closely related host species, and it is a general fact that a few hosts harbour the majority of the parasites, while the rest are clean or have only few parasites. This makes sense – males and females are needed together in a host to produce “babies”. But *H. aduncum* can live in almost any teleost, both as larva and adult. This indicates that it is an abnormal parasite.

The genera *Anisakis*, *Pseudoterranova* and *Contracaecum/Phocascaris* are all parasites of warm-blooded hosts – whales, seals and fish-eating birds. They have three lips, excretory pore at base of lips, and a large excretory organ. Their 3rd stage larvae become encapsulated in fish. Until fairly recently, the genera *Anisakis*, *Pseudoterranova* and *Contracaecum/Phocascaris* were each considered to have only a few species, each of wide geographical distribution, and their larvae in fish could only be identified to genus on morphological characters.

Anisakis and *Pseudoterranova* have similar lips, no interlabia, both have a large glandular ventricle (Fig. 4B), but only *Pseudoterranova* has an intestinal caecum. These anatomical structures have evolved over time and may be functionally important. *Anisakis* species infect several species of whales, but are also known from seals.

Anisakis simplex, known as the whale or herring worm, is known from the stomach of many toothed and baleen whales. Its 3rd stage larva, arriving with captured fish, is about 2.5 cm long. In small hosts, such as the porpoise (*Phocaena phocaena*) it grows to reach a mean body length of about 6–7 cm, in the larger long-finned pilot whale (*Globicephala melas*) it is slightly larger, and in the much larger minke whale (*Balaenoptera acutorostrata*), the mean female body length is 12.6 cm, range 6.4 – 24 cm (Ugland *et al.*, 2004). The hosts must have means of “informing” the worms to keep their growth potential in check.

The new tools in molecular biology have revealed several new species in the genus *Anisakis* (Mattiucci *et al.*, 1997, 2001), their number has risen to nine (Mattiucci & Nascetti 2006, Valenti *et al.*, 2006). That means that there are as many *Anisakis* larval types, but they are probably morphologically very similar. The *A. simplex* larva in the North Atlantic was by Berland (1961a) described as *Anisakis* sp. larva (I), to distinguish it from *Anisakis* sp. larva (II), that is now known to be the larva of *A. physeteris* in the sperm whale.

Pseudoterranova, formerly known as *Terranova* and *Phocanema*, is known from seals only. *Pseudoterranova* is similar to *Anisakis*, but with a short intestinal caecum (Berland 1989). *P. decipiens*, known as seal worm or cod worm, was considered a parasite of many species of seals, with a cosmopolitan distribution. However, by enzyme electrophoresis *P. decipiens* in the North Atlantic was shown to be comprised of three species, provisionally named A, B and C, and their larvae in fish could also be identified as A, B and C by enzyme electrophoresis (Paggi *et al.*, 1991). The provisional species C, parasitic in the bearded seal, *Erignathus barbatus*, was already known as *P. bulbosa* (Cobb, 1888) from the Pacific, and its larva occurs in the liver of long rough dab, *Hippoglossoides platessoides*, in northern Norwegian waters (Bristow & Berland 1992). The species A, mainly from the grey seal, *Halichoerus grypus*, was redescribed and named *P. krabbei* by Paggi *et al.* (2000), in honour of Krabbe who originally described these parasites as *Ascaris decipiens* Krabbe, 1878. The species B, parasitic mainly in the harbour seal, *Phoca vitulina*, retained the name *P. decipiens* (*sensu stricto*). New *Pseudoterranova* species are becoming known from the southern hemisphere (George-Nascimento & Urrutia 2000); the genus comprises at least seven species in seals in all seas (Nadler *et al.* 2005).

Species in the genera *Contracaecum* and *Phocascaris*, parasites of marine birds and seals, also have opposed intestinal caecum and ventricular appendix, which explain the name *Contracaecum*. *Contracaecum* species also have three lips, but in addition have between them well-developed interlabia. Adult specimens from seals and fish-eating birds can be identified to species, but not their larvae in fish. The genus *Phocascaris*, with only three described species, are known from arctic seals. As they have only small interlabial knobs, they do not fit into the genus *Contracaecum*. As the larvae of both *Contracaecum* and *Phocascaris* lack the adult characters, they can only be named *Contracaecum/Phocascaris*.

The species *Contracaecum osculatum*, from several seals in the Atlantic Arctic-Boreal region, were shown by Nascetti *et al.* (1993) to be composed of three sibling species. In fish-eating birds *Contracaecum rudolphii* was similarly split into three sibling species by Nascetti *et al.* (1988). The phylogenetic relationships, among species in the genera *Contracaecum* and

Phocascaris, based on nuclear rDNA sequence data, were studied by Nadler *et al.* (2000, 2005).

Head, oesophagus and feeding of adult worms

What do the worms do, and eat, in the gut of their hosts? Visible food particles are never seen in their intestines so we may conclude that they drink nutrient soup. As they have glands in their oesophagus and ventricular appendix, do they digest their way into decomposing prey? Some of these nematodes also have a large excretory gland that opens by a pore on the head; its secretions could equally well play a role in “softening” food or host tissue. By comparison, spiders cannot chew their prey, but puncture and “spit” into it and by squeezing the corpse between their gnathobases, drink the liquefied digested tissues.

The nematodes dealt with here, all being ascarids, have three large lips (Fig. 3B). The sucking action of the oesophagus draws fluid towards the mouth opening, but the presence of conspicuous interlabia in some genera may play a role in keeping large food particles from entering the mouth radially. Further, in the genera *Contracaecum* and *Phocascaris*, the cuticle on the head is thrown into large transverse folds that may form concentric valves to keep digestive secretions near lips from leaking away and to keep large particles from entering the mouth.

In many whales and seals, nematodes may be lodged in clusters in craters in the stomach wall. Do they “attack” the host when little or no food is present? Seals have fasting periods, when they haul out on land or ice to breed, mate or moult. Are these fasting stints a clever way to reduce the number of stomach nematodes?

What happens when there is no food in the stomach and intestine – when the host is starving? The worms probably feed on mucus and fluids, but for *H. aduncum* it seems that during winter their numbers may be reduced, and reproduction is reduced or stopped. Möller (1976) showed that fish kept without food in aquaria lost their gut nematodes.

Hysterothylacium and related genera have an intestinal caecum and a short oesophageal ventricle with a fairly large posterior appendix (Soleim & Berland 1981) (Fig. 3A). The caecum increases the intestine’s inner surface, but the size of the ventricular appendix may indicate the species’ ability to bore into prey. Note that their 3rd stage larvae, commonly encapsulated in fish, also have a caecum and an appendix, and these structures are probably also active in the larvae. Species in the genera *Contracaecum* and *Phocascaris*, parasites of marine birds and seals, also have an intestinal caecum and a ventricular appendix. *Anisakis* has a large ventricle, but neither appendix nor caecum (Fig. 4B).

Life cycles and larvae

In the North Atlantic there are four anisakid larval types in marine teleosts: *Hysterothylacium aduncum* (Fig. 3), *Anisakis simplex* (Fig. 4 A, B & C), *Pseudoterranova decipiens* (sensu lato) and *Phocascaris/Contracaecum* (Fig. 5) (see Berland 1989). *H. aduncum* larvae, found on the

stomach and between pyloric caeca of their fish intermediate hosts, have obviously moved only a short distance from the gut. *Anisakis* larvae, forming tight coils 3–4 mm in diameter (Fig. 4A) on the viscera, are very conspicuous against the light-coloured liver surface, and sometimes also in the belly flaps. The yellow-brown *Pseudoterranova* larvae bore into the dorsal somatic muscles of cod and other fish, and may cause a stir at dinner table.

Phocascaris/Contracaecum larvae of more irregular shape are encapsulated on the viscera.

All these larvae, being 3rd stage, have the oesophageal-intestinal characters of the adults, but they are all without lips, and they have a so-called boring tooth on the head, ventral to the mouth (Fig. 3C). *H. aduncum* larva has the excretory pore at the level of the nerve ring; in the others it is located far forward near the boring tooth. As *H. aduncum* matures in cold-blooded fish, the anlagen of the gonads and their ducts are visible in the larvae, and it is possible to tell their future sex: the male with a single zigzag ventral cord, the female with two wavy lateral cords. Aspects of its life cycle and morphology were studied by Kjøie (1993). In the other larvae, destined to reach maturity in warm-blooded hosts, there is no visible trace of future gonads.

As the encapsulated larvae may live for a long time in their fish intermediate or transport hosts, they must strike a balance with the host to remain viable; they need nutrients and oxygen. The connective tissue capsule, produced by the host, is histologically very similar to the mammalian epidermis, being a stratified squamous epithelium. Kahl (1938–1939), studying the various larvae in fish, described and depicted the larvae in their capsules. If the capsule wall becomes too thick, the larvae may die *in situ* and slowly be resorbed; dead and “wilted” larvae are sometimes seen. Larsen found, by TEM, fragments of host white blood cells in the gut of encapsulated *H. aduncum* larvae (Larsen & Berland 1979, Larsen 1980). The encapsulated 3rd stage larvae of *H. aduncum* feed and grow, and as the other larvae are similarly encapsulated, they also must feed and grow, albeit slowly, and they may remain alive for years. The likely explanation is that the larval head, with its protruding boring tooth, lacerates the inside of the capsule, which becomes damaged, permitting its body to grow, and host cells coming to repair the damage, are consumed.

Are the oesophageal glands, glandular ventricle and appendix active in the larvae, helping to soften or digest lightly the capsule inner wall? The nematodes also have an excretory organ, which in some, such as *Anisakis*, is very well developed. If it does not function in excretion, its secretions, leaving the pore on the head, may have a “softening” effect on surrounding host tissue? As the excretory organ is well developed also in the adult nematodes, its secretions may have a similar function.

A bony fish may harbour several larval types (Fig. 5). What happens when such a fish is devoured by any of the warm-blooded fish-eating birds or mammals? In a whale only *Anisakis* larvae will develop to maturity, while others may start to develop and possibly moult to the 4th stage, but they do not make it to maturity. In a suitable seal the *Contracaecum* and *Pseudoterranova* larvae may develop to maturity. Seals are also known as hosts for *Anisakis* species, but if they moult to the 5th stage, do they produce eggs as they would in a whale?

Anisakis larvae are also common in saithe, *Pollachius virens* (Højgaard 1995, Strømnes & Andersen 1998, 2000, 2003, Klimpel *et al.*, 2004)). Strømnes (2006), studying the seasonal

variation in infection with *Anisakis* larvae in saithe (*Pollachius virens*), cod (*Gadus morhua*), and redfish (*Sebastes marinus*), found the prevalence to be close to 100 % in saithe, and for all three a “spring rise” in abundance occurred. He found a positive correlation between fish length and worm length. Although most *Anisakis* larvae were found in the viscera in the fish species, the muscles of the fatter redfish were rather more infected. Strømnes experimentally found that *Anisakis* larvae moved toward fat in agar with fish oil added.

From my own experience, and that of others, the pelagic blue whiting, *Micromesistius poutassou*, is very heavily infected with *Anisakis* larvae, not only in the viscera, but also in the somatic muscles, mainly in the belly flaps (Højgaard 1980, 1998b, Karasev 1990). This pelagic fish feeds on krill, which are known as first intermediate hosts for *Anisakis* (see Smith 1971, 1983); it is thus not strange that this fish becomes loaded with *Anisakis* larvae, not only in the viscera but also in the muscles. The blue whiting is further of interest as host to coccidian oocysts, loaded with spores, in their livers. This was originally believed to be an *Eimeria* sp. (Berland & Højgaard 1981), but is probably a *Goussia* sp. (see Costa & MacKenzie 1993, 1994).

The flesh of gadoid fish is lean; most of the fat is stored in the liver. During winter and periods when food may be scarce, and when the fish prepares to reproduce, the liver store may shrink to provide metabolic energy and “building material” for ovaries and testes and their gametes. What happens to the many coccidian oocysts in the liver tissue and the multitude of encapsulated *Anisakis* larvae on and in the liver of the blue whiting if and when the liver shrinks in size? Is there so little liver tissue left that the fish dies? If yes, the parasites have an effect on fish mortality. Or are these parasites eliminated or purged and the fish starts with a small, but clean liver after spawning? Possibly yes, but not very likely.

Why do *Anisakis* larvae sometimes occur in the fish flesh, mainly in the belly flaps? As the whole fish is swallowed by the whale final host, there is no reason for the larvae to migrate longer than necessary in the fish intermediate host. In a normal gadoid - the cod and saithe are good examples - the two large liver lobes lie between the body wall and the stomach-intestine. Larvae migrating from stomach-intestine to the body cavity “meet” the soft liver when emerging, and after a little wandering they coil up and become encapsulated. I have sometimes seen saithe in spring/early summer with almost vestigial livers. During winters, when food is scarce, the energy reserve in the liver may be drawn upon, thus shrinking its size. In such fish the stomach and intestine are in intimate contact with the body wall, i.e. the belly flaps, and larvae migrating through the gut wall do not meet the liver lobes, but continue straight into the belly flaps where they coil up and become encapsulated. In 1987 the Barents Sea was in crisis, and the cod, being very lean, became infected with many *Anisakis*, and some *P. decipiens*, larvae, in the flesh (Berland & Hemmingsen 1991).

Pseudoterranova decipiens larvae are mainly found as light brown “screws” in the flesh of cod and other fish, sometimes also in their livers. Keeping in mind that seals, as final hosts, prefer the flesh and liver of large fish, it makes sense for the larvae to wander much further in their fish intermediate hosts than *Anisakis* does.

Can parasites sometimes be useful for the host?

I have often been struck by the presence of *H. aduncum* specimens in the stomachs of fish having recently ingested a whole fish. The worms wriggle or bore into the dead fish, and in so doing make holes and openings in it, mechanically loosening it up and making way for the digestive fluids to penetrate deep into the prey, thus speeding up digestion. Digestive fluids may liberate encapsulated larvae in the ingested fish, and these may “tear down their prisons”. This nematode may thus be a “benign” one by breaking down large ingested prey.

I have also seen the same in birds, once in a cormorant with 3 or 4 sculpins (*Myoxocephalus scorpius*) stacked on top of each other in the oesophagus, the “bottom” one in the stomach riddled with *Contracaecum rudolphi* specimens, boring deeply into the fish. In my opinion, these so-called parasites do useful work for the hosts; they “pay for board and lodging” by mechanically breaking down and fragmenting ingested prey (Berland 1980, 1998a & b).

The symbiotic relationship is then not parasitism, but **mutualism**. This I cannot prove, but the idea is fascinating. Another example is a large milky-white copepod, *Ommatokoita elongata*, attached to the eyes of the Greenland shark, *Somniosus microcephalus*. These may act as lures, attracting the attention of seals and fish to approach this voracious, but sluggish, shark (Berland 1961b). Again, the animals cannot read, they do what is strategically wise.

Seals, whales and large fish are known to have large numbers of nematodes in their stomachs. As these animals swallow their prey whole, stomach nematodes may also in these hosts bore into prey and “swim” in the stomach contents. Their behaviour is “probing”. It is quite common to find a nematode that has been stuck in a narrow opening in a fish bone, as shown in Fig. 2B.

By contrast, sharks and skates have only few stomach nematodes, attached to, or bored into, the stomach mucosa. Without teeth to chew and fragment food, and no nematodes to “dig into prey”, digestion is a surface phenomenon. Some sharks, at least in cool seas, seem to have slow digestion (Berland 1960). Large snakes, swallowing very large entire prey, which may take months to digest, might benefit from gastric nematode tenants to fragment food, but this solution has apparently not been chosen.

Anderson (2000 p. 391), using marked food, found that nematodes of the genera *Physaloptera* and *Turgida*, parasitic in carnivores and birds of prey, and neotropical mammals respectively, do not normally feed on the gastric mucosa, but detach and feed on food in the stomach. The development of *P. maxillaris* from 3rd to later stages depends on the presence of ample food in the stomach. In the unfed host, the nematode remains in the 3rd stage, attached to the gastric mucosa.

Fish, whales, seals and birds may gorge themselves when food is plentiful, and their internal tenants share in the feast. If they mechanically fragment the hosts' food, they pay for board and lodging into the bargain.

Typical plankton feeders filter out small organisms; their food is already “fragmented” and is easily digested. For animals feeding on larger organisms, it is important to mechanically fragment their food. This can be done by teeth cutting, grinding or chewing prey, but animals without teeth (birds and baleen whales) or with pointed teeth (toothed whales and seals), still need to fragment ingested food quickly.

Mammals generally have specialized teeth, sharp incisors and pointed canines to grab, hold and pierce prey or cut plant material, and premolars and molars to chew or fragment food before it is swallowed. This is strikingly seen in plant feeders – horses, cows and rodents. Fish-eating birds and mammals, on the other hand, swallow entire fish whole or in large chunks. Some animals are known to ingest pebbles or grit to act as grinders – a gastric mill – in their stomachs; this is known for some reptiles and plant eating birds. I have often found pebbles and clay in the stomachs of hooded seals (*Cystophora cristata*), but is this universal?

The toothed whales – dolphins and porpoises – have pointed conical teeth, which are excellent for grabbing slippery prey, but they cannot be used for chewing. The prey has to be swallowed whole. Seals also have sharp pointed teeth, which grab and hold prey. Seals also do not chew food, but hauling out on land or ice they may land their prey and tear off chunks of muscles and viscera, leaving the head and most of the skeleton of large fish. The baleen whales, and all birds, lack teeth, and they have to swallow their prey whole. Note that the birds swallow their prey head first – this probably applies also to the other fish eaters. Do they rely to some extent on parasitic nematodes to fragment ingested prey?

Speciation and life cycles

The interesting fact is that the larvae in fish, which can only be identified to genus on their morphology, can be identified to species using enzyme electrophoresis and DNA analysis. This also applies to sexually mature adults which may be very difficult to identify on morphological characters. The new species are ecological species, their eggs and larvae are channelled, via invertebrate and fish intermediate or transport hosts, to the “correct” final hosts. Perhaps in the future the species may evolve characters that permit morphological identification

The life cycles of these nematodes involve marine invertebrates as 1st intermediate hosts, which transfer them up the food chain to fish. The infective 3rd stage larva, arriving in the gut of a fish, has a so-called boring tooth on its head, but it is not known if this is used to bore its way through the gut wall. Having glandular tissue in their ventricle and oesophageal appendix, and a large excretory organ, they may well use their secretions to ease the penetration. The larvae migrate through the gut wall into the body cavity, and some even penetrate into the somatic muscles. How far they wander may also part of their strategy – there is energy parsimony. To the fish host, they are foreign bodies that have to be “wrapped up” by its immune system; the larvae become encapsulated mainly in and on the viscera and to a lesser extent in the flesh. The immune system in very young fish is probably poorly developed, because in very young fish “naked” nematode larvae may be found in its body cavity; the encapsulation response developing slowly. Such “naked” *H. aduncum* larvae in the

body cavities of very young halibuts (*Hippoglossus hippoglossus*) made one of their eyes pop out, killing the fish (Bristow 1990).

When a fish, harbouring a 3rd stage larva of *H. aduncum*, or the viscera of a gutted one thrown into the sea, is eaten by another fish, the larva may, depending upon its size, grow to maturity in the new definitive host (Køie 1993), or become re-encapsulated. The larvae of *Anisakis*, *Pseudoterranova*, *Contracaecum* and *Phocascaris*, similarly ingested by a fish, become digested free in its stomach, and the migrations into the body cavity and re-encapsulations are repeated. Thus large and old fish may over the years accumulate enormous numbers of encapsulated larvae in their viscera. The repeated challenges may lead to increasing immune resistance to new larvae, which may be stopped halfway through the thickened stomach wall, or even be lodged in clusters in craters in the stomach, their tails sticking out (Berland 1981) (Fig. 2A). The fish is an intermediate or transport host; when it is captured and eaten by a warm-blooded mammal or bird (Fig. 5), the worms arrive in a warm stomach and, if the host is correct, grow, moult twice and reach maturity, mate, reproduce and die. The eggs, and the spent worms, are shed with the host's faeces.

The worms in the warm-blooded host have in principle two strategy options: a) become mature early at small body size, live long with modest total egg out-put, or b) grow to large body size before becoming mature, copulate, produce many eggs and shed them all in a few days at the end of their short lives. For *Anisakis* in whales the second option has been "chosen". Their entire stay in the stomach of the definitive host may last 2–3 months, and when they are spent new recruits "are waiting in the wings" to replace them. A large *A. simplex* female may contain close to 2 million eggs (Ugland *et al.*, 2004). No development takes place in the eggs in the host's warm intestine, until they are voided into the cold and oxygen rich sea (Højgaard 1998a).

The larvae with boring tooth are all 3rd stage, and lips are absent. In the final host they grow and moult to the 4th stage, which has lost the boring tooth and has developed lips. Interlabia are not present in genera and species which have such characters in their 5th stage, thus 4th and 5th stage worms can be distinguished. The 4th stage grows in size, and at the final fourth moult, the last 5th stage appears. This is not fully grown, but reproductive organs develop, the worms mate and in due course the female's ducts are filled with ova.

Nematodes and human health

Starting as a student, more than fifty years ago, to study nematodes of marine fish, the attitude of my fellow students at the time was that the subject is academically interesting, but knowledge gained would never be of any practical use. In the 1950s consumption of very lightly salted herring led to acute stomach pains, caused by live *Anisakis* larvae, in a few people in the Netherlands, making human anisakiasis/anisakidosis a hot medical topic in the 1960s (Berland 2003). In the 1970s aquaculture started modestly in Norway, and lo and behold, the farmed fish became infected by parasites already known from wild fish, both in fresh water and in the sea. In the 1980s live *Anisakis* larvae from marinated herring wriggled across millions of German TV screens, leading to instant fish market collapse. This prompted H. Möller to organize a workshop on "*Nematode problems in North Atlantic fish*", held in

Kiel, Germany 3–4 April 1989. All contributions were published by convener Möller (1989), including mine on identification of the larval nematodes (Berland 1989).

The fish nematodes, collectively known as *kveis* in Norwegian, have in Scandinavia never been a health hazard, because fish are cooked, fried, dried or properly salted. The closest we get to raw fish is lightly marinated salmon – *gravlaks*. But in the Far East, raw fresh fish is considered a delicacy. The accumulated human cases of *anisakidosis* worldwide was stated by Ishikura *et al.* (1998) to be 35 000 by the end of 1997, about 32 300 of them from Japan. Since then, the numbers are likely to have risen: searching for *Anisakis* on the Internet yields many more recent reports. If the western world adopts a taste for sushi and sashimi Japanese style, human cases of acute stomach pains, caused by live larvae of *Anisakis* and *related genera*, may increase.

As the food health authorities, and the general public, are becoming aware of the possible presence of nematode larvae in fish flesh (they are aesthetically unpleasant) practical and cheap measures to find and remove larvae must be adopted. By so-called candling with strong light from below, larvae in fresh and frozen fillets can be spotted, but many are easily missed. Dead larvae fluoresce under UV light, permitting larvae in frozen fish fillets to be spotted, but the UV-energy does not reach deep-seated larvae, which are easily missed. After normal candling, fillets and bones with attached fish flesh were by Levsen *et al.* (2005) digested in pepsin-HCl to find possible remaining larvae missed by visual inspection. By freezing the digested left-overs briefly, the remaining missed larvae stood out brightly under UV-light.

It is almost axiomatic that when a fish dies its encapsulated nematode larvae start to wriggle in the body cavity and into the flesh. However, as long as the capsules are intact, the worms are “locked”, but of course autolysis eventually “softens” the viscera and the larvae then start to move. In modern fisheries, when hundreds or thousands of fish are hoisted out of water in net-bags, and transported in fishing vessels’ holds, the “bottom” fish are under great pressure, which causes the capsules to rupture, thus freeing the larvae (Berland 2003). Roepstorff *et al.* (1993) handled very gently small samples of live herring and kept them ungutted for several days on ice; there was no significant *post mortem* migration. In whole salted fish such as herring, larvae in the viscera liberated by autolysis or rough handling, try to evade the increasing salt concentration by moving into the less salty muscles, where they eventually are overtaken and die.

The nematode larvae in fish are killed by proper freezing for several days, and it is important that the core temperature is sufficiently low for all worms to die (search www.lovddata.no for details). In continental Europe there is a market for unfrozen, salted and marinated fish, particularly herring that is consumed as is. (The British have not acquired a taste for such “raw food”, which is strange in view of the close contact for several hundred years between the British Isles and the European Continent.) The combination of salt, acid, temperature and time are crucial to make sure that nematode larvae are dead before consumption (Karl *et al.*, 1995).

BERLAND: Musings on nematode parasites

The public, and the food safety authorities of fish in several countries, are now aware of the aesthetic and health effects of fish parasites. There is much more work waiting to be done and to keep the public informed about the results obtained.

Further, in recent years strong immune reactions to fish nematodes have been reported in the literature, mainly in Spain. Search the web, using *Anisakis* and *allergy* as cue words, and see Levsen *et al.* (2005) for recent references. In Spain, several studies on effects of ingestion of aromatic monoterpenes with larvicidal properties against *Anisakis simplex* 3rd stage larvae have been successfully carried out, damaging or killing the worms (Hierro 2004, Hierro *et al.*, 2006, Valero *et al.*, 2006).

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FIGURES

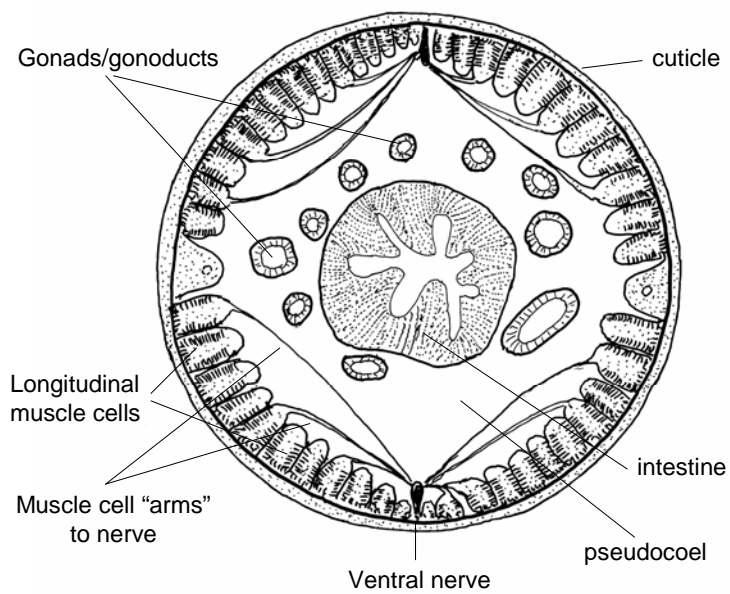


Fig. 1. Schematic transverse section of a nematode.

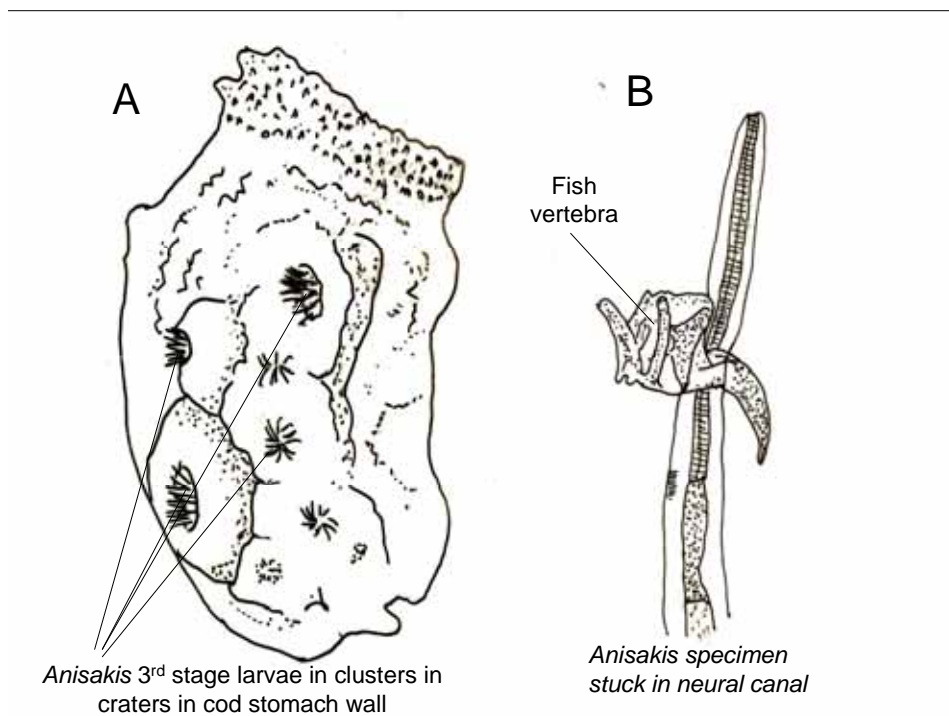


Fig. 2. **A.** Clusters of *Anisakis* larvae in craters in stomach wall of a very large cod. (redrawn after Berland 1981). **B.** An *Anisakis* specimen from a whale stomach stuck in the neural canal of a fish vertebra.

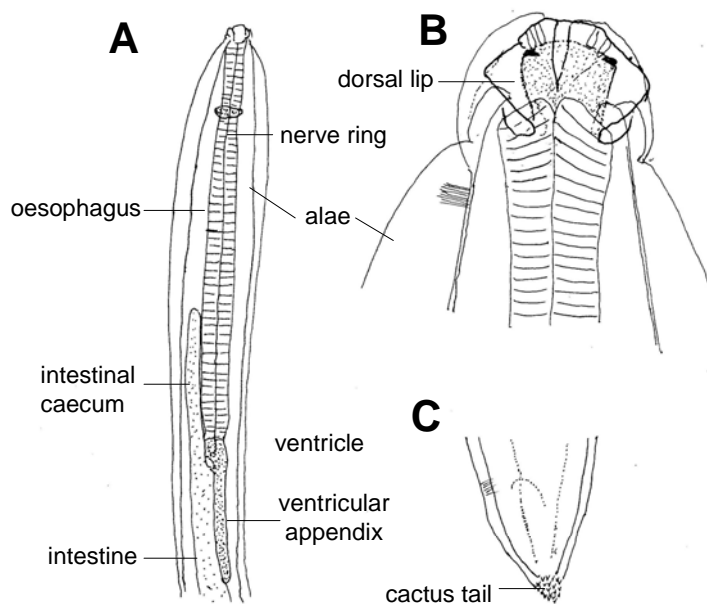


Fig. 3. *Hysterothylacium aduncum*. **A.** Anterior, showing oesophagus and opposed ventricular appendix and anterior intestinal caecum. **B.** Head with three lips, note cervical alae. **C.** Tail in both sexes with characteristic terminal "cactus".

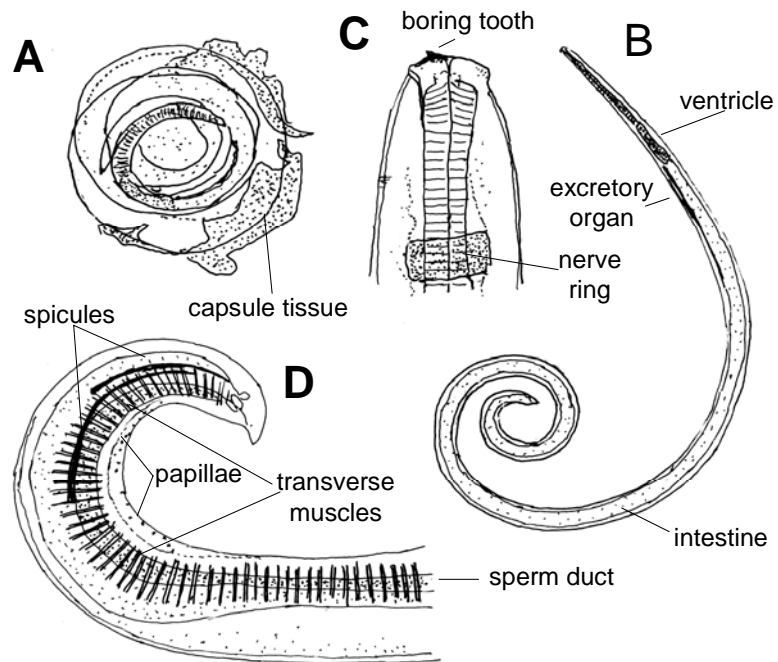


Fig. 4. *Anisakis*. **A.** *A. simplex* larva as found encapsulated on and in fish viscera. **B.** Larva removed from capsule and killed in hot alcohol or glacial acetic acid, note shape with head facing out. **C.** Head, note boring tooth. (A, B and C redrawn after Berland 1989). **D.** Posterior of adult male *Anisakis* sp. from whale stomach; note caudal papillae, two unequal spicules and transverse ventro-lateral muscles.

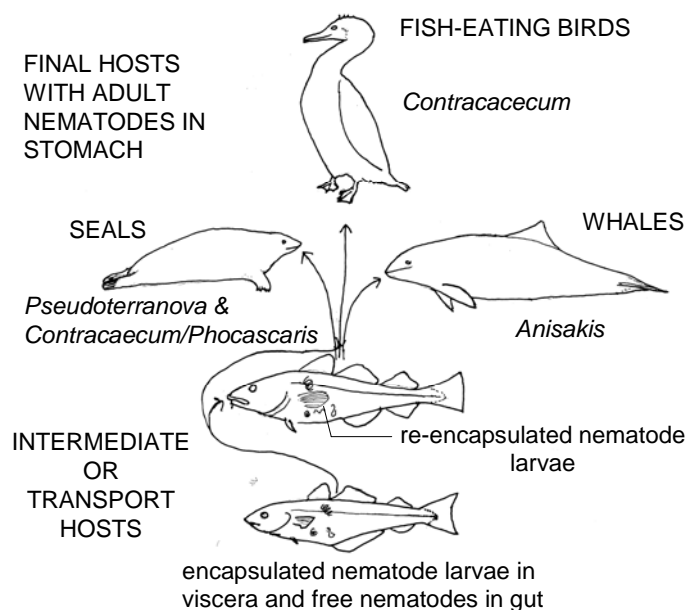


Fig. 5. Schematic illustration of the life cycles of ascarid nematode parasites. Many fish serve as intermediate or transport hosts for their larvae, which are carried up the food chain to fish-eating birds and marine mammals, which harbour the sexually mature males and females.

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