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Variations in Abundance of Larval Anisakines,Sealworm(*Phocanema decipiens*) and Related Species in Cod and Flatfish from the Southern Gulf of St.Lawrence (4T) and the Breton Shelf (4Vn)

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Canadian Technical Report of Fisheries and Aquatic Sciences No. 1201

October, 1983

VARIATIONS IN ABUNDANCE OF LARVAL ANISAKINES, SEALWORM (PHOCANEMA DECIPIENS) AND RELATED SPECIES IN COD AND FLATFISH FROM THE SOUTHERN GULF OF ST. LAWRENCE (4T) AND THE BRETON SHELF (4Vn)

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ABSTRACT

McClelland, G., R.K. Misra and D.J. Marcogliese. 1983. Variations in abundance of larval anisakines, sealworm (Phocanema decipiens), and related species in cod and flatfish from the southern Gulf of St. Lawrence (4T) and the Breton Shelf (4Vn). Can. Tech. Rep. Fish. Aquat. Sci. No. 1201, ix + 51 p.

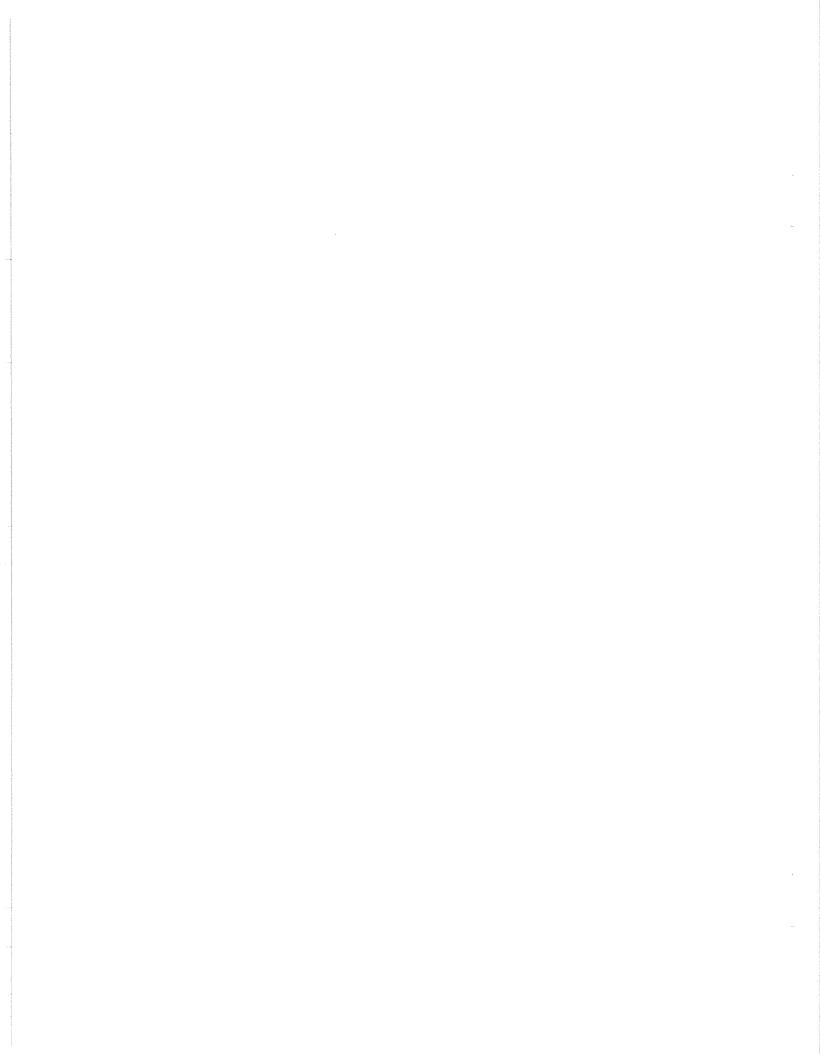
The life cycle, medical importance, and geographic distribution of "sealworm" (Phocanema decipiens) are reviewed and methods for controlling the parasite are discussed. Also reported here are current records of the abundance of sealworm and related species of anisakine nematode (Anisakis sp. and Contracaecum sp.) in cod (Gadus morhua), American plaice (Hippoglossoides platessoides), and gray sole (Glyptocephalus cynoglossus) from the southern Gulf of St. Lawrence (4T) and Breton Shelf (4Vn). Sealworm occurred mainly in the fillets, although they were also found in the "flaps" (hypaxial musculature of the abdomen) and in the body cavity of the fish host. The parasite was most numerous in inshore cod but was uniformly abundant in offshore cod from 4T and from 4Vn summer and winter fisheries. While worm abundances in 4T cod were similar to those recorded 25 years ago, infections in 4Vn cod, plaice, and gray sole were far heavier than previously reported. Anisakis sp. and Contracaecum sp. larvae were encysted on visceral organs and mesenteries but occurred infrequently in the flesh. These latter nematode species were most abundant in cod and plaice in the southern Gulf. Contracaecum sp. larvae were found infrequently in local 4Vn cod and were not detected in 4Vn plaice or sole. Because of the disparity in abundance of Contracaecum larvae in 4T and 4Vn cod, this parasite is an ideal tag for migrant 4T stocks. Prevalence and abundance of Tarval anisakines invariably increased with length of fish host. Analysis of variance revealed that variations in abundance with host length and geographic origin were highly significant, but abundance did not vary significantly with host sex. When fish were stored in the round on ice, adult and immature specimens of a fourth ascaridoid nematode, Hysterothylacium aduncum, migrated from the gastrointestinal tract of the fish and were found in the body cavity and flesh or leaving the host via the gills and mouth. There was no evidence, however, that larval Anisakis migrate from the viscera to the flesh of iced round cod.

Key words: Sealworm, <u>Phocanema decipiens; Anisakis sp.; Contracaecum sp.; Hysterothylacium aduncum</u>, nematodes; <u>parasitic anisakine; cod, Gadus morhua; American plaice, Hippoglossoides platessoides;</u> gray sole, <u>Glyptocephalus cynoglossus; southern Gulf of St. Lawrence; Breton Shelf; prevalence;</u> abundance; <u>variations; geographic; host length; and host sex.</u>

RÉSUMÉ

McClelland, G., R.K. Misra and D.J. Marcogliese. 1983. Variations in abundance of larval anisakines, sealworm (Phocanema decipiens), and related species in cod and flatfish from the southern Gulf of St. Lawrence (4T) and the Breton Shelf (4Vn). Can. Tech. Rep. Fish. Aquat. Sci. No. 1201, ix + 51 p.

Nous passons en revue, dans l'article qui suit, le cycle de vie, l'importance médicale et la distribution géographique du ver de phoque (Phocanema decipiens) et examinons les méthodes de contrôle du parasite. Nous mentionnons également les données courantes sur l'abondance du ver de phoque et espèces apparentées de nématodes anisakines (Anisakis sp. et Contracaecum sp.) dans la morue (Gadus morhua), la plie canadienne (Hippoglossoides platessoides) et la plie grise (Glyptocephalus cynoglossus) du sud du golfe du Saint-Laurent (4T) et du plateau du Cap-Breton (4Vn). Le ver de phoque se trouve surtout dans les filets, bien qu'il y en ait également dans les "volets" (musculature hypaxiale de l'abdomen) et dans la cavité du corps du poisson hôte. Le parasite est plus abondant dans la morue côtière, mais il est uniformément abondant dans la morue du large de 4T et dans celle des pêches d'été et d'hiver de 4Vn. Bien que l'abondance des vers dans la morue de 4T soit semblable à celle signalée il y a 25 ans, les infestations dans la morue, la plie canadienne et la plie grise de 4Vn sont de beaucoup supérieures à celles rapportées antérieurement. Les larves d'Anisakis sp. et de Contracaecum sp. sont enkystées sur les organes et les mésentères viscéraux, mais se rencontrent rarement dans la chair. Ces espèces de nématodes se trouvent en plus grande abondance dans la morue et la plie canadienne du sud du Golfe. Les larves de Contracaecum sp. observées dans la morue locale de 4Vn et ne l'ont pas été dans la plie canadienne ou la plie grise de cette division. A cause de la différence d'abondance des larves de Contracaecum dans la morue de 4T et 4Vn, le parasite est une marque idéale pour les stocks migrateurs de 4T. La prévalence et l'abondance des larves anisakines augmentent invariablement en fonction de la longueur du poisson hôte. L'analyse de variance indique que les variations dans l'abondance des vers en fonction de la longueur de l'hôte et de son origine géographique sont très significatives, mais que l'abondance des vers ne varie pas notablement en fonction du sexe de l'hôte. Quand des poissons entiers sont entreposés dans la glace, les sujets adultes et immatures d'un quatrième nématode ascaridoide, Hysterothylacium aduncum, émigre du tractus gastro-intestinal du poisson, et on l'a trouvé dans la cavité du corps et la chair, ou encore quittant l'hôte par voie des branchies et de la bouche. Il n'y a toutefois pas de preuves que des larves d'Anisakis émigrent des viscères à la chair du poisson conservé rond dans la glace.



INTRODUCTION

AN OVERVIEW OF THE SEALWORM PROBLEM

The occurrence of larval parasitic nematodes commonly known as "sealworm," <u>Phocanema</u> (<u>Terranova</u>, <u>Porrocaecum</u>) <u>decipiens</u> in the <u>flesh of cod (Gadus</u> <u>morhua)</u> and other groundfish species is a chronic cosmetic problem in eastern Canadian fisheries. Now, in light of recent evidence that sealworm ingested in raw, marinated, or undercooked fish may infect humans (Jackson 1975; Margolis 1977), the problem had developed medical overtones.

As Margolis (1977) points out, however, infection with sealworm "Phocanemiasis" can hardly be considered a major public health problem in North America. Of the 46 confirmed cases documented throughout the world, 37 occurred in Japan where fish is traditionally eaten raw. In North America, where fish is usually deep frozen and/or well cooked beforehand, a mere handful of cases (six in the U.S.A., one in Canada) have been reported. While symptoms such as severe epigastric pain, nausea, and vomiting frequently occurred in Japanese cases, North American cases were virtually asymptomatic. When the infection is allowed to run its course, the partially embedded nematodes either withdraw or are expelled from the tissues of the stomach wall and the symptoms quickly disappear ("self cure"). Recovery can be expedited by removing the worm with gastrofiberoptic biopsy forceps.

Although sealworm would seem to have little public health significance in North America, the elevation of the parasite to a status of medical importance can only aggravate the existing cosmetic problem. Now, processed fish will be subject to much closer scrutiny, not only by those government agencies who regulate the quality of seafood products, but also by wholesalers, retailers, and consumers alerted to the problem through government publications, trade journals, and the popular media. Thus, it becomes increasingly important to reduce the number of worms in fillets to the satisfaction of potential customers and not merely to comply with government regulations. Indeed, under existing tolerances for maximum numbers of worms in fish fillets (one worm per three pounds of fillet in Canada, one per two pounds of fillet in the U.S.A.), the average consumer of cod stands a good chance of finding sealworm. By selling fish which merely complies with government standards regarding worm infestation, retailers could run into numerous complaints from consumers and, as a consequence, they would seek out suppliers whose products have the fewest worms. In the end, the marketplace decides what level of worm infestation is acceptable and products free of worms and other flaws find the most lucrative markets.

Unfortunately, the renewed concern over sealworm has come at a time when eastern Canadian fisheries are attempting to expand operations and enter new markets as a result of the extension of Canada's offshore jurisdiction (Appy 1978). In North American markets, eastern Canadian cod is reputed to be wormy (Chitwood 1970), even to the extent the problem is perceived as uniquely Canadian (Odense 1978). Odense considers this a myth as many offshore cod stocks in eastern Canada are rather lightly infected, while sealworm has become a problem in competing European cod fisheries, particularly those of Iceland, Britain, and Norway (Young 1972; Platt 1975; Bjorge et al. 1981). Odense feels that the Europeans, however, have been coping with their sealworm problem somewhat better than eastern Canada through superior quality control and promotion.

LIFE CYCLE

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Ova of P. decipiens are 45 m to 50 m in diameter and partially embryonated when passed with the faeces of the seal host (Scott 1955). The ova settle in sea water and adhere to the substrate. Development to ensheathed second- (third-?) stage larva occurs within the ova, with mean development time to hatch varying from eight days at 20°C to 52 days at 5°C (McClelland 1982). The lowest temperature at which embryonic development and hatching are known to occur is 2°C, while temperatures \geq 24°C are lethal to ova.

Ensheathed Second- (Third- ?) Stage Larvae

The larval nematodes which hatch from the eggs are approximately 200 μ m in length and have retained the cuticle of the previous larval stage as a sheath (Scott 1955). The larvae anchor themselves to the substrate by their caudal extremities and oscillate vigorously at temperatures >10°C; at 5°-10°C the larvae are rather sluggish and at <5°C, virtually inactive. As stored food reserves of the non-feeding larvae are consumed, activity declines; and, if not ingested by a suitable host, the nematodes ultimately become inactive and die. The post-hatch survival period for ensheathed larvae varies from \leq 48 h at 20°C to 140 days at 5°C (McClelland 1982).

First Intermediate Host

When ingested by benthic, epibenthic and natant copepods of the Harpacticoida and Cyclopoida, recently hatched larvae of P. decipiens ensheath in the gut of the crustacean and penetrate to the haemocoel (McClelland 1982). Mature female copepods develop the heaviest infections, while light infections occur in adult males and fifth copepodite females. In the copepod haemocoel the larval nematodes begin to grow, body length increasing at an exponential rate which varies with temperature. Within the life span of its copepod host, P. decipiens grows an average of 60% and a maximum of 130% in length; such growth occurs within seven days at 15°C, 15 days at 10°C, and 35 days at 5°C. However, the nematode does not moult or undergo significant morphological change in copepods; and this indicates that these particular crustaceans are transfer or paratenic hosts, not true intermediate hosts. Although copepods have frequently been infected with P. decipiens in the laboratory and appear to be suitable hosts, natural infections in copepods have not yet been detected.

Second Intermediate Host

Benthic macroinvertebrates, including crustaceans (mysids, isopods, amphipods, cumaceans, and decapods), errant polychaetes, and molluscs (<u>Nudibranchia:Coryphilla</u> sp.), become infected with P. decipiens by ingesting copepods (McClelland T983a). The nematodes invade the haemocoels of crustaceans, the coelom of polychaetes, and the visceral mass of molluscan hosts. Laboratory experiments have shown that the most suitable of the above hosts are gammaridean amphipods, Unciola irrorata and Gammarus lawrencianus; and natural infections have been detected in the latter species. When groups of G. lawrencianus were fed copepods infected with P. decipiens, 100% became infected with the parasite; the mean intensity of infection was 60 nematodes per amphipod. However, in a natural population of G. lawrencianus, only 3 (0.15%) of 2,000 amphipods were infected and each case was a single-worm infection. Natural infections have also been found in the amphipod, Caprella septentrionalis (Val'ter 1978), and in the polychaete, Lepidonotus squamatus (Val'ter and Popova 1974).

Larval P. decipiens undergo significant growth and morphological development in the amphipod haemocoel (McClelland 1983a). Initially, growth is rapid, with body length increasing at an exponential rate; the nematodes reach 2-3 mm in length within 30 days at 15°C, 60 days at 10°C, and 140 days at 5°C. The nematodes subsequently enter an asymptotic growth phase, reaching 7-10 mm in length after 90 days at 15°C. While in amphipods, P. decipiens also develops lip primordia and the characteristic intestinal caecum and, with rapid growth and differentiation of the genital primordium, become sexually dimorphic. Evidently, <u>P. decipiens</u> reaches the infective stage, i.e., the stage at which they can infect the definitive seal host, in amphipods: and hence the life cycle may be completed without a fish host.

Fish Hosts

Larval <u>P. decipiens >2 mm in length are</u> infective to marine fish (McClelland 1983b). Smelt (<u>Osmerus mordax</u>), juvenile cod, and various flatfish species which feed primarly on invertebrates probably become infected via amphipods or other macroinvertebrate hosts. However, large piscivorous fish such as mature cod may also accumulate the parasite by feeding on smaller fish hosts (Scott 1953). Sealworm infects a broad range of fish species (Margolis and Arthur 1979); and variations in abundance of the parasite with host species or population are probably related to host diet, proximity to seal colonies, and environmental temperature.

When infected amphipods are ingested by fish, larval P. decipiens escape from the haemocoel of the partially digested crustaceans and penetrate the gut wall of the fish; early penetrations occur in the stomach, but subsequent penetrations may occur in the intestines (McClelland 1983b). The nematodes traverse the coelom, often by burrowing through the liver or other visceral organs, and enter the hypaxial musculature. Although some nematodes may remain in the viscera or hypaxial musculature, many ultimately migrate into the epaxial musculature. In smelt maintained at 15°C, sealworm 2-7 mm in length escape from amphipod haemocoeles and penetrate the stomach wall within 2-3 h of ingestion; the larvae reach the hypaxial muscles within 12 h and the epaxial muscles within 24 h. However, the migration rate varies with size of sealworm, size of fish being invaded, and ambient temperature.

Once established in the tissues of a fish host, small sealworm larvae continue to grow, with the rate of increase in body length being linear at first but ultimately becoming asymptotic. The average length of sealworm in smelt maintained at 15°C increased from 4 mm (2-7 mm) to 28 mm (25-31 mm) over an eight-week period (McClelland 1983b). Sealworm in the tissues of fish maintained at temperature ≥ 10 °C usually remain fully extended and active, while at ≤ 10 °C they assume a spirally coiled position. The capsule of host connective tissues surrounding some nematodes presumably develops after the parasite has been in residence for some time and/or has completed larval growth. Although sealworm larvae >5 mm in length are infective to seals, they may grow to 60 mm in length in fish hosts.

Seal Host

When fish or crustaceans infected with sealworm are ingested by seals, the parasite escapes from the tissues (or haemocoel) of the intermediate host during digestion and partially embeds itself in the stomach wall (McClelland 1980a). Usually, only the cephalic extremity of the worm is embedded, with the head being anchored in the submucosa by a "hyaline cap." Here the nematode performs its third and fourth larval moults, the third moult occurring after 2-5 days in the seal stomach and the fourth and final moult after 5-15 days. Sealworms reach sexual maturity after 15-25 days in seals, and eggs may be laid and subsequently passed in the faeces of seals as early as the 16th day. During development in seals, the worms occasionally leave the stomach wall to feed on gut contents and reattach themselves between meals.

After 35-50 days in seals, adult females and adult males of P. decipiens may reach 82 mm (70-104 mm) and 64 mm (54-73 mm) in length, respectively; adult females may contain 200,000-500,000 eggs and lay several thousand eggs daily (McClelland 1980b). The average life span of the worms in seals is approximately 35 days, and the maximum is approximately 75 days.

The grey seal (Halichoerus grypus) appears to be the most important definitive host of P. decipiens in the North Atlantic (Young 1972; Platt 1975; Mansfield and Beck 1977; McClelland 1980b; Bjørge et al. 1981). Heavy sealworm infestations in the cod stocks of Great Britain, Norway, Iceland and eastern Canada are clearly related to the distribution and density of grey seal populations, although infestations in some areas where grey seals are rare, e.g. the southern Nova Scotia inshore and lower Bay of Fundy, are probably related to harbour seal (Phoca vitulina) populations. The average intensity of P. decipiens infection in eastern Canadian grey seals (approximately 600 worms per seal) is about ten times greater than in harbour seals (approximately 60 worms per seal) from the same region. Further, laboratory experiments have shown that survivorship, growth, and fecundity of <u>P</u>. decipiens are greater in grey seals than in harbour seals (McClelland 1980<u>b</u>), while at the same time the parasite is less pathogenic in the former host than in the latter (McClelland 1980c). All these factors suggest that the sealworm has an affinity for grey seals. In spite of their large numbers, harp seals (Phoca groenlandicus) evidently do not have a significant influence on the abundance of sealworm in eastern Canada; intensity of infection in this seal is quite low (<10 worms per seal).

As a rule, <u>P. decipiens</u> is non pathogenic in free-living seals (Young and Low 1969); but it can often be pathogenic in captive seals which

presumably are under stress (McClelland 1980c). In captive seals, lesions or localized inflammatory areas are frequently found in association with dense clusters of worms embedded in the stomach wall.

SEALWORM IN EASTERN CANADIAN COD

According to the major surveys of Scott and Martin (1957) and Templeman et al. (1957), sealworm was most abundant in the fillets of cod from the lower Bay of Fundy, the southwestern Gulf of St. Lawrence, and southwestern Newfoundland. Scott and Martin simply recorded the numbers of nematodes recovered from graded fillets by routine candling at processing plants, and there was only a rough indication of where the fish were caught. Templeman et al., on the other hand, recorded length, weight and sex of cod as well as sampling locations; in this study fillets were sliced to facilitate detection of the parasites. Neither study reports the time of year when samples were taken, a factor of considerable importance in areas where there is seasonal migration of cod, such as in the southern Gulf of St. Lawrence (4T) and Sydney Bight (4Vn).

Subsequent to the surveys above, there have been few attempts to document sealworm abundance in eastern Canadian cod. Scott and Martin (1959) recorded the numbers of nematodes in the fillets of young cod from southwestern Nova Scotia (Lockeport inshore) and the southern Gulf (northern New Brunswick and the Magdalen Islands); in this study, cod samples were stratified by age. Wiles (1968) examined cod from Newfoundland water previously surveyed by Templemen et al. (1957) in an effort to determine whether the imposition of a bounty on harbour seal had alleviated the sealworm problem. Finally, during a research cruise in 1975, Appy (1978) observed the prevalence (percentage of hosts infected in a sample or population) of sealworm infection in the fillets of cod from Fundy, Scotian Shelf and southern Gulf regions, and noted that geographic variations in prevalence of the parasite were similar to those reported by Scott and Martin (1957). Unfortunately, Appy examined only a few fish from each sampling location, used a very crude method for candling the fillets and did not record the numbers of nematodes present in the fillets.

Canadian records of worm infestation in cod are clearly inadequate and out of date. This is particularly true in light of the apparent growth of the grey seal population over the past two decades (Mansfield and Beck 1977; Zwanenburg et al. 1981). As this seal appears to be the most important definitive host of the parasite, corresponding increases in abundance of sealworm might be anticipated. Processors interviewed during the preparation of this report noted that worm infestations had become a problem in Scotian Shelf (4Vs, 4W) cod and that this problem seemed to be getting worse. According to the records of Scott and Martin (1957) and Templeman et al. (1957), Scotian Shelf cod were lightly infected 25 years ago.

SEALWORM IN EUROPEAN COD

European records show heavy worm infestations in cod from the west coast of Scotland, Irish Sea, Bristol Channel, west English Channel and northeast English coast (Young 1972), as well as in cod from the Faroe Plateau and most Icelandic fisheries (Platt 1975). However, these records document the abundance of the parasites in the flesh, including worms in the flaps (hypaxial musculature surrounding the coelomic cavity) with those in the fillets. Significant numbers of worms occur in the flaps and they become increasingly prevalent in this location in older cod. The abundance of sealworm, i.e., mean number of worms per fish, in western Icelandic cod, for example, is higher (8.4) than that reported in cod from the southern Gulf of St. Lawrence; but in terms of numbers of worms per unit fillet weight, the infections may be similar.

The abundance of sealworm in cod from southern Norwegian coastal waters (8.5) (Bjørge et al. 1981) is similar to that reported in western Icelandic cod, and hence worm infections in Norwegian cod may also rival those in cod from the southern Gulf of St. Lawrence. Unfortunately, Norwegian records do not indicate lengths and weights of cod sampled or the distribution of worms in host tissues.

DISTRIBUTION OF SEALWORM IN TISSUES OF COD

As Canadian records to date document numbers of sealworm in cod fillets alone, they do not provide an accurate indication of the true abundance of the parasite. It is clear from European records that significant numbers of sealworm also occur in the flaps (Young 1972; Platt 1975) and visceral mesenteries (Pälsson 1979). Further, the distribution of the parasite in host tissues varies with the age of the host. In yearling cod from western Iceland, 92% of the sealworms occur in the fillets; but in four-year-old cod from the same area, only 70% of the worms occur in the fillets. The flaps are the primary site of infection in large British and Icelandic cod (>50 cm in length) (Young 1972; Platt 1975). For example, in Icelandic cod >100 cm in length, >80% of the parasites occur in the flaps.

VARIATIONS IN SEALWORM INFECTION WITH AGE (LENGTH, WEIGHT) OF COD

In a recent survey of eastern Canadian fish processors, Odense (1978) found that there was some confusion as to whether small or large cod were now heavily infected with sealworm. Odense speculates that large cod generally have more worms on a per-fish basis but fewer worms on a per-unit-weight basis than small cod. Certainly, records of worm infestations in eastern Canadian cod fillets (Scott and Martin 1957, 1959; Templeman et al. 1957) seem to support this view. Prevalence and abundance in cod fillets generally increase while numbers of worms per unit fillet weight decrease with size (age) of host. There are exceptions, namely in southern Gulf of St. Lawrence and Cape Breton cod where the numbers of parasites per unit fillet weight remain constant or actually increase with size of host.

Scott and Martin (1957) and Templeman et al. (1957) point out that the parasites are more difficult to detect in larger fillets; and this may explain, in part, the apparent decrease in numbers of worms per fillet weight in larger fillets. Another explanation for this phenomenon would be the tendency of the nematodes to locate in the flaps or viscera of larger cod as reported by European investigators (Young 1972; Platt 1975; Pälsson 1979). Abundance of sealworm clearly increases with host size (age) in British and Icelandic cod, but this is largely due to accumulation of the parasites in the flaps. The abundance of sealworm in the fillets remains more or less constant or, at least, does not increase in proportion to fillet weight. Consequently, the number of nematodes per unit weight declines.

OTHER FISH HOSTS OF SEALWORM

P. decipiens or Phocanema-type larvae have been reported in more than 30 species of marine and anadromous fish in eastern Canada (Margolis and Arthur 1979). Most of the host species are groundfish belonging to the cod, flatfish, and sculpin families. In inshore areas near seal colonies, sealworm may be quite abundant in many of these fish hosts, even in brook trout caught in freshwater several miles inland. As a rule, however, widespread infestations seem to be limited to cod and three or four other species.

Eastern Canadian smelt are generally more heavily infected with sealworm than cod on a per-unit-weight basis, and on a per-fish basis in some areas (Scott 1955; Templeman et al. 1957). According to fish processors interviewed during the preparation of this report, sealworm has recently become a problem in American plaice and witch flounder (gray sole) throughout the southern Gulf of St. Lawrence (4T), Sydney Bight (4Vn), and Scotian Shelf (4Vs and 4W) regions. Infestations in plaice are extremely severe (as many as 70 worms per fillet) in the Cabot Strait and Chedabucto Bay areas. Evidently, the parasite has also increased in abundance in haddock from some areas of the Breton and Scotian shelves to the extent that the occasional catch has to be candled.

There are few European records of sealworm infestation in marine fish species other than Atlantic cod. Wootten and Waddell (1977) found infections with larval Phocanema in long rough dab (American plaice), witch flounder, haddock, whiting (Merlanguis merlangus), poor cod (Trisopterus minutus), and dragonet (Callionymus lyra) from Scottish waters; but occurrences in these latter fish hosts were limited to isolated inshore locations and in each case the prevalence of infection was extremely low.

OTHER ASCARIDOID NEMATODES IN COD

Cod and other marine fish from eastern Canadian and European waters are host to a number of ascaridoid nematodes, such as <u>Anisakis spp.</u>, <u>Contracaecum spp.</u>, <u>Phocascaris spp.</u> larvae and adults of <u>Hysterothylacium</u> (<u>Thynnascaris</u>, <u>Contracaecum</u>) <u>aduncum</u> (Wootten 1978; Margolis and <u>Arthur 1979</u>; <u>Pälsson 1979</u>). These nematodes bear a superficial resemblance to sealworm and microscopic examinations may be required to separate the species.

Anisakis spp. larvae commonly known as "herring worm" belong to the anisakinae, the same ascaridoid sub-family as the sealworm. The life cycle is similar to that of the sealworm, with the definitive host again being a marine mammal but, in this case, usually a cetacean (Smith and Wootten 1978). The first intermediate hosts of herring worm are believed to be krill (Euphausiidae) (Smith 1971). Infective larvae of Anisakis spp. are found encysted both in the flesh and on the visceral mesenteries of marine fish but are often more prevalent in the latter location (Parsons and Hodder 1971; Wootten 1978; Pälsson 1979). Herring worm are important fish parasites in their own right as they can be extremely pathogenic to humans when ingested in raw, undercooked and marinated fish. Herring worm disease "Anisakiasis" has been a public health problem in the Netherlands and Japan (Van Thiel et al. 1960; Ruitenberg 1970; Oshima 1972; Smith and Wootten 1978) although it has not been reported frequently in North America (Jackson 1975; Margolis 1977).

Occurrences of <u>Anisakis</u> larvae on the visceral mesenteries of herring in eastern Canada were extensively surveyed by Parsons and Hodder (1971), but little is known of the distribution of the parasite in eastern Canadian cod. Templeman et al. (1957) documented abundances of <u>Anisakis</u> larvae in the fillets of eastern Canadian cod but, as the flaps and viscera were not examined, these records are incomplete. In this study, the prevalence of the parasite in fillets of cod was generally <2%, the abundance <0.01. <u>Anisakis</u> larvae were greatly outnumbered by sealworm in the fillets except in cod from some Newfoundland fisheries such as Flemish Cap and the Grand Banks, which were lightly infected with <u>Phocanema</u>. <u>Anisakis</u> larva were not found in cod from the southern Gulf of St. Lawrence.

Evidently, the flesh of European cod is often heavily infested with <u>Anisakis</u> larvae, although the parasite is far more prevalent in the flaps than it is in the fillets (Young 1972; Platt 1975). Larval <u>Anisakis</u> is particularly abundant in Arcto-Norwegian cod which is lightly infected with sealworm.

Contracaecum and Phocascaris spp. are anisakine nematodes which mature in the gastro-intestinal tract of piscivorous birds and marine mammals (Berland 1963; Huizinga 1967). Experimental evidence indicates that the first intermediate hosts of these nematodes are copepods (Huizinga 1967; Davey 1969). Infective larvae usually encyst on the visceral mesenteries of the fish host, with mesenteries of the pyloric caecae apparently being the preferred site in Atlantic cod (Palsson 1979). According to Berland (1963), infective larvae of Contracaecum and Phocascaris spp. are morphologically inseparable, and hence specific identification of the larvae would be extremely difficult. Contracaecum osculatum, for example, is a species which occurs in seals throughout the world and yet there has been only one positive identification of C. osculatum larvae in a fish host, that being in Baltic Sea cod, (Fägerholm 1982).

Hysterothylacium (Thynnascaris, Contracaecum) aduncum is an ascaridoid which utilizes marine fish as intermediate and definitive hosts (Berland 1961). Larvae of this nematode occur primarily in the body cavity (rarely in the flesh), while adults are found in the gastro-intestinal tract (Pälsson 1979). Hysterothylacium larvae are also found in a broad range of invertebrate hosts including coelenterates, molluscs, annelids, crustaceans, echinoderms, and chaetognaths (Norris and Overstreet 1976).

POST-MORTEM MIGRATIONS OF ASCARIDOID NEMATODES IN FISH TISSUES

Many eastern Canadian fishemmen and fish processors interviewed during the present study claimed that sealworm moves from the flesh to the body cavity of cod stored (in the round) on ice; thus, it would follow that round cod reaching processing plants would have fewer worms in the fillet than cod gutted at sea. Although there is no documented evidence to support this rationalization, it has frequently been cited as justification for the continued acceptance of round cod at fish plants in some areas, particularly in the southern Gulf of St. Lawrence.

As the viscera decay more rapidly than the flesh of fish, it is far more likely that parasitic nematodes would migrate from the body cavity to the flesh rather than in the opposite direction. Cheng (1976) found that <u>Hysterothylacium</u> (Contracaecum) aduncum escaped from the gastro-intestinal tract of dead cod and summer flounder (Paralichthys dentatus) via the anus and gills or by migrating through the disintegrating gut wall to the body cavity and the flesh. As nematodes found in the coelom and flesh were morphologically identifiable as enteric forms (fourth-stage larvae and adults), there was no doubt that migration had occurred. Normally, only the third-stage larvae of <u>Hysterothylacium</u> occurs in the coelom or flesh (Palsson 1979).

There is some controversy as to whether there is a significant migration of medically important Anisakis spp. larvae from the body cavity to the flesh of round fish. Some European investigators report experimental evidence of such a migration occurring in mackerel (Vik 1966) and herring (Smith and Wotten 1975). In these studies, relative abundances of <u>Anisakis</u> larvae in the flesh of fish stored round for periods of several hours to several days were compared with abundances of the parasite in the flesh of fish gutted at time of catch. Other investigators (Khalil 1969; Davey 1972) performing similar experiments found no evidence of a significant migration.

CONTROL OF THE PROBLEM

Odense (1978) described a number of possible solutions to the sealworm problem in the areas of biological control, technological innovations in quality control of fish processing and better promotion. Many of these solutions were subsequently discussed at a "Codworm (Sealworm) Workshop" conducted at the Department of Fisheries and Oceans Halifax Laboratory in March, 1979.

Biological Solutions

As sealworm reproduce in the stomachs of seals (see life cycle above), the primary biological solution to the problem is control of seal populations. Grey seals (Halichoerus grypus) appear to be the most important definitive host, and there appears to be a strong correlation between the distribution of grey seals and heavy sealworm infestations in cod stocks on both sides of the North Atlantic (Young 1972; Platt 1975; Mansfield and Beck 1977; McClelland 1980b]; other North Atlantic seals, such as harbour (Phoca vitulina) and harp seals (Phoca groenlandicus), are of relatively minor importance as sealworm hosts.

Annual culls have been conducted in grey seal breeding colonies in eastern Canada since 1967. The positive aspect of this program is that it is a humane kill by commercial sealers, with pelts and other products being utilized. However, as the seals breed in mid winter and most of the population is widely dispersed and inaccessible, only a few hundred animals, mainly pups, have been taken each year (Mansfield and Beck 1977; Zwanenburg et al. 1981). The harvest is neither commercially feasible nor effective as a control of the seal population or the sealworm problem. Pups normally suffer a high mortality rate and require several years to reach the reproductive age. The seal population can be reduced more efficiently by culling mature animals which, as it happens, also support the heaviest sealworm infestations (McClelland 1980b). A bounty was placed on the grey seal in 1976, resulting in an additional 600-1,000 seals being eliminated each year between 1976 and 1980. The total number of seals taken through the bounty and culling programs during the same years ranged from 1,300-2,000. Yet according to the most recent stock assessment, the population continues to grow (Zwanenburg et al. 1981).

Odense (1978) suggested that as an alternative to killing seals the animals might be treated with "worm medicine," anthelmintics or vaccines, thus rendering them unsuitable hosts for sealworm. Such treatments are used routinely for control of parasitic diseases in domestic or zoo animals, where the patients are available for follow-up treatment and reexposure to the parasites can be prevented but, unfortunately, would not be practical for controlling sealworm in free-living seals. Only unweaned or recently weaned grey seal pups in large island colonies are accessible and approachable for the treatment. The pups do not become infected with sealworms until they have left the breeding sites and learned to catch fish. They are subsequently exposed to reinfection with the parasite throughout their lives. In many parasitic diseases, the host normally develops a resistance to reinfection; but, in the case of sealworm, seals appear to adapt to the presence of the parasite and support progressively heavier infections as they get older (McClelland 1980b). As seals do not develop "effective immunity" to the parasite in nature, it is unlikely that such immunity could be induced artificially with a vaccine.

Selective fishing for worm-free cod stocks is another solution to the sealworm problem. But clearly, this is only a temporary solution which merely avoids the problem and in no way controls it. Selective fishing can put excessive pressure on lightly infected stocks and ultimately it would be necessary to exploit wormy stocks again (Rae 1972). Further, wormy stocks are usually found in close association with seals (Scott and Martin 1959; Bjørge 1979), probably serving to reinfect these hosts. Failure to exploit fish stocks serving as reservoirs for larval sealworm together with the apparent growth of the seal population can only lead to increasing abundance of the parasite. As a result, the problem could spread to adjacent cod stocks and other fish species.

Young (1972), noting that abundance and distribution of sealworm in the flesh of cod varied with the age of the host, suggested that selective fishing for lightly infected age classes of cod was a possible solution to the worm problem. Platt (1975) subsequently pointed out, however, that concentration of the fishing effort on a particular age group (in this case, older cod) might not be economically feasible. Nevertheless, the manner in which worm abundance varies with age of cod could be taken into consideration when managing the stocks. The goals of maintaining healthy, productive stocks and minimizing worm infestations need not conflict. Biological approaches to the sealworm problem may offer, at best, reductions in the abundance of worms in fish stocks over the long term. Nevertheless, these measures are necessary to ensure that processors are provided with fish that can be processed into high-quality products, at a reasonable cost.

Technological Solutions

Unlike biological approaches to the sealworm problem, technological solutions, many of which should be applied at present, offer immediate relief.

First, the potential health hazard can be eliminated through proper cooking, freezing, salting, smoking or drying of fish, although further investigation is needed to determine what degree of processing is adequate to destroy the worm in each case (Margolis 1977). Unfortunately, while a certain number of parasites may leave the flesh during some of these processes, many persist, nonviable, but still unacceptable from the aesthetic point of view. Although many of these procedures can be regulated at the processing level, cooking, for instance, is usually performed by the consumer or retailer and it follows that these parties should be apprised of the potential hazard. This, of course, draws attention to the parasite. More rigorous cooking standards are necessary to prevent potential parasitic infections from beef, pork, and mutton; parasites, such as tapeworms and trichinella are far more pathogenic than sealworm but, unlike sealworm, are not plainly visible to the naked eye.

The candling procedure used for detection and removal of the parasites from fillets has seen little or no improvement during the decades it has been in use. A number of innovations to improve the efficiency of the procedure have been proposed, such as thin slicing of fillets, optimizing transmitted and incident light conditions, and frequent rotation of candlers to avoid eyestrain, fatigue, and boredom. However, these practices were either unacceptable to processors or were practiced for a time but subsequently fell into disuse. The time has come, perhaps, to give these procedures another look. Odense (1978) has devised a new candling table incorporating crossed polarizing filters, which may provide improved opportunities for detecting the parasites and at the same time reduce eyestrain. This device is being tested currently.

There have been a number of recent attempts to develop sophisticated ultrasonic or photosensitive devices to detect worms, bones, and other flaws in fillets. Such devices would not eliminate the need for candlers but would screen out flawed fillets for subsequent candling or, perhaps, processing into fish meal or other products, while fillets free of flaws could bypass the candling operation. At present, however, use of automated worm detectors appears to be impractical under industrial conditions and perhaps too expensive for smaller processing plants.

OBJECTIVES

The main objective of the present study was to document current abundances of sealworm and related species of parasitic nematode in southern Gulf of St. Lawrence (4T) and Sydney Bight (4Vn) cod, plaice, and gray sole, indicating distributions of the parasites in the flesh and viscera of the host and variations in their abundance with sex, body length, and geographic origin of the host. A second objective was to investigate, by experimental means, possible migration of these parasites from the viscera to the flesh (or vice versa) of iced round cod.

MATERIALS AND METHODS

COLLECTION AND EXAMINATION OF FISH SAMPLES

Samples of round cod (Gadus morhua), American plaice (Hippoglossoides platessoides), and witch flounder (Glyptocephalus cynoglossus) were collected from commercial inshore and offshore draggers, Danish seiners, and long liners in the southern Gulf of St. Lawrence (4T) and Sydney Bight (4Vn). Usually the fish were measured as they were selected, in an effort to stratify the samples into 5-cm length groups containing equal numbers of fish. Cod samples, for example, were divided into ten length groups: ≤30 cm, 31-35 cm, 36-40 cm, 41-45 cm, 46-50 cm, 51-55 cm, 56-60 cm, 61-65 cm. 66-70 cm, and \geq 71 cm. However, because of the type of gear being used, variations in the size of fish caught in different fisheries, or the small size of the catch, particularly from inshore boats, it was often difficult to fill the smaller and/or larger length categories. Fish in some samples, collected for the investigators by fishermen, were of random length.

The samples were transferred to DFO. Halifax Fisheries Research Laboratory, and stored either on ice for examination in the fresh condition or frozen at -17°C and examined at a later date. Parasitological examinations were similar to those described by Templeman et al. (1957), Young (1972), and Platt (1975). The fish were measured (to the nearest cm in length), weighed (to the nearest 0.1 kg), gutted, sexed and filleted. The visceral organs, mesenteries and peritoneum were scanned with the naked eye, and nematodes detected therein were tentatively identified and counted. The nematodes were placed in labelled vials of 0.9% saline and their identities subsequently verified by microscopic examination. The fillets and flaps of cod and plaice were inspected by systematic destruction of the flesh (Wiles 1968); witch fillets were simply candled. Flesh adhering to the frames was also inspected for nematodes or portions of nematodes severed during filleting. Nematodes in the flesh, clearly identifiable as sealworm, were simply counted, while those of uncertain identity were examined microscopically.

INVESTIGATION OF MIGRATIONS OF LARVAL ANISAKINES IN ROUND COD

The following experiments were performed in order to determine if larval anisakines migrate from the viscera to the flesh (or vice versa) of cod when it is stored in the round after capture. In February, 1980, 334 cod, 45-65 cm in length, were collected from a side dragger on the "Edge of Ground" (4Vn). Of these, 187 were eviscerated immediately after capture while the remainder were divided into three groups of about 50 fish each and were then stored on deck for periods of 6 h, 12 h and 24 h, respectively, prior to being eviscerated. The viscera and bodies of individual fish were stored in separate labelled polyethylene bags and were subsequently examined for nematodes as above.

In a similar experiment, 164 cod, 50-60 cm in length, were collected from a long liner on Scatari Bank (4Vn) in October, 1981. The sample was separated into three groups, each containing 50-60 randomly selected fish. Cod from one of these groups were eviscerated immediately after capture; the viscera and bodies of individual fish were stored in separate labelled polyethylene bags. The other two groups were stored round, on ice, until examination in the laboratory four days later. After a routine examination was made of viscera and flesh for larval anisakines, flesh from the flaps and fillets was retained in individual labelled polyethylene bags and subsequently digested in a pepsin-HCl solution. The digestion procedure was employed in order to detect small or unencysted nematodes which might have been overlooked during routine examinations and, at the same time, test the efficiency of the routine examination.

DIGESTION PROCEDURE

The digestion technique used herein was adapted from procedures described by Novotny and Uzmann (1960) and Smith and Wootten (1975). The solution consisted of 5 g of 1:10,000 pepsin per litre of 1% hydrochloric acid. Fillets of individual cod were placed in 4-L beakers containing 2 L of solution, the flaps in 1-L beakers with 0.5 L of solution. These mixtures were incubated in a water bath at 35°-40°C, coupled with frequent stirring. After 2-3 h of incubation, they were strained through a series of sieves ranging from 5-mm to 0.3-mm mesh. Nematodes recovered from the sieves were identified by microscopic examination.

STATISTICAL ANALYSIS

Counts of larval anisakines in individual fish were transformed by $\log_{10}(X+1)$. Variations in worm counts with sex, sample and length of host were analyzed by three- and two-way ANOVAs (Type III) using the GLM procedure (SAS 1982). Program PIV of BMDP-79 (BMDP-79 1979) was used for regression analysis of worm count/host length relationships.

Cells with zero frequency were eliminated from the analysis of cod parasites by including all fish <45 cm in length in a single length stratum and by deleting one sample (Chéticamp, N.S.) lacking cod \geq 61 cm in length. For the same reason gray sole 35 cm in length were grouped in a single length stratum. Due to a scarcity of male plaice \geq 41 cm in length, ANOVAs involving variations in worm counts with host sex were computed for three host length strata: ≤30 cm, 31-35 cm, and 36-40 cm. All six length strata, from <30 to <50 cm inclusive, were used, however, in two-way analyses of variance related to sampling and length of plaice. Where blank cells persisted in spite of those precautions, their influence on the results were investigated by repeating analyses with Type IV ANOVAs.

RESULTS

LARVAL ANISAKINES IN 4T AND 4Vn COD

Three species of larval anisakine, Phocanema decipiens, Anisakis sp., and Contracaecum (Phocascaris) sp. were found in 4T and 4Vn cod. Prevalences and abundances of each nematode species are summarized by host sample and length group in Table 1, and sampling locations are indicated in Fig. 1.

Frequency distributions of untransformed worm counts shown for combined cod samples herein (Figs. 2, 3 and 4) were in each case skewed to the right to varying degrees. Graphic tests employing normal probability plots, however, show that the distributions are brought remarkably close to normal by the log transformation. This was also the case for frequency distributions of worm counts in plaice and gray sole (Figs. 8 and 10).

P. decipiens

Of the more than 10,000 sealworm, recovered from 4T and 4Vn cod, 90% were found in the fillets, 7% in the flaps and 3% in the coelomic cavity (Table 2). Infections in the flaps and coelom were most common in large market and steak cod, particularly in specimens with heavy infestations in the fillets. Sealworm from the fillets and flaps were 15-60 mm in length while those found on the visceral organs and mesenteries and on the peritoneum were 8-42 mm in length. Nematodes in the flesh and coelom were usually encysted; however, unencysted nematodes occurred with increasing frequency when samples were stored on ice for prolonged periods.

Abundance of sealworm in 4T and 4Vn cod varied from sample to sample, with inshore fish generally being more heavily infected than offshore fish (Table 1; Fig. 5). The heaviest infections occurred in samples collected from the 4T inshore near Souris, Prince Edward Island, in the spring of 1981 and from Frenchman's Shoal (4Vn) in the summer of 1981. The lighest infections were found in 4T offshore cod collected near St. Paul's Island in November, 1980.

Invariably, worm abundance increased with host length, not only on a per-fish but often on a per-unit-weight basis (Table 1; Fig. 5). In most samples, steak cod (\geq 71 cm in length) had the greatest number of worms per unit fillet weight.

Although sealworm appeared to be slightly more abundant in male cod than in female cod (Table 3), a three-way ANOVA revealed that variations in worm abundance attributable to host sex were not significant in interactions or as a main effect (Table 4). Variations attributable to host length and sampling, on the other hand, were highly significant both in interaction and as main effects (Tables 4 and 5).

A priori contrasts of samples differing in respect to geographic origin and/or season of capture show that variations attributable to sampling in two-way ANOVAs were largely a result of differences in worm abundance in inshore and offshore fish (Table 5; Fig. 5). Not only did inshore and offshore fish differ significantly in mean worm count ($P \le 0.0001$), but also in the worm count/host length interaction. Mean worm counts in cod from 4T and 4Vn offshore, on the other hand, did not differ significantly, although worm counts in 4Vn winter and summer samples differed at $P \le 0.05$.

Plots of cell means of transformed worm counts versus host length seemed to indicate the existence of a linear relationship between sealworm abundance and host length (Fig. 5). Regression analysis, however, revealed that although regression coefficients were invariably highly significant ($P \le 0.0001$), the resultant regressions often explained less than 10% of the variance. Distributions of residuals (error) as shown in scatter plots were far from normal; and in fact, the departure from normality was so severe that tests for significance of regression coefficients would be meaningless (see Underwood 1981). Hence, it was not possible to compare samples by covariant analysis of worm count/host length regressions.

Anisakis sp.

Anisakis larvae found in 4T and 4Vn cod varied from 12 mm to 35 mm in length and closely resembled A. simplex as described by Palsson (1979). The nematodes were usually encysted on the surface of the liver (61%) and on the mesenteries surrounding the pyloric caecae (31%) (Table 2); in cod \geq 71 cm in length, they occurred more frequently on the pyloric caecae (49%) than on the liver (42%). Anisakis larvae were found infrequently in the flaps (T.21%) and fillets (1.11%).

Prevalence and abundance of worms invariably increased with host length, and cod collected in 4T were more heavily infected than cod from 4Vn (Table 1; Fig. 6). The three-way ANOVA (Table 4) indicates that the influences of host length and sampling on worm abundance were highly significant as main effects and in interaction, while host sex (Table 3) was not a significant factor. A priori contrasts (Table 5; Fig. 6) reveal that 4T offshore cod differed significantly from 4Vn offshore cod in mean worm count ($P \le 0.0001$) and the worm count/host length interaction ($P \le 0.0001$). In an overall comparison of inshore cod with offshore cod, the difference in sample means and interactions was not significant, while the difference between mean worm counts for inshore and offshore fish from 4T alone was significant at P≤0.05.

Contracaecum (Phocascaris) sp.

Larval <u>Contracaecum</u> from 4T and 4Vn cod were 7-23 mm in length and closely resemble larvae of C. osculatum cultivated in vitro by McClelland and Ronald (1974b). The nematodes were encysted on visceral organs and mesenteries with most (96%) occurring on the mesenteries of the pyloric caecae (Table 2); only a few (0.11%) were found in the flesh. Unlike encysted larvae of P. decipiens and Anisakis which were spirally coiled, <u>Contracaecum</u> larvae were either fully extended or <u>sharply</u> recurved (similar to a hairpin).

While they were found infrequently in small cod, Contracaecum larvae were often quite abundant in cod ≥61 cm length (Table 1; Fig. 7). Cod from 4T, especially offshore fish, were far more heavily infected than cod from 4Vn. According to the three-way ANOVA, worm abundance was influenced significantly by host length and sampling not only in the two-way interaction and as main effects but also in a three-way interaction with host sex (Table 4). Host sex, however, was not a significant factor in two-way interactions with length or sample or as a main effect. As indicated by a priori contrasts, variations in the ANOVA related to sampling were attributable to differences in worm abundance in inshore and offshore cod and also to differences between 4T and 4Vn cod (Table 5; Fig. 7).

LARVAL ANISAKINES IN 4T AND 4Vn PLAICE

Abundances of larval anisakines in 4T and 4Vn plaice are summarized by host sample and length group in Table 6, and sampling locations are indicated in Fig. 1. Frequency distributions for untransformed counts of P. decipiens are shown for the combined samples in Fig. 8.

P. decipiens

Sealworm in 4T and 4Vn plaice (Table 7) were found almost exclusively (98%) in the fillets. Prevalence and abundance of the parasites increased with host length in samples from the southwestern Gulf; however, in the 4Vn samples, there was a decrease in intensity of infection in fish \geq 51 cm in length (Table 6; Fig. 10). The sample from Shediac Valley (4T) was the only one in which the parasite increased in abundance with host length on a per-unit-weight basis.

While male plaice seemed to be more heavily infected than females of corresponding length (Table 8), a three-way ANOVA for fish <40 cm in length indicates that variations in worm abundance attributable to host sex were not significant (Table 9). Variations related to host length and sampling were again highly significant, but the sample x length interaction became significant only when host length strata \geq 41 cm were included in the analyses (Table 10). As shown by contrasts (Table 10; Fig. 9), the significance of variations in sample means and sample mean x host length interactions was mainly related to differences between southwestern Gulf (Pt. Escuminac, New Brunswick, and Shediac Valley) and southeastern Gulf (Chéticamp) and 4Vn samples. Mean worm counts and worm count/host length interactions did not differ significantly in inshore samples collected near Chéticamp (4T) and Cape Smokey (4Vn) in the fall. These inshore samples, in turn, did not differ in either of the above respects from an offshore sample taken on the "Edge of Ground" (4Vn) in winter.

Anisakis and Contracaecum (Phocascaris) sp.

Significant numbers of <u>Anisakis</u> and <u>Contracaecum</u> larvae were found in plaice from western 4T (Shediac Valley and Pt. Escuminac), but only a few specimens were found in samples from Chéticamp and 4Vn (Table 6). In fact, only one <u>Contracaecum</u> larva was found in the Cheticamp sample and this parasite was not detected in 4Vn samples. Only two <u>Anisakis</u> larvae were found in the fillets; the remainder occurred in the body cavity, most frequently (56%) on the liver (Table 7). <u>Contracaecum</u> larvae were found primarily on the mesenteries of the pyloric caecae, and only two specimens were detected in the fillets. The two-way ANOVA indicates that variations in abundance of these two nematode species related to host length and sampling were highly significant (Table 10).

LARVAL ANISAKINES IN 4Vn WITCH (GRAY SOLE)

All P. decipiens larvae found in 4Vn witch occurred in the fillets. Only two Anisakis larvae were found in the three samples, both in the coelom, and no Contracaecum larvae were detected. Prevalence and abundance of <u>P. decipiens</u> and the abundance of the parasite per unit fillet weight all increased with host length (Table 11). While variations in worm abundance related to host sex (Table 12) were not significant, those associated with host length and sampling were highly significant (Table 13).

HYSTEROTHYLACIUM ADUNCUM IN 4T AND 4Vn COD AND FLATFISH

A fourth species of ascaridoid nematode was found in the coelom and occasionally in the flesh of 4T and 4Vn cod, plaice, and witch. However, aside from a few encysted third-stage larvae found in small cod and plaice from 4T, most of the specimens were unencysted fourth-stage larvae or adults of enteric origin. Records of these nematodes in the coelom and flesh (Table 14) do not reflect their overall abundance as many specimens were found protruding from the mouth, gills and anus, or free of the fish host. Further, the gastro-intestinal lumen of the host was not inspected. They are cited here nevertheless as evidence of migration of parasitic nematodes from the gut lumen to the coelom and flesh after the death of the host.

MIGRATION OF LARVAL ANISAKINES IN ROUND COD

There was no evidence of a migration of larval anisakines from the viscera to the flesh of round cod within 24 hours of catch (Table 15). Sealworm occurred primarily in the flesh, with only two (0.5%) of 408 larvae being found in the viscera of freshly gutted cod and three (1.8%) of 171 larvae in the viscera of cod left ungutted for 6-24 hours after catch. Conversely, Anisakis occurred mainly in the viscera and only four (3.0%) of 131 were found in the flesh of freshly gutted cod and three (1.9%) of 159 in the flesh of round cod.

The experiment with Scatari Bank cod (Table 16) also failed to provide conclusive evidence of migrations of larval anisakines in round cod. In this case, sealworm did not occur in the viscera of either freshly gutted cod or cod stored on ice for four days prior to evisceration. The distribution of Anisakis sp. larvae between viscera and flesh was similar in freshly gutted and round cod; nine (21%) of 43 Anisakis larvae occurred in the flesh of the former, 13 (24%) of 55 in the flesh of the latter. In respect to infections in flesh alone, however, round cod had relatively fewer nematodes in the flaps than freshly gutted cod; four (2%) of 203 sealworm and five (38%) of 13 Anisakis larvae in the flesh of round cod occurred in the flaps; while of the nematodes in the flesh of freshly gutted cod, nine (7%) of 123 sealworm and six (67%) of nine Anisakis were in the flaps. As the total numbers of nematodes were small and as respective distributions of sealworm and Anisakis in tissues of freshly gutted and round cod varied by only a few nematodes in each case, the results were not subjected to a further analysis.

Following digestion of the flesh of Scatari Bank cod, it became clear that a number of nematodes had escaped detection by routine examination of fillets and flaps (Table 16). Of the nematodes in the flesh of freshly gutted and round cod, 45 (13%) of 339 sealworm and 15 (68%) of 22 <u>Anisakis</u> sp. larvae were recovered by digestion.

DISCUSSION

Before the results of the present study can be discussed or compared with results of similar studies, several factors must be considered. Fillets, flaps, and viscera were examined for ascaridoid nematodes in our study, whereas only the fillets were examined in earlier surveys of eastern Canadian groundfish (Scott and Martin 1957, 1959; Templeman et al. 1957; Wiles 1968; Appy 1978). In our study, nematodes in the fillets and flaps were recovered by systematic destruction of the flesh. As shown in experiments in which the flesh of cod was digested following routine examination, some P. decipiens (13%) and most Anisakis sp. larvae (68%) in the fillets and flaps were not detected by systematic destruction of the flesh. Templeman et al. (1957) found P. decipiens and Anisakis sp. larvae in the fillets of eastern Canadian groundfish by cutting the fillets into thin slices prior to candling. This latter approach appears to be nearly as reliable for detection of sealworm as systematic destruction of the flesh (Power 1961) and has subsequently been adopted by European investigators (Young 1972; Platt 1975). Experiments conducted by Power (1961), however, indicate that commercial candling procedures employed in the Scott and Martin (1957) survey of eastern Canadian cod were probably less than half as efficient for finding worms as the methods above.

Records of sealworm infestation are usually reported as average numbers of worms per fish, per fillet, or per unit fillet weight; but average or mean worm counts are often misleading. Frequency distributions of worm counts are invariably skewed to the right with most fish either uninfected or lightly infected (Platt 1975; Pälsson 1979; herein). When sample sizes are small, the mean worm count may be greatly influenced by occurrences of heavy infestations in a few fish. As worm abundance varies with length or age of host (Platt 1975; herein), the mean count is also influenced by the structure of the sample with regard to length or age classes present and relative numbers of fish included in each class. The influence of length or age structure of sampling remains when mean counts are computed for broad categories such as scrod, market, or steak cod. In other studies discussed here, samples appear to have been chosen at random and have normal frequency distributions for age or length of fish. Our samples, however, were selected according to a forced orthogonal design in which they were stratified into length groups containing equal numbers of fish. Most of these samples conform with the design to some degree.

The difficulty of comparing the findings of various surveys is compounded by the fact that fish length or age strata chosen for data summaries often differ. Moreover, growth rates of cod and flatfish populations vary geographically (Kohler 1964; Powles 1965; Pitt 1975; Beacham 1982) and, hence, fish of a given year class vary in size from population to population. Conversely, fish in corresponding length strata may differ in age.

Although sampling locations in the present and earlier surveys often coincide, the times of year at which samples were collected may differ. With seasonal migrations of cod and plaice, particularly 4T stocks (Martin and Jean 1964; Powles 1965), various transient populations may occur in a given location at different times of year. In the event that the same cod or flatfish population has in fact been sampled in current and past surveys, fish in coincident length strata may differ in age due to changes in growth rates over the intervening years (Kohler 1964; Powles 1965).

Frequency distributions of worm counts in the present study are positively skewed but brought remarkably close to normality by the \log_{10} (n+1) transformation. Because of the complexity of design and the lack of sufficient numbers of observations in some cells, the possibility that the distributions are negative binomials was not tested. Platt (1975) applies the above transformation to data on sealworm and Anisakis larvae in northern European cod, having first established that worm count distributions lie between Poisson and negative binomials. In Pälsson's (1979) study of larval anisakines in cod from the Icelandic inshore area, worm count distributions are in fact negative binomials.

ANOVAs reported here are of non-orthogonal design, i.e. unbalanced with unequal numbers of observations in the individual cells, and main effects (host length, sample and sex) are assumed "fixed." The disparity in cell size is often severe, with some cells, albeit a small minority, having no observations. With Type II ANOVAs offered in the SPSS and BMDP packages used for preliminary analyses here, cross-contamination of interaction and main effects may occur in analyses of unbalanced data (Fruend and Littel 1981). This problem was resolved by reanalyzing the data with Type III and Type IV ANOVAs, which subsequently became available in the GLM procedure (SAS 1982). With the Type III ANOVA, each effect is adjusted for all other effects and significance of main effects can be considered in the presence of interactions. Type IV functions have been designed for analyses in which cells of zero frequency are involved. In the few cases where this situation applied to our analyses, results of Type III and Type IV ANOVAs were similar.

Distributions of error terms were not tested for normality for the following reasons (see Underwood 1981). First, most tests for normality require large samples and, as indicated above, some cells contained few observations. Secondly, for complex designs such as the one used in the present study, none of the tests for normality are practicable. Finally, non-normality has little effect on statistical procedures which compare treatment means, and thus it may be safe to disregard this assumption.

In all likelihood, the assumption of homogeneity of variances has also been violated in this analysis. When heterogeneity of variance is severe, the probability of Type I error is greater than specified in the table of F-ratios, and the factors in ANOVA are prone to false significance (Bliss 1970; Underwood 1981). In the present study, however, the data were not tested for homogeneity of variance. According to arguments presented in Underwood's (1981) review, ANOVA is robust to many types and magnitudes of departure from homogeneity of variance and may be more valid than tests of the assumption. Hence, prior tests for homogeneity of variance may not be necessary.

A priori contrasts are non-orthogonal and consequently prone to results of false significance (as is the case with heterogeneity of variance, the probability of Type I error is greater than specified in the table of F-ratios). For descriptive purposes, it is often more convenient to confine such analyses to orthogonal contrasts; but this should not prevent the investigator from testing those contrasts of greatest theoretical importance (Harris 1975).

Given the above limitations, the results of our analysis must be interpreted with caution. While non-significance, e.g., effect of host sex, is a reliable result, significant results may be dubious (Underwood 1981). Underwood infers that one way of offsetting the possibility of false significance would be to test significance at a lower probability level; i.e., lower than the P<0.05 level normally employed in such tests. Our analysis indicates that apparent variations in worm abundance related to host length and geographical origin are for the most part highly significant (P \leq 0.0001).

SEALWORM IN 4T AND 4Vn COD AND FLATFISH

It would be difficult to conclude that sealworm abundances in the fillets of 4T cod reported here differ from those reported 25 years ago (Scott and Martin 1957; Templeman et al. 1957). Although there were great variations in abundance of sealworm in 4T cod examined in present and past studies, infestations generally fall within the same range. In the recent Bradelle Bank sample, steak cod had heavier infections, while scrod and market cod had lighter infections than previously reported. Infections in small inshore cod collected off Chéticamp in the fall of 1980 were light in comparison with earlier records from this area; and yet cod samples from the neighbouring Souris, P.E.I., inshore in the spring of 1981 had extremely heavy infections, rivalling those found in cod from Caraquet, N.B., in 1946.

Sealworm seems to be somewhat more abundant in the fillets of local 4Vn cod than previously reported by Scott and Martin (1957). Worm counts were consistently greater (three- to tenfold) in the 1981 samples, regardless of sampling location or grade of cod. Our analysis revealed that differences of lesser magnitude among our own samples were often highly significant. The possibility that worm counts have inflated by inclusion of heavily infected migrant cod from 4T applies only to Scott's and Martin's study. In our study, a distinction has been made between local 4Vn cod, collected from late May through early October. and cod collected in winter which was presumably a mixture of local 4Vn and migrant 4T cod. Methods used to find nematodes in the fillets in our study, however, may have been two to five times as efficient as the commercial candling procedures employed by Scott and Martin (see Power 1961). When this factor is taken into consideration, evidence that sealworm has become more numerous in 4Vn cod is less convincing.

The 4T plaice examined in our study were heavily infected with sealworm, generally having more worms per unit fillet weight than cod. According to processors, the worm problem in 4T plaice, particularly off the Chéticamp to Pleasant Bay area of Cape Breton, is a recent development. Given the lack of historical records from this area, however, it is difficult to confirm this claim. The records of Templeman et al. (1957) show significant numbers of worms in plaice from the 4T offshore. In fact, the parasite appears to have been as abundant in plaice sampled from Bradelle Bank in this early study as it was in our sample from Shediac Valley. On the other hand, the heavy sealworm infections in 4Vn plaice reported in this study are without precedent. According to Templeman et al. (1957), infections in 4V plaice were extremely rare; the prevalence of the parasite in a sample from Cape North 4Vn was only 4%. Similarly, sealworm infections in 4Vn gray sole (witch flounder) were much heavier in our study than reported by Templeman et al. (1957), who found only one worm in 116 witch from the "Edge of Ground" (4Vn). While current infections in sole are much lighter than those in cod and plaice, they still exceed government standards (one worm for every three pounds or 0.73 worms per kg of fillets), and candling would be required in processing.

Sealworm appears to be no more abundant in 4T and 4Vn cod than it is in many European cod stocks (Young 1972; Platt 1975; Bjørge et al. 1981). However, in European cod, the parasite accumulates more frequently in the flaps and becomes increasingly prevalent in this location in larger fish. In British cod, for example, P. decipiens is equally abundant in the flaps and fillets of fish <50~cm in length; in fish >50~cm in length, the numbers of worms in the fillets remain more or less constant while numbers in the flaps increase at an exponential rate with host length (Young 1972). As a consequence, European cod generally have fewer worms in the fillet and fewer worms per unit fillet weight than 4T and 4Vn cod, herein. Sealworm infections in European fisheries are confined mainly to cod; and infections in other groundfish species, such as witch or plaice (long rough dab), are rare (Wootten and Waddell 1977).

SEALWORM ABUNDANCE IN GROUNDFISH AND GREY SEAL DISTRIBUTION

The persistence of heavy sealworm infestations in 4T cod and plaice and the apparent increase in abundance of the parasite in 4Vn cod and flatfish are difficult to explain in terms of grey seal distribution, as records of the seal in the southern Gulf and Sydney Bight have been rather infrequent. Recent records of grey seal distribution, however, are based largely on frequencies of bounty kills and gear entanglements, usually involving gill nets (Zwanenburg et al. 1981). An apparent scarcity of grey seals in a given area may merely indicate that fishermen who might normally hunt the seal are more profitably occupied or, possibly, the lack of an active gill net fishery.

Comparisons of current information on worm infestations in groundfish with recent records of grey seal distribution and abundance may be misleading, as the relationship between the two phenomena is not an instantaneous one. Depending on environmental temperature, hatching and development of the parasite in poikilothermic intermediate hosts may require periods of several months to several years duration (McClelland 1982, 1983b). Upon reaching the "infective" stage (i.e., infective to seals) in the fish host, the sealworm may persist indefinitely in encysted form. Hence, there would be a considerable time lag between the growth or decline of the seal population in a given area and corresponding changes in the abundance of sealworm in groundfish.

Mansfield and Beck (1977) speculate that the ice-breeding grey seal colony in Georges Bay may have grown in recent years as a result of increased ice stability in the area following the construction

of the Canso Causeway. The heavy worm infestations currently found in Souris cod and Chéticamp plaice may be related to the existence of this colony and/or increased numbers of transient seals migrating to and from the colony via the Souris/Chéticamp area. Apparent increases in sealworm abundance in 4Vn groundfish may also be attributable to the more frequent occurrence of transient seals. Grey seals migrating between the eastern Nova Scotia/Sable Island area and the Gulf of St. Lawrence via the Cabot Strait would pass through 4Vn en route. This migration route has probably been used with increasing frequency in recent years as a result of the growth of the seal population and/or the causeway having blocked access to the Gulf via the Strait of Canso. Heavy infections in cod from Frenchman's Shoal have probably resulted from the increased abundance of grey seals on Scatari Island and along the Cape Breton coast between Louisbourg and Fourchu. Numerous grey seals were also sighted on drift ice over the "Edge of Ground" 4Vn at the time we were sampling from commercial draggers in February 1980 and 1981.

ANISAKIS SP. LARVAE IN 4T AND 4Vn COD AND FLATFISH

The abundance of larval <u>Anisakis</u> in whole 4T and 4Vn cod was 1.65 in our study, with the parasite generally being most numerous in 4T fish. Abundance in the flesh was 0.038; 0.018 in the fillets, and 0.020 in the flaps. Significant numbers of the parasite also occurred in plaice from the southwestern Gulf, but infections were rare in Chéticamp and 4Vn plaice. The overall intensity of infection in plaice was 0.040, and only two larvae were found in the fillets of 1,681 plaice. In an earlier survey (Templeman et al. 1957) in which only the fillets of groundfish were examined, the overall abundance of <u>Anisakis</u> in eastern Canadian cod was 0.014. The parasite was most abundant in cod from Newfoundland waters but was not detected in either cod or plaice from the 4T offshore.

Anisakis larvae appear to be far more numerous in European cod. In fact, infections in the flesh of cod from many European fisheries are heavier than those found in whole 4T and 4Vn cod herein. According to Young (1972), abundances in the flesh of cod from the northeast and southeast British coasts and from the North Sea were 2.60, 2.54 and 1.92 respectively. Platt (1975) reports abundances in cod flesh ranging from 2.0 to 4.4 on Faroe Island Bank, from 1.7 to 2.8 in Icelandic fisheries, and from 4.1 to 10.2 in eastern Arctic and Arcto-Norwegian waters.

Since they are somewhat smaller than sealworm and greyish to yellowish-white in colour, Anisakis larvae are difficult to detect in the flesh of groundfish (Wootten and Waddell 1977). As shown in our digestion experiment, 68% of these nematodes in the flaps and fillets of average size (50-60 cm) cod escaped detection by slicing and systematic destruction of the flesh. Techniques used for routine examinations of the flesh in the present study, however, were similar in efficiency to those employed in surveys of European cod (Young 1972; Platt 1975). Hence, the apparent difference in abundance of Anisakis in the flesh of eastern Canadian and European cod is not attributable to differences in examination procedures. One may further conclude that if numbers of Anisakis in the flesh of eastern Canadian cod have been greatly underestimated, they have likewise been

underestimated, for European cod. Given that this parasite can be pathogenic to humans (Smith and Wootten 1978) and at the same time difficult to detect by procedures routinely employed in fish processing, the fact that Canadian cod has much lighter infections than European cod should be of advantage in the marketplace.

Of course, it is possible that <u>Anisakis</u> may not be as abundant in areas we surveyed as they are in other eastern Canadian fisheries. According to Parsons' and Hodder's (1971) survey of herring, larval <u>Anisakis</u> infections in 4T stock were somewhat lighter than those occurring in the Scotian Shelf, southwestern Nova Scotian, and various Newfoundland stocks; herring from Gabarus Bay (4Vn), however, had fairly heavy infections. Appy (1978), on the other hand, reported that the prevalence of "mesenteric nematodes" (mainly larval <u>Anisakis</u>) was higher in cod from the southern Gulf of St. Lawrence than in Scotian Bank and Bay of Fundy cod, while abundance was fairly uniform throughout these areas.

The distribution of Anisakis larvae in the tissues of the fish host appears to vary. In the present study, these nematodes occured mainly on the liver and visceral mesenteries and only two or three percent were found in the flesh during routine examinations. When additional nematodes, however, were recovered from flaps and fillets of cod by digestion procedures, the frequency of the parasite (in the flesh) was 22% - 11% in the flaps and 11% in the fillets. Young (1972) found Anisakis larvae to be more numerous in the flesh (69%) than in the viscera (31%) of North Sea and British cod, but in a later study of North Sea cod (Wootten and Waddell 1977), mean intensity of infection in the flesh was only eight (12%) compared to 23 (88%) in the viscera. According to Pälsson (1979) the parasite occurred more frequently in the viscera of Icelandic cod, the frequency in the flesh being about 25% in one- to two-year-old and about 10% in three-year-old fish. European investigations invariably report, however, that most of the Anisakis larvae occurring in the flesh were concentrated in the hypaxial musculture of flaps rather than being evenly distributed between flaps and fillets as was the case herein. Of the Anisakis larvae found in the flesh of European cod, 90% (Young 1972) to 100% (Wootten 1978) were in the hypaxial musculature.

CONTRACAECUM (PHOCASCARIS) SP. LARVAE IN 4T AND 4Vn COD AND FLATFISH

Contracaecum larvae on the visceral mesenteries of cod and plaice herein closely resemble infective-(third-?) stage larvae of C. osculatum reared in vitro by McClelland and Ronald (1974b). The fact that they were more numerous by far in 4T fish is probably attributable to the seasonal occurrence of harp seals in the southern Gulf, as mature C. osculatum are most commonly found in this seal species (Berland 1963; Mansfield and Beck 1977; Pälsson 1979; McClelland 1980b). Although they visit there but a few months of the year, harp seals greatly outnumber the combined populations of other resident and migrant seal species in the southern Gulf. Moreover, among seal species of the Northwest Atlantic, harp seals are most heavily infected with C. osculatum on a per-seal basis.

There is less likelihood that the larval nematodes reported here are <u>Phocascaris</u> spp., although third-stage larvae of <u>Contracaecum</u> and <u>Phocascaris</u> spp. may be morphologically inseparable (Pälsson 1979). Adults of <u>Phocascaris</u> spp. occur in the gastro-intestinal tracts of various seal species in the Northwest Altantic, but the principal host in the southern Gulf of St. Lawrence would be the hooded seal (<u>Cystophora cristata</u>); infections in harp and grey seals are extremely light (Berland 1963; McClelland 1980b). The hooded seal population in the southern Gulf is small, numbering in the hundreds, and like the harp seal, occurs there only during the breeding season.

Unfortunately, it was not possible to make a positive identification of <u>Contracaecum</u> larvae on the basis of their morphology or geographical distribution. It may be necessary to rear some of these nematodes to maturity in order to confirm their specific identity. This can be accomplished, as shown with sealworm (Scott 1953; McClelland 1980a), by transmitting the larvae to the suspected definitive host. Alternatively, the parasite may be reared to maturity or near maturity in vitro (McClelland and Ronald 1974b). Recently, larval nematodes from the viscera of Baltic cod were identified as <u>C. osculatum</u>, following surgical implantation and maturation in the peritoneal cavities of laboratory rats (Fägerholm 1982).

VARIATIONS IN ABUNDANCE OF LARVAL ANISAKINES WITH LENGTH OF FISH HOST

Larval anisakine infections usually increase in intensity with length, weight or age of fish host (Scott and Martin 1957, 1959; Templeman et al. 1957; Parsons and Hodder 1971; Young 1972; Platt 1975; Wootten and Waddell 1977; Wootten 1978; Pälsson 1979; herein). These nematodes ultimately become encysted in fish tissues, emerging only upon ingestion by a subsequent host. Hence, they may persist throughout the life span of the fish, becoming increasingly numerous through cumulative reinfections. On the other hand, as indicated by occurrences of cysts containing dead and disintegrating worms, the parasites may eventually be eliminated, possibly as a consequence of a host reaction. Even under these latter circumstances, however, the nematodes would be more numerous in larger fish as the frequency of reinfection would increase with the appetite of the host.

Of course, the rate at which fish accumulate larval anisakines would depend not only on the quantity of prey consumed, but on diet preferences as well. Cod become increasingly piscivorous as they mature, and 4T and 4Vn cod are no exception in this regard (Kohler and Fitzgerald 1969). Cod may become infected with larval anisakines by feeding on invertebrate or smaller fish hosts (Scott 1954; Smith 1974; McClelland 1983b), but the parasite would probably occur more frequently in a diet of fish. For example, young plaice which become increasingly important in the diet of large cod (Powles 1965; Waiwood et al. 1980) are themselves heavily infected with sealworm.

As shown in present and earlier studies (Scott and Martin 1957; Templeman et al. 1957), increases in sealworm abundance in 4T and 4Vn cod are either proportionate to or greater than increases in host weight; and large cod have as many or more worms per unit fillet weight than smaller cod. Prevalence and abundance of worms, on a per-fish or per-fillet basis, increase dramatically with the size of the fish, while efficiency of candling procedures used to detect the parasites declines with increasing thickness of fillet (Power 1961). Given the above factors, it would seem that the worm problem in 4T and 4Vn cod might be alleviated by fishing for smaller cod and, by management practices, maintaining young cod populations in these areas. According to processors, however, the worm problem is not the only concern. Market cod are generally preferred for processing into fillets, steak and market cod for saltfish markets. Scrod may have fewer worms but take longer to process (per unit weight) and deteriorate more rapidly than market cod.

As previously discussed, sealworm tend to accumulate more frequently in the flaps than in the fillets of large European cod (Young 1972; Platt 1975). As a consequence, the parasites become less numerous per unit fillet weight with increasing length or age of host. Young (1972) suggests that, under these circumstances, selective fishing for large cod, together with removal and separate processing of flaps, might be a useful approach to the worm problem. While dismissing selective fishing as an uneconomic option, Platt endorses the separate processing of flaps, noting that infections with <u>Anisakis</u> sp. larvae as well as sealworm are concentrated in these tissues.

On a per-unit-fillet-weight basis, sealworm is most numerous in small plaice from 4T and 4Vn (Templeman et al. 1957; this study). The apparent decrease in the frequency of reinfection in mature plaice is probably attributable to change in diet. Crustaceans and annelids serving as precursor hosts of the parasite (Val'ter 1978; Val'ter and Popova 1974; McClelland 1983a) make up the greater part of the diet of young plaTce, while the mature fish feed mainly on echinoderms and molluscs (Powles 1965; Minet 1973).

Unfortunately, we were not able to establish mathematical relationships between variations in worm abundance and host length. Plots of mean transformed worm counts versus mean host length seem to indicate the existence of linear regression, and a relationship is also implied by the significance of host length and host length/sample mean interactions in two-way ANOVAs. Regression analysis, however, reveals gross violations of the assumption of normality of the distribution of error, and under this circumstance, tests for significance of regression coefficients are meaningless (Underwood 1981). When this analysis was completed for a few samples, the regressions explained <10% of the variance, although regression coefficients were apparently highly significant $(P \le 0.00001)$. The above assumption also applies to tests of significance of correlation coefficients but may have been overlooked by Platt (1975) in computing significance of correlations between transformed worm counts (P. decipiens and Anisakis sp. larvae) and Tength, weight and age of host European cod.

VARIATIONS IN ABUNDANCE OF LARVAL ANISAKINES WITH SEX OF FISH HOST

According to three-way ANOVAs in this study, abundances of larval anisakines in 4T and 4Vn cod and flatfish did not vary significantly with host sex. In the case of <u>Contracaecum</u> sp. larvae in cod, the three-way interaction of host sex, length and sample was highly significant ($P \le 0.0001$). The significance of this particular interaction, however, was probably attributable to the influences of sample and length, which were also highly significant ($P \le 0.0001$) in two-way interaction and as main effects. Sex was not significant as a main effect or in respective two-way interactions with length and sample. As previously surmised by Templeman et al. (1957) and Appy (1978) with regard to larval <u>Phocanema</u> and <u>Anisakis</u> infections in cod, variations in intensity of larval anisakine infections can be considered without reference to host sex.

LARVAL ANISAKINES AS BIOLOGICAL TAGS

Our analyses (fixed-factor design - a priori contrasts) indicate that <u>P. decipiens</u>, <u>Anisakis</u> sp. and <u>Contracaecum</u> (<u>Phocascaris</u>) sp. larvae could be used as biological tags for 4T and 4Vn cod and flatfish stocks. Variations in abundance of <u>P. decipiens and Contracaecum</u> larvae would be useful in discriminating between inshore and offshore stocks. Quantitative comparisons of <u>Anisakis</u> sp. and, particularly, <u>Contracaecum</u> sp. infections might also be employed in distinguishing 4T from 4Vn stocks.

As they occur mainly on the visceral organs and mesenteries, Anisakis and Contracaecum are certainly more convenient to study than sealworm. With a trained eye they are readily identified and counted. If it is not possible to examine samples at sea. only the viscera need be collected for subsequent examination in the laboratory. Quantitative studies of sealworm, on the other hand, are laborious and time consuming (Appy 1978). They require that the fish be boned and skinned and that a variety of procedures such as slicing, candling or systematic destruction of the flesh be employed in detection of worms embedded in fillets and napes. Of course, the efficiency of worm detection can vary greatly with the technique employed (Power 1961). Given that proper facilities for these procedures are usually not available at sea, hundreds of kilograms of fish must be collected, stored, and transported to the laboratory.

As shown here and in earlier studies (Scott and Martin 1957; Templeman et al. 1957; Appy 1978), sealworm is most numerous ($P \le 0.0001$) in the inshore cod of 4T and 4Vn. Once a distinction has been made between inshore and offshore samples, however, it becomes apparent that worm abundances in our 4Vn cod samples were similar to those in our 4T samples. In the past, when sealworm infections in local 4Vn cod were relatively light (Scott and Martin 1957), seasonal immigrations of 4T cod must have had a dramatic impact on the worm problem in 4Vn fisheries. With the parasite uniformly abundant in 4T and local 4Vn cod, as indicated in our current samples, this should no longer be the case. Indeed, Cape Breton processors interviewed here remarked that at one time the worm problem in 4Vn cod fisheries was most severe in winter and spring when migrant 4T cod were present; but in recent years there were no seasonal variations in worm infestations.

Sealworm counts in our samples of cod from the 4Vn winter fishery were similar to those found in offshore cod from 4T and 4Vn summer fisheries. With regard to abundances of Contracaecum and Anisakis larvae, on the other hand, 4Vn winter cod samples differed greatly ($P \le 0.0001$) from 4T offshore cod, while appearing more similar to local cod collected from the 4Vn offshore in summer. On the basis of nematode counts, then, one would conclude that although some migrant 4T cod were included in these

samples they were mainly comprised of local offshore fish. These findings are somewhat surprising in light of tagging experiments (Martin and Jean 1964; Kohler 1975) which indicate that cod overwintering along the Laurentian Channel in 4Vn are mainly migrant 4T cod. Moreover, distribution of tag recoveries from 4T show that this migrant stock is a mixture of inshore and offshore fish.

As discussed above, Contracaecum sp. larvae probably belong to <u>C. osculatum which</u>, like sealworm, mature and reproduce in the gastro-intestinal tract of seals. The fact that adults of this nematode abound in harp seals breeding in the southern Gulf of St. Lawrence would explain why the larvae are so numerous in 4T cod while rarely occurring in local 4Vn cod. In 4T offshore cod ≥ 61 cm in length (n = 261), prevalence of <u>Contracaecum</u> infection was 53%, abundance, 4.94. However, in cod \geq 61 cm in length from the 4Vn winter fishery ($\eta = 768$), prevalence and abundance of Contracaecum were 12% and 0.91, respectively. One might therefore conclude that only a small proportion (less than one-quarter) of the cod in the 4Vn winter samples were migrants from 4T. At present, a conclusion of this nature would be presumptuous, since it is based on nematode abundances in a few samples of cod which may not be typical of commercial catches. With more extensive sampling and a larger data base, however, it may be possible to estimate (by reference to larval Contracaecum infections) what proportion of cod caught in the 4Vn winter fishery are migrant 4T fish. Harp seals are even more numerous off Labrador and northeastern Newfoundland than they are in the southern Gulf of St. Lawrence (Sergeant 1965, 1966), and larval Contracaeum might also prove to be a useful biological tag for cod stocks in these areas.

Fish parasites are most useful as tags when there is radical variation in their abundance in different host populations (Smith and Wootten 1978). With regard to larval <u>Contracaecum</u> infections in 4T and 4Vn cod, <u>this criterion</u> has been met. Similarly, Platt (1976) has shown that Icelandic and Greenland cod stocks can be distinguished by differences in sealworm abundance; the prevalence of the parasite is high in the former and low in the latter stocks. Seasonal mixing of Icelandic and migrant Greenland cod on the spawning grounds south of Iceland is indicated by sealworm prevalences intermediate to those found in the component stocks.

Our analysis of larval anisakines in 4T and 4Vn plaice illustrates the perils of attempting to define a given fish stock by its parasitic infections. Worm counts in plaice from the southeastern Gulf (Chéticamp) were consistent with those in 4Vn plaice while differing significantly from those found in plaice from the southwestern Gulf (Pt. Escuminac, N.B. and Shediac Valley). This was particularly evident with Anisakis sp. and Contracaecum sp. larvae which occurred in significant numbers in samples from the southwestern Gulf but were rare or lacking in Cheticamp and 4Vn samples. According to Powles (1965), southern Gulf plaice are a discrete stock, meristically distinct from 4Vn plaice. Powles' tagging experiments indicate, however, that the 4T stock consists of two main groups, a northern or "Miscou-Magdalen" group and a southern or "Cape Breton" group. Our samples from Pt. Escuminac and the Shediac Valley would belong to the northern group and our Chéticamp sample to the southern group.

MIGRATIONS OF ASCARIDOID NEMATODES IN ICED ROUND FISH

As indicated here and in Cheng's (1976) study, adult and immature Hysterothylacium (Thynnascaris, Contracaecum) aduncum typically found in the gastro-intestinal tract of marine fish migrate through the gut wall to the body cavity and flesh after the death of the host. These nematodes are also found escaping from the mouth, gills, and anus of fish stored on ice, in the round. The allegation that sealworm leave the fillets of round cod can probably be traced to the presence of migrant H. aduncum free among the bodies or in the body cavities of the fish. While the adults of H. aduncum are often too large (>10 cm in length) to be confused with sealworm, the immature stages of the former are similar in size, shape and colour to the latter; to the layman, the two species would be inseparable. As some H. aduncum invade the flesh after the death of the host, there would be an increase rather than a decrease in the numbers of nematodes in the fillets of round cod.

We found that the larval anisakines on the visceral organs and mesenteries were usually encysted and remained so, long after the death of the fish host, even as surrounding host tissues decayed. However, occurrences of unencysted larvae and vacated capsules among host viscera indicate that some anisakines may excapsulate and these would be free, like <u>H. aduncum</u>, to migrate to the flesh. Experiments in which the distributions of larval anisakines in the flesh of round and freshly gutted cod were compared (Table 16) neither proved nor disproved this possibility. Round cod had more Anisakis sp. larvae in the flesh (24% of the total) than freshly gutted cod (21% of the total), and the ratio of Anisakis in the fillets to those in the flaps was greater in the former (1.6:1) than in the latter (0.5:1). No sealworm were found in the viscera of round or freshly gutted cod, but the ratio of worms in fillets to worms in flaps was again greater in the former (49.0:1) than in the latter (13.7:1). While these results suggest that some larval anisakines may move to the fillets in iced round cod, they are far from conclusive. Distributions of the nematodes in the tissues of round and gutted cod differed by only a few worms in each case, and such differences could be attributed to natural variations.

Similar experiments performed by Smith and Wootten (1975) indicated significant migrations of Anisakis sp. larvae from the viscera to the flesh of iced round herring. While only 4% of these nematodes occurred in the flesh of freshly gutted herring, 12%-20% were found in the flesh of iced round herring examined 37 hours after capture. As the fish used in this study were heavily infected (mean intensities of infection ranging from 9 to 22 in various samples), there were noticeable increases in the numbers of nematodes in the flesh, although the shift of worms from viscera to flesh was actually rather small on a percentage basis (8%-16%). Migrations of this order would be impossible to detect in the present study because of the low abundance of Anisakis (0.77-0.96). The rate of such migrations would also be influenced by host species and size of host; cod used in our study were considerably larger (averaging 1500 g) than the herring (60-250 g) employed in Smith's and Wootten's experiments.

		15

	Host					· Larv	al anisakines			
		<u> </u>			Phocanema		Anis	<u>akis</u>	Contrac	aecum
Sample	Length range (cm)	Mean weight (kg)	n	Prevalance ^a	Abundance ^b	No. per kg fillet	Prevalence	Abundance	Prevalence	Abundanc
Shediac	<u>≤</u> 40	0.49	22	27	0.41	2.63	41	0.50	5	0.05
/alley (4T)	41-50	0.99	52	44	0.62	1.54	77	1.63	15	0.25
Sept. 1980	51-60	1.47	37	51	1.41	2.67	62	1.16	33	0.95
1900 (1900	61-70	2.60	14	79	2.71	2.95	64	1.71	57	3.14
	≥71	4.49	10	100	10.50	6.76	90	8.00	50	4.20
Pt∙ Escuminac,	<u>≤</u> 30	0.17	46	26	0.26	4.80	11	0.15	0	0
I.B., (4T)	31-35	0.34	37	22	0,30	2.77	16	0.16	0	0
June 1981	36-40	0.56	35	54	0.69	3.87	40	0.60	3	0.03
	41-45	0.75	48	54	1.10	4.51	50	0.77	0	0
	46-50	1.10	44	55	1.14	3.26	48	1.20	2	0.05
	51-55	1.40	40	55	1.33	2.99	63	1.65	15	0.20
	56-60	1.40	34	82	2.29	3.63	91	2.62	44	1.74
	<u>></u> 6I	2.60	23	100	5.09	5.72	96	6.09	70	5.26
Bradelle Bank	<u>< 45</u>	0.75	31	58	1.06	4.46	32	0.45	0	0
(4T) Nov. 1981	46-50	0.98	33	21	0.42	1.36	55	0.94	õ	ŏ
	51-55	1.45	28	61	0.86	1.79	75	2.71	14	0.32
	56-60	1.72	35	69	1.31	2.42	77	2.57	20	0.40
	61-65	2.16	28	61	1.54	2.15	86	3.79	25	1.54
			25	84						
	66–70 <u>≥</u> 71	2.84 5.50	29 29	84 97	2.84 7.3	2.86 7.76	76 86	4.04 5.52	48 62	1.48 3.69
Souris, P.E.I.,	36-40	0.59	20	30	0.45	2.42	60	1.20	0	0
(4T) April 1981	41-45	0.75	38	58	1.39	5.90	45	0.79	3	0.05
	46-50	1.00	27	63	1.93	5.96	30	0.52	7	0.07
	51-55	1.44	26	77	3.38	6.82	81	1.96	4	0.35
	56-60	1.91	36	81	5.22	8.51	69	1.28	17	0.58
	61-65	2.27	31	84	7.16	9.26	77	2.48	13	0.19
	66 - 70 ≥ 71	2.74 4.69	13 13	92 100	13.92 25.31	13.84 13.84	46 77	1.08 6.38	15 54	0.85 4.38
Souris, P.E.I.,	36-40	0.60	11	36	0.91	4.80	18	0.27	0	0
(4T) May 1981	41-45	0.78	33	61	1.79	7.15	30	0.36	0	0
	46-50	1.06	34	79	2.91	8.60	35	0.88	9	0.15
	51-55	1.46	33	76	3.09	6.45	45	1.15	б	0.18
	56-60	1.86	36	75	5.28	8.76	64	1.81	22	0.86
	61-65	2.38	26	85	8.38	10.26	54	1.58	23	0.50
	66-70 ≥71	2.81 4.63	17 20	94 95	16.35 15.60	16.92 9.19	41 70	0.82 4.15	12 25	0.18 0.70
					,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		,.			00,0
) AT) Nov. 1980	<u><</u> 35 36-40	0.32 0.53	20 63	35 33	0.40 0.56	3.52 3.34	20 19	0.20 0.25	0	0 0
417 NOV . 1900										
	41-45	0.78	62	35	0.65	2.62	32	0.37	0	0
	46 - 50 ≥ 51	1.04 1.43	106 38	43 58	0.79 1.24	2•29 2•73	43 50	0.63 0.82	2 0	0.02 0
t. Paul's Island	≤ 50	1.01	13	8	0.08	0.24	62	1.46	8	0.23
4T) Nov. 1980	51-55	1.30	55	40	0.71	l•50	62	2.00	22	0.44
	56-60	1.57	55	38	0.55	1.06	73	2.91	25	1.51
	61-65	1.95	40	35	0.63	0.77	78	2.73	60	4.63
	66 - 70	2.48	29	38	1.28	1.50	86	3.41	59	3.03
	≥.71	3.87	29	72	3.10	2.13	97	8.93	62	13.17
St. Paul's Island	≤ 50	1.04	84	36	0.45	1.34	45	0.83	4	0.06
(4T) Dec. 1980	51-55	1.36	99	39	0.73	1.65	64	1.40	6	0.12
	56-60	1.70	55	55	1.53	2.77	62	1.62	16	0.58
	61-70	2.39	33	61	2.88	3.45	76	2.27	48	3.09
	≥ 71	4.75	24	96	8.46	4.47	83	6.75	- 50	10.79

TABLE 1. Abundances of larval anisakines in 4T and 4Vn cod.

	Host					Larv	al anisakines	detected		
					Phocanema		Ants	akis	Contrac	aecum
Sample	Length range (cm)	Mean weight (kg)	n	Prevalance ^ä	Abundance	^b No. per kg fillet	Prevalence	Abundance	Prevalence	Abundance
Edge of Ground	<u><</u> 30	0.18	29	14	0.14	2.38	10	0.24	0	0
(4Vn) Feb. 1980	31-35	0.31	58	36	0.84	8.53	7	0.07	3	0.03
	36-40	0.48	50	30	0.60	3.83	8	0.10	2	0.06
	41-45	0.17	53	19	0.36	1.60	13	0.34	2	0.02
	46-50	1.00	70	24	0.74	2.27	33	0.91	0	0
	51-55	1.34	100	45	1.40	3.12	33	0.95	3	0.12
	56-60	1.67	105	57	2.51	4.53	26	0.63	2	0.07
	61-65	2.09	56	48	2.16	3.10	30	1.11	9	0.46
	66 - 70 ≥71	2.56 3.34	29 27	59 63	2.41 5.22	2.73 4.20	21 22	1.55 1.26	14	1.76 0.59
Edge of Ground	≤35	0.35	40	38	0.53	4.58	15	0.18	0	0
(4Vn) Feb. 1981	36-40	0.50	58	40	0.84	5.37	34	0.62	5	0.16
	41-45	0.72	76	47	1.03	4.38	45	0.78	1	0.03
	46-50	1.02	58	41	1.34	2.75	50	1.03	3	0.19
	51-55	1.37	69	46	1.13	2.42	55	1.42	6	0.13
	56-60	1.76	54	46	2.22	3.78	48	1.24	2	0.02
	61-65	2.18	61	67	4.18	5.79	59	1.33	15	0.52
	66–70 ≥71	2.71 4.74	47 43	81 95	3.68 15.58	4.09 8.69	74 74	2.13 3.88	30 19	1.06 1.21
	2	44/4	45		12.50	0.09	74	2.00	15	1•21
dge of Ground	≤ 35	0.31	52	15	0.19	1.78	10	0.12	0	0
(4Vn) Mar. 1981	36-40	0.50	80	13	0.15	0.79	45	1.10	0	0
	41-45	0.68	74	34	0.74	3.32	55	1.18	0	0
	46-50	0.98	74	39	0.76	2.35	57	1.43	5	0.18
	51-55	1.34	79	46	1.38	3.17	62	1.87	13	0.23
	56-60	1.79	71	62	2.56	4.25	63	1.65	10	0.46
	61-65	2.29	66	71	2.86	3.65	71	2.65	20	2.32
	66-70	2.71	58	83	4.07	4.58	64	2.52	33	3.43
	≥71	3.78	52	96	7.79	5.37	81	2.79	29	2.08
ngonish, N.S.,	36-40	0.55	11	45	1.00	5.70	55	1.09	9	0.18
(4Vn) May 1981	41-45	0.77	22	73	1.59	6.36	45	0.82	9	0.14
	46-50	1.04	37	65	1.49	4.42	59	2.14	5	0.11
	51-55	1.34	38	63	2.66	5.74	79	2.61	18	0.71
	56-60	1.72	33	61	2.88	5.13	70	2.00	6	0.06
	61-65	2.13	36	64	2,58	3.63	72	1.89	0	0
	66-70	2.54	27	81	4.07	4.55	78	2.63	7	0.11
	≥71	4.72	26	96	13.92	6.91	77	5.23	12	2.65
renchmen's	31-35	0.38	9	44	0.56	4.64	22	0.56	0	0
Shoal (4Vn)	36-40	0.58	33	42	1.03	5.65	12	0.15	0	0
July 1981	41-45	0.74	34	59	1.88	7.02	18	0.18	0	0
	46-50	1.03	36	58	2.11	6.29	14	0.39	0	0
	51-55	1.29	36	- 89	3.83	9.15	44	0.56	0	0
	56-60	1.69	31	84	3.68	6.80	48	0.71	0	0
	61-65 66-70	2.13 2.60	33 38	82 87	9.24 9.32	10.80 10.34	55 45	0.94 1.21	0 3	0 0.26
	≥71 ≥71	3.57	33	97	11.24	8.60	76	4.36	18	1.21
dae of Ground	< 45	0.65	13	23	0.23	1.12	23	0.54	0	0
(4Vn) June 1981	<u>46</u> -50	0.97	46	46	0.25	3.17	25	0.33	0	0
	51-55	1.30	49	51	1.55	3.76	37	0.74	2	0.02
	56-60	1.67	43	49	0.67	1.32	51	1.02	2	0.33
	61-65	2.05	45	62	2.07	3.15	44	0.87	2	0.02
	66-70	2.46	45	73	3.04	3.56	56	1.38	2	0.02
	<u>></u> 71	3.87	46	87	6.46	4.16	61	2.72	0	0

Host length	Larval anisal	cines	Distr	ibution of r	nematodes in	host tissues	. (%)
range (cm)	sp	n	Fillets	Flaps	Liver	Pyloric caecae	0ther ^a
<u><</u> 30	Phocanema	18	94.44	0	0	0	5.55
	Anisakis	7	0	0	57.14	0	42.86
	Contracaecum	0	0	0	0	0	0
31-35	Phocanema Anisakis Contracaecum	50 31 0	96.00 0 0	2.00 0 0	0 83.87 0	0 6.45 0	2.00 9.68
36-40	Phocanema	196	97.96	0.51	0	0.51	1.02
	Anisakis	212	1.42	1.89	77.83	13.21	5.66
	Contracaecum	13	0	7.69	0	92.31	0
41-45	Phocanema	457	95.84	1.53	0.44	1.31	0.88
	Anisakis	342	1.45	0.88	78.07	15.50	4.09
	Contracaecum	6	0	0	0	100.00	0
46-50	Phocanema	663	97.44	1.96	0.15	0.30	0.15
	Anisakis	622	1.45	1.13	74.60	19.61	3.22
	Contracaecum	61	0	0	0	95.08	4.92
51-55	Phocanema	943	95.33	3.71	0	0.11	0.85
	Anisakis	946	1.27	1.06	69.45	23.04	5.18
	Contracaecum	136	0	0	0	96.32	3.68
56-60	Phocanema	1202	96.76	2.75	0.08	0.08	0.33
	Anisakis	864	0.69	1.27	69.21	24.31	4.51
	Contracaecum	312	0.32	0	0.32	97.44	0.96
61-65	<u>Phocanema</u>	1591	90.70	5.03	0.63	2.58	1.07
	Anisakis	868	1.15	1.27	60.14	29.84	7.60
	Contracaecum	551	0	0	1.63	89.29	9.07
66-70	Phocanema	1670	91.68	6.41	0.36	0.66	0.90
	Anisakis	773	0.65	1.29	54.20	36.09	7.76
	Contracaecum	550	0	0	0.36	96.36	3.27
<u>></u> 71	Phocanema	3576	82.24	11.97	1.31	2.63	1.85
	Anisakis	1549	1.23	1.23	41.83	48.81	6.91
	Contracaecum	1120	0	0.09	0.09	98.57	1.25
Totals	Phocanema	10366	89.89	6.80	0.65	1.51	1.15
	Anisakis	6214	1.11	1.21	60.67	31.01	6.00
	Contraceacum	2749	0.04	0.07	0.47	95.93	3.38

TABLE 2. Distribution of larval anisakines in the tissues of 4T and 4Vn cod (n = 3,760).

^aIncludes unencysted nematodes in the body cavity as well as nematodes encysted on the peritoneum and mesenteries of the stomach, intestines, spleen, gallblader, gonads, etc.

	Host			Larval a	nlsakines detect	ed			
			Phoca	nema	Anisa	ik is	Contracaecum		
Length range	Sex	n	Prevalence	Abundance	Prevalence	Abundanœ	Prevalence	Abundanc	
< 30	m	32	28	0.31	16	0.34	0	0	
	f	57	19	0.21	7	0.07	0	0	
31-35	៣	61	33	0.61	10	0.10	0	0	
	f	137	28	0.45	16	0.20	ł	0.01	
36-40	m	145	34	0.68	29	0.55	3	0.06	
	f	237	31	0.53	31	0.56	ł	0.03	
41-45	m	246	46	1.10	39	0.76	2	0.02	
	f	256	40	0.80	41	0.68	0	0	
46-50	m	361	43	0.93	44	1.06	4	0.11	
	f	325	47	1.19	45	0.91	4	0.06	
51-55	m	350	52	1.63	59	1.65	9	0.24	
	f	364	51	1.41	52	1.26	7	0.15	
56-60	m	286	64	2.58	57	1.45	13	0.59	
	f	315	58	2.31	59	1.63	13	0.47	
61-65	m	199	71 ,	4.84	65	1.96	15	0.84	
	f	264	61	2.83	61	2.06	23	1.55	
6 6- 70	m	154	79	4.94	66	2.40	27	1.73	
	f	202	73	4.84	59	2.23	26	1.66	
<u>></u> 71	m	130	93	11.81	69	4.03	23	2.93	
	f	216	90	10.12	75	4.53	31	3.38	
Totals	m	1964	56	2.71	51	1.50	10	0.57	
	f	2373	53	2.51	49	1.51	11	0.72	

TABLE 3. Variations in abundance of larval anisakines with sex of host 4T and 4Vn cod.

Source of	Nematode	Degrees of	Mean	F ^a
variation	sp.	freedom	square	
Main effects				
Host sex	<u>Phocanema</u>	1	0.081	0.82
	Anisakis	1	0.090	1.16
	Contracaecum	1	0.108	2.54
Host sample	Phocanema	12	3.791	38.29****
	Anisakis	12	2.045	26.33****
	Contracaecum	12	1.302	30.48****
Host length	Phocanema	6	10.195	102.97****
	Anisakis	6	3.163	40.74****
	Contracaecum	6	2.356	55.15****
Two-way interaction	<u>s</u>			
Sex x sample	Phocanema	12	0.141	1.42
	Anisakis	12	0.067	0.86
	Contracaecum	12	0.056	1.31
Sex x length	Phocanema	6	0.073	0.73
	Anisakis	6	0.057	0.74
	Contracaecum	6	0.079	1.86
Sample x length	<u>Phocanema</u>	71	0.284	2.87****
	Anisakis	71	0.189	2.43****
	Contracaecum	71	0.277	6.49****
Three-way interacti	ons			
(Sex x sample x length)	Phocanema Anisakis Contracaecum	71 71 71	0.119 0.089 0.074	1.20 1.14 1.74****
Error	<u>Phocanema</u> Anisakis Contracaecum	3,889 3,889 3,889	0.099 0.078 0.043	

TABLE 4. Three-way ANOVA for variations in abundance of larval anisakines with sex, sample and body length of host 4T and 4Vn cod.

aSignificance at P<0.05*, \leq 0.01**, \leq 0.001***, and \leq 0.0001****.

		Source of variation						
		Sar	nple means	Host length		Sample means x host length		
Contrast	Nematode spectes	d.f.	Fa	d.f.	Fa	d.f.	Fa	
Two-way ANOVA	Phocanema	12	34.01****	6	88.31****	72	2.96****	
	Anlsakis Contracaecum	12 12	24•79**** 24•72****	6 6	37•4 **** 58•90****	72 72	2•74**** 7•17****	
Inshore vs. offshore	Phocanema	1	187.63****	-	-	6	3.01**	
	Anisakis Contracaecum	1	0.14 12.13****	-	-	б б	₊0 3₊26**	
4T inshore vs. offshore	Phocanema	1	99.05****	-	-	б	3.52**	
	Anisakis Contracaecum	I I	4.66* 18.16****		-	6 6	0•99 2•94**	
4T offshore vs. 4Vn	Phocanema	I	2.54	-	-	б	1.66	
offshore (winter)	Anisakis Contracaecum	1	66•59**** 42•25****	-	- -	6 6	7•95**** 20•84****	
4Vn offshore (winter)	Phocanema	I	4.03*	-	-	6	1.43	
vs. 4Vn offshore (summer)	Anisakis Contracaecum	1	7 .90** 19 . 31 *** *	-	-	6 6	I∙17 3∙59**	
4Vn offshore (summer)	Phocanema	1	68.51****	-	-	б	0.85	
vs. 4Vn inshore (spring and summer)	Anlsakis Contracaecum	1	17.86**** 2.11	-	-	6 6	l • 14 l • 14	

TABLE 5. Two-way ANOVAs for variations in abundance of larval anisakines with sample and length of host 4T and 4Vn cod and contrasts of samples grouped according to geographic origin and/or season of capture.

^aSignificance at P \leq 0.05*, \leq 0.01*, \leq 0.001*, and \leq 0.0001****.

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Host				Larval anisakines detected							
	Length range (cm)	Mean weight (kg)	n	Phocanema			Anisakis		Contracaecum		
Sample				Prevalance	Abundance	No. per kg fillet	Prevalence	Abundanœ	Prevalence	Abundano	
Shed I ac	< 30	0.18	57	16	0.19	3.64	0	0	0	0	
Valley (4T)	31-35	0.29	374	24	0.30	3.79	2	0.02	ł	0.03	
Nov. 1980	36-40	0.42	228	28	0.39	3.38	4	0.04	4	0.10	
	41-45	0.64	54	56	1.00	5.65	11	0.13	6	0.94	
	<u>></u> 46	1.21	15	93	2.47	7.42	20	0.27	7	0.20	
Pt. Escuminac,	<u><</u> 30	0.20	56	43	0.77	14.65	2	0.02	0	0	
N.B., (4T)	31-35	0.30	56	50	0.96	11.84	4	0.04	2	0.02	
June 1981	36-40	0.52	49	45	0.73	4.99	4	0.04	4	0.04	
	41-45	0.76	63	59	1.33	6.20	3	0.08	3	0.27	
	46-50	1.10	21	90	2.95	9.46	19	0.33	14	0.33	
	<u>></u> 51	1.44	8	100	3,50	5.15	38	2.88	25	2.13	
Chéticamp (4T)	<u><</u> 25	0.11	93	18	0.35	12.14	I	0.01	0	0	
Nov. 1980	26-30	0.19	87	32	0.84	16.89	0	0	0	0	
	31-35	0.29	33	45	1.00	12.76	0	0	0	0	
	<u>></u> 36	0.83	27	44	1.19	5.37	0	0	4	0.04	
Ingonish, N.S.,	31-35	0.32	58	55	2.00	23.43	0	0	0	0	
(4Vn)	36-40	0.44	75	53	1.77	15.03	0	0	0	0	
0ct. 1980	41-45	0.66	68	57	2.38	13.55	0	0	0	0	
	46-50	0.96	39	51	1.97	7.59	3	0.03	0	0	
	<u>></u> 51	1.40	21	48	0.81	2.18	0	0	0	0	
Edge of Ground	31-35	0.31	25	32	0.96	11.68	0	0	0	0	
(4Vn) Feb, 1981	36-40	0.49	34	41	0.91	6.95	3	0.03	0	0	
	41-45	0.69	54	63	2.67	14.41	4	0.04	0	0	
	46-50	1.01	46	59	2.48	9.22	2	0.02	0	0	
	<u>></u> 51	1.40	41	68	1.71	4.58	2	0.02	0	0	

TABLE 6. Abundances of larval anisakines in 4T and 4Vn plaice.

Host length	Larval anisak	Larval anisakines		Distribution (\$) of nematodes in host tissues						
range (cm)	sp	n	F11 lets		Unencysted in body					
				Liver	Gastro-Intestinal mesenteries	Other visceral tissues	cav1ty			
< 25	Phocanema	36	100.00	-	_	_	-			
	Anisakis	I I	-	-	-	-	100.00			
	Contracaecum	0	-	-	-	-	-			
26-30	Phocanema	124	98.39	-	-	-	1.61			
	Anisakis	I	-	100.00	-	-	-			
	Contracaecum	0	-	-	-	-	-			
31-35	Phocanema	337	98.81	-	-	0.30	0.89			
	Anisakis	8	12.50	50.00	12.50	-	25.00			
	Contracaecum	13	7.69	-	84.62	-	7.69			
36-40	Phocanema	300	97.67	-	-	1.00	1.33			
	Anisakis	11	9.09	63.64	18.18	-	18.18			
	Contracaecum	25	4.00	-	80.00	4.00	12.00			
41-45	Phocanema	455	98.68	0.22	-	-	1.10			
	Anisakis	12	-	50.00	16.67	8,33	25.00			
	Contracaecum	69	-	-	97.10	-	2.90			
46-50	Phocanema	284	97.89	0.70	-	-	1.41			
	Anisakis	9	-	88.89	11.11	-	-			
	Contracaecum	7	-	-	100.00	-	-			
<u>></u> 51	Phocanema	135	95.56	2.22	-	0.74	1.48			
	Anisakis	26	-	46.15	53.85	-	-			
	Contracaecum	20	-	10.00	90.00	-	-			
Totals	Phocanema	1671	98.14	0.36	-	0.30	1.20			
	Anisakis	68	2.94	55.88	29.41	1.47	10.29			
	Contracaecum	134	1.49	1.49	91.79	0.75	4.48			

TABLE 7. Distribution (\$) of larval anisakines in the tissues of 4T and 4Vn plaice (n = 1681).

Host			Phocanema	detected
Length range (cm)	Sex	n	Prevalence	Abundance
<u><</u> 25	m	52	23	0.48
	f	50	14	0.22
26-30	m	93	32	0.68
	f	104	28	0.59
31-35	m	113	38	0.93
	f	426	30	0.54
36-40	m	50	46	1.36
	f	345	35	0.67
41-45	m	21	48	1.52
	f	228	59	1.85
46-50	m	2	50	2.50
	f	118	64	2.36
<u>≥</u> 51	m f	- 79	- 60	1.70
Totals	m	331	36	0.90
	f	1350	41	1.01

TABLE 8. Variations in abundance of larval $\underline{Phocanema}\ \underline{decipiens}\ with$ sex of host 4T and 4Vn plaice.

TABLE 9. Three-way analysis of variations in abundances of larval Phocanema with sex, sample and body length of host 4T and and 4Vn plaice⁴.

Source of variation	Degree of freedom	Mean square	Fb
<u>Main effects</u> Host sex Host sample Host length	1 4 2	0.004 0.261 0.296	0.09 5.70*** 6.47**
Two-way interactions Sex x sample Sex x length	4 2 8	0.059	1.28
Sample x length <u>Three-way interaction</u> Sex x sample x length	8	0.040	0.87
Error	1204	0.162	

^aFor host length strata $\leq\!40\,$ cm. b Significance at P $\!\leq\!0.05^{*},\,\leq\!0.01^{**}$ and $\leq\!0.001^{***}.$

Contrast	Nematode species	Sample means		Host length		Sampe means x host length	
		d.f.	Fa	d.f.	Fa	d•f•	Fa
Two-way ANOVA	Phocanema	4	3.96**	5	11.30****	20	3.40****
·····, ·····	Anisakis	4	27.58****	5	14.91****	20	9.30****
	Contracaecum	4	11.73****	5	4.4 ***	20	3.40****
West 4T vs. east	Phocanema	I	4.31*	-	-	5	6.84****
4T and 4Vn	Anisakis	1	90.63****	-	-	5	22.69****
	Contracaecum	1	31.81****	-	-	5	6.78****
West 4T, inshore vs.	Phocanema	1	9.94**	-	-	5	0.93
offshore	Anlsakls	1	9.94**	-	-	5	3.57*
	Contracaecum	1	9.84**	-	-	5	3.41**
47 vs. 4Vn	Phocanema	I	0.16	-	-	5	4.08**
4T inshore vs. offshore	Phocanema	I	1.28	-	-	5	1.39
Pt. Escuminac, N.B., (4T) vs. Cheticamp, N.S., (4T)	Phocanema	I	8•89**	-	-	5	1.71
Cheticamp (4T) vs. Ingonish, N.S., (4Vn)	Phocanema	1	0.92	-	-	5	0.69
Cheticamp-Ingonish vs. 4Vn offshore	Phocanema	1	0.20	-	-	5	1.57

TABLE 10. Two-way ANOVAs for variations in abundance of larval anisakines with sample and length of host 4T and 4Vn plaice and contrasts of samples.

asignificance at P \leq 0.05*, \leq 0.01**, \leq 0.001*** and \leq 0.0001****.

TABLE 11. Larval anisakines in 4Vn witch.^a

Host				Phocanema detected			
Sample	Length range (cm)	Mean weight (kg)	n	Prevalence	Abundance	No. per kg fillet	
Ingonish Oct. 1980	31-35 36-40 41-45 46-50 ≥51	0.25 0.41 0.58 0.83 1.12	33 99 135 49 8	6 10 18 43 50	0.06 0.16 0.19 0.53 0.88	0.91 1.22 1.25 2.11 2.92	
Edge of Ground Feb. 1980	≤40 41-45 46-50 ≥51	0.29 0.50 0.73 1.08	55 66 56 19	2 12 16 26	0.02 0.12 0.21 0.32	0.24 0.90 1.10 1.10	
Edge of Ground Mar. 1981	<u><</u> 35 35-40 41-45 46-50 <u>≥</u> 51	0.22 0.36 0.49 0.76 1.04	45 55 65 39 9	0 2 11 26 67	0 0.04 0.12 0.28 1.78	0 0.37 0.92 1.39 6.38	

^aSingle Anisakis larvae were found in the Cape Smokey and the Edge of Ground (Feb. 1980) samples; both larvae occurred in the body cavity.

TABLE 12. Variations in abundance of larvae $\underline{Phocanema}\ \underline{decipiens}$ with sex of host 4Vn witch.

Host			Larval <u>Phoc</u>	Larval <u>Phocanema</u> detected		
Length range (cm)	Sex	n	Prevalence	Abundance		
<u><</u> 35	m	41	2	0.02		
	f	45	2	0.02		
36-40	m	114	5	0.10		
	f	87	7	0.09		
41-45	m	102	19	0.21		
	f	163	12	0.13		
46-50	m	35	23	0.40		
	f	108	30	0.32		
<u>></u> 51	m	3	33	0.33		
	f	33	42	0.85		
Totals	m	295	12	0.16		
	f	436	17	0.21		

TABLE 13. Two- and three-way analyses of variations in abundances of Phocanema decipiens with sex, sample and length of host 4Vn gray sole.

Source of variation	Degree of freedom	Mean square	F ^a
Three-way ANOVA main effects	an an an an ann an San <u>Ann an Ann an Ann an Ann a</u> n an Ann		
Host sex Host sample Host length	1 2 4	0.003 0.120 0.217	0.22 8.25*** 14.96****
Two-way interactions			
Sex x sample Sex x length Sample x length	2 4 8	0.021 0.003 0.054	1.47 0.22 3.69***
Three-way interaction	6	0.005	0.37
Error	703	0.015	
Two-way ANOVA main effects			
Host sample Host length	2 4	0.131 0.365	9.06**** 25.30****
Two-way interaction			
Sample x length	8	0.057	3.92***
Error	717	0.014	

aSignificance at P<0.05*, <0.01**, <0.001*** and <0.0001****.

	Host	Hysterothyl	Hysterothylacium detected			
Length range (cm)	n	Prevalence	Average no.			
≤30 31-35 36-40 41-45 46-50 51-55 56-60 61-65 66-70 >71	95 201 386 504 686 714 601 471 356 353	7 8 7 8 11 11 8 8 8 12	0.13 0.23 0.09 0.09 0.10 0.19 0.15 0.16 0.11 0.24			
Totals	4367	9	0.15			

TABLE 14. Migrant adults and fourth-stage larvae of Hysterothylacium aduncum in the body cavity and flesh of 4T and 4Vn cod.

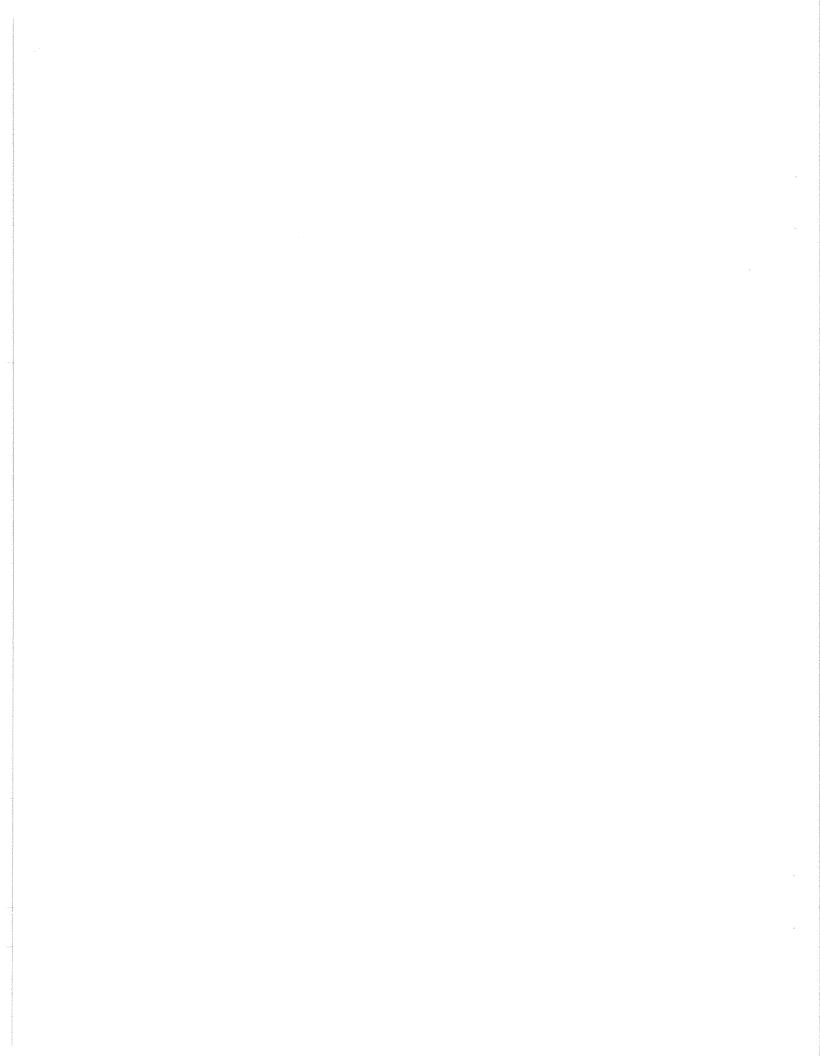
TABLE 15. Larval anisakines in viscera and flesh of freshly eviscerated cod and round cod.

Host				Hysterothylacium detected			
Time ^a (hr)	'n	Mean length (cm)	Mean weight (kg)	Sp	Prevalence	Abundance	% in flesh
0	187	55	1.51	P. <u>decipiens</u> Anisakis sp	49 26	2.18 0.70	99.51 3.05
6	49	55	1.42	P. <u>decipiens</u> Anisakis sp	45 20	1.33 0.88	98.46 0
12	48	54	1.45	P. decipiens Anisakis sp	33 50	1.21 1.27	98.27 3.28
24	50	55	1.53	P. <u>decipiens</u> Anisakis sp	40 34	0.96 1.10	97.92 1.82

 $^{\rm a}{\rm Hours}$ elapsed between time fish were caught and time they were eviscerated.

		Gutted cod (n = 56; mean weight = 1.54 kg)		Round cod (n = 57; mean weight = 1.53 kg)		Control (n = 51; mean weight = 1.55 kg)	
		Phocanema	Anisakis	Phocanema	Anisakis	Phocanema	Anisakis
Larval anisakines	Prevalence	55	41	77	42	63	53
detected by	Abundance	2.07	0.66	3.11	0.81	2.06	1.51
destruction of flesh	No. per kg fillet	4.02	0.04	6.36	0.07	4.10	0.20
	\$ In fillets	95	3	99	4	98	6
	≸ in flaps	5	5	I	4	I	0
	% in viscera	0	92	ł	91	I	94
Larval anisakines	Prevalence	61	50	81	49		
detected after	Abundance	2.36	0.77	3.63	0.96		
digestion of flesh	No. per kg flllet	4.50	0.11	7.37	0.29		
	\$ in fillets	93	7	98	15		
	% in flaps	7	14	2	9		
	<pre>% in viscera No. detected by digestion as % of total</pre>	0	79	ο,	76		
	no, in flesh No, detected by digestion as	12	67	14	69		
	% of total no.	12	14	14	16		

TABLE 16. Abundances of larval anisakines in the viscera and flesh of gutted and round cod (50-60 cm length range) from Scatari Bank (4Vn), October 1981.



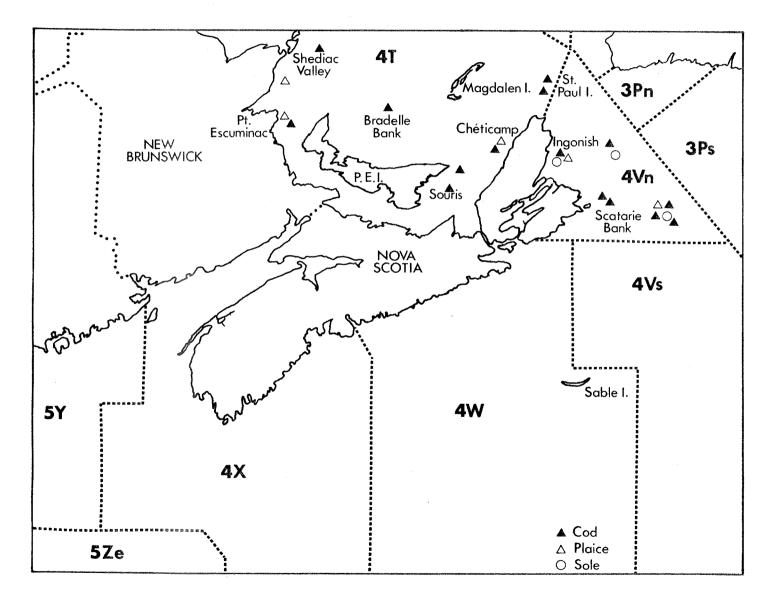


FIG. 1. Sampling locations.

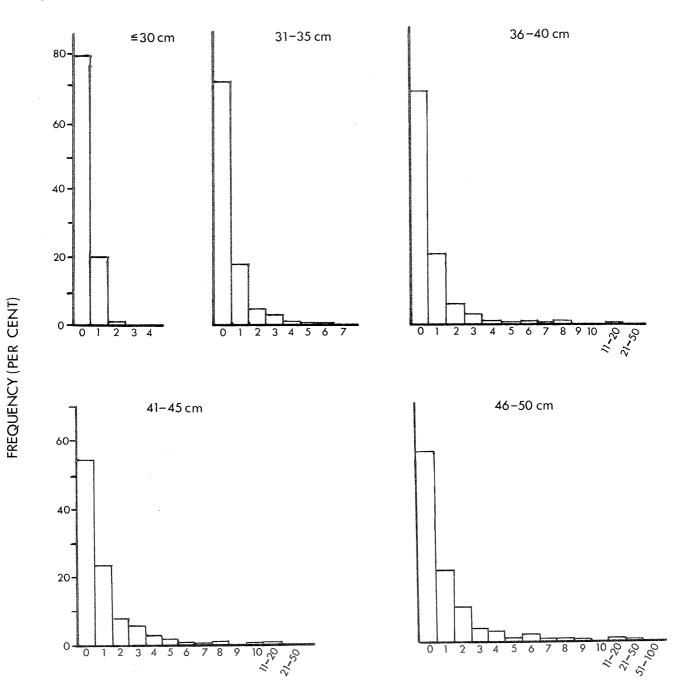
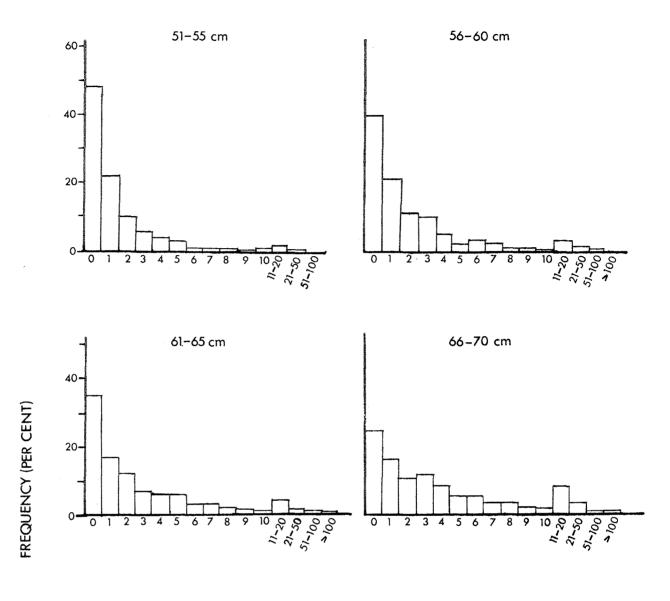
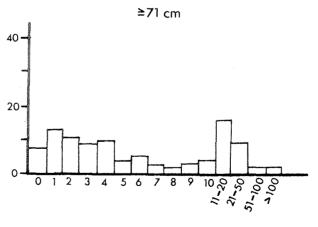




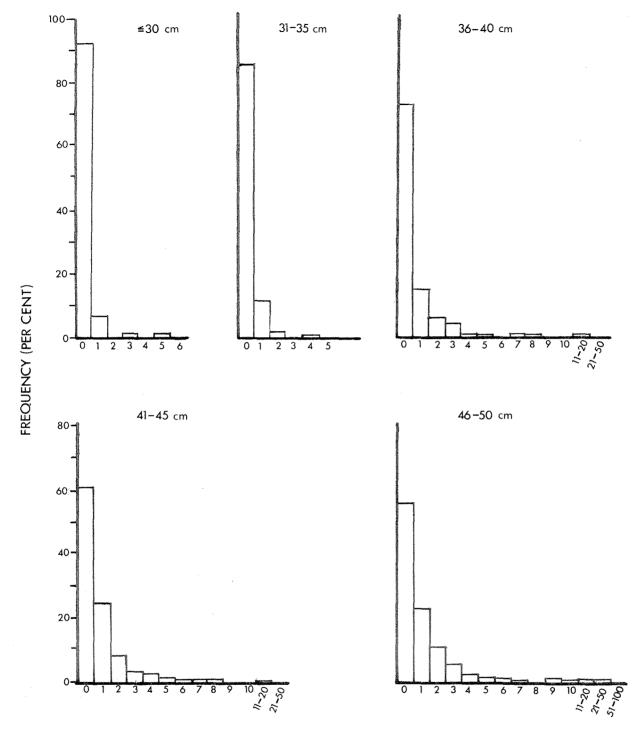
FIG. 2. Frequency distributions of worm counts for <u>Phocanema</u> <u>decipiens</u> in 4T and 4Vn cod: cod stratified into 5-cm length groups.





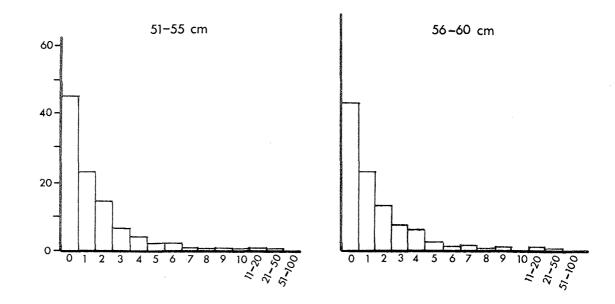
NO. OF PHOCANEMA IN COD

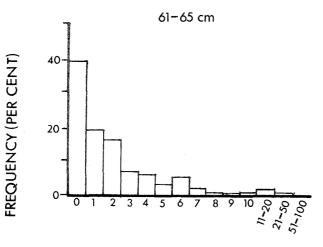
FIG. 2. Continued.

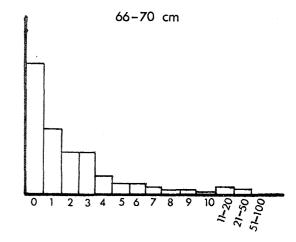


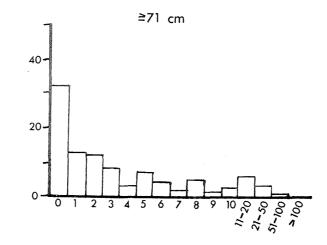
NO. OF ANISAKIS IN COD

FIG. 3. Frequency distributions of worm counts for Anisakis sp. in 4T and 4Vn cod: cod stratified into 5-cm length groups.



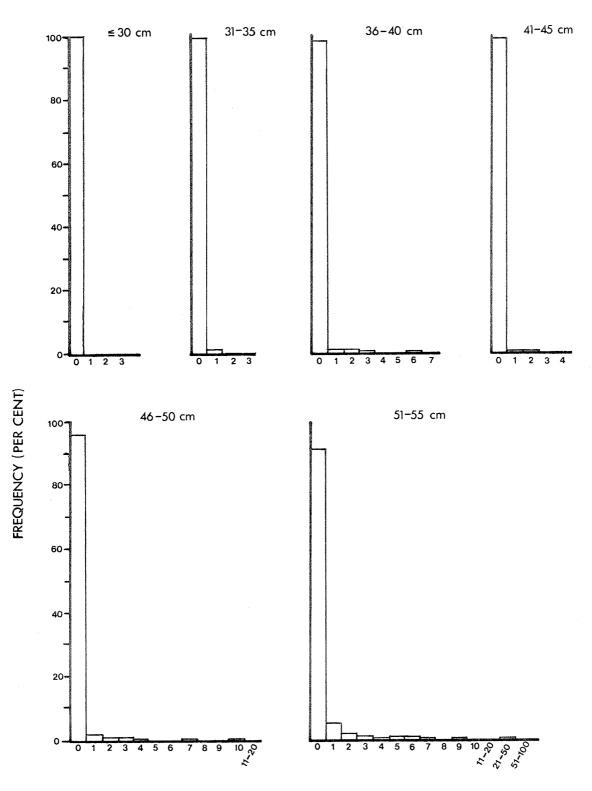






NO. OF ANISAKIS IN COD

FIG. 3. Continued.



NO. OF CONTRACAECUM IN COD

FIG. 4. Frequency distributions of worm counts for Contracaecum (Phocascaris) sp. in 4T and 4Vn cod: cod stratified into 5-cm length groups.

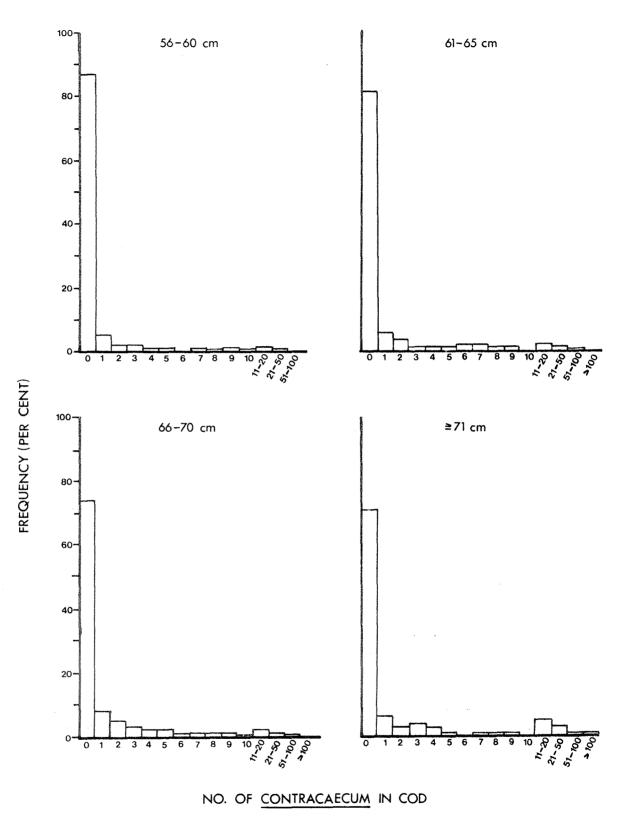


FIG. 4. Continued.

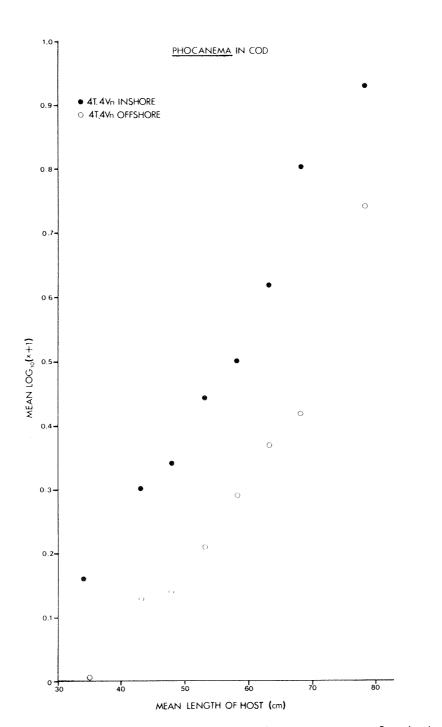
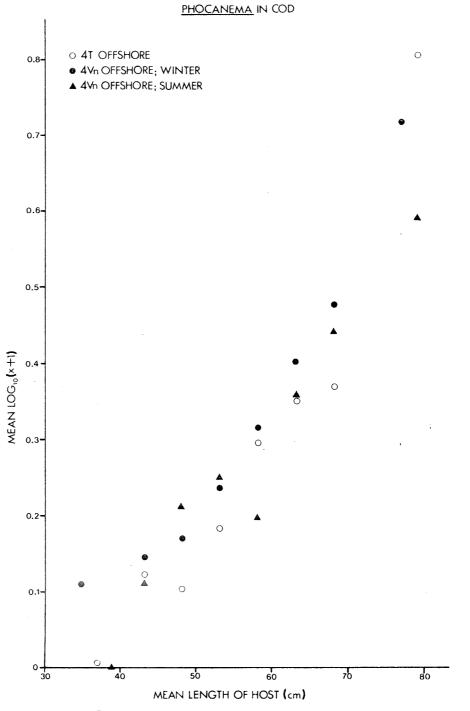
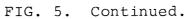


FIG. 5. Mean transformed counts of <u>Phocanema</u> <u>decipiens</u> versus mean host length in 5-cm length groups of 4T and 4Vn cod.





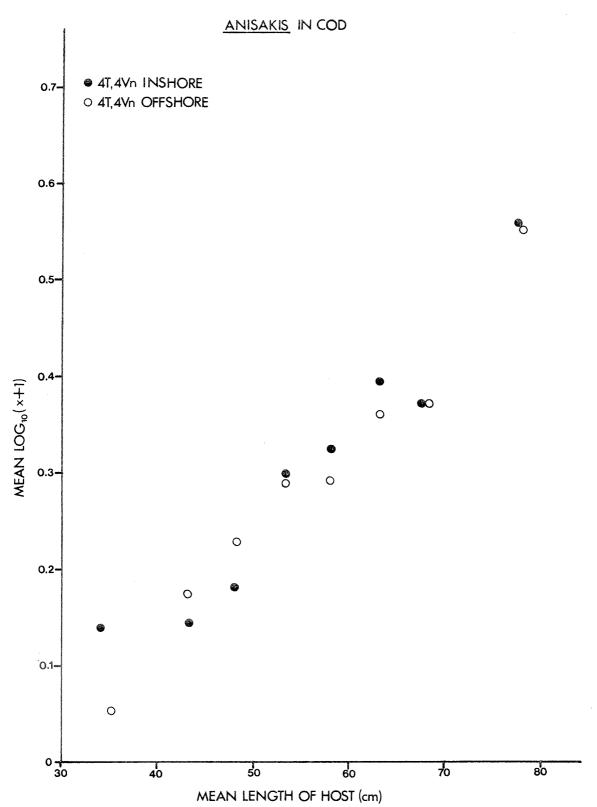


FIG. 6. Mean transformed counts of <u>Anisakis</u> sp. versus mean host length in 5-cm length groups of 4T and 4Vn cod.

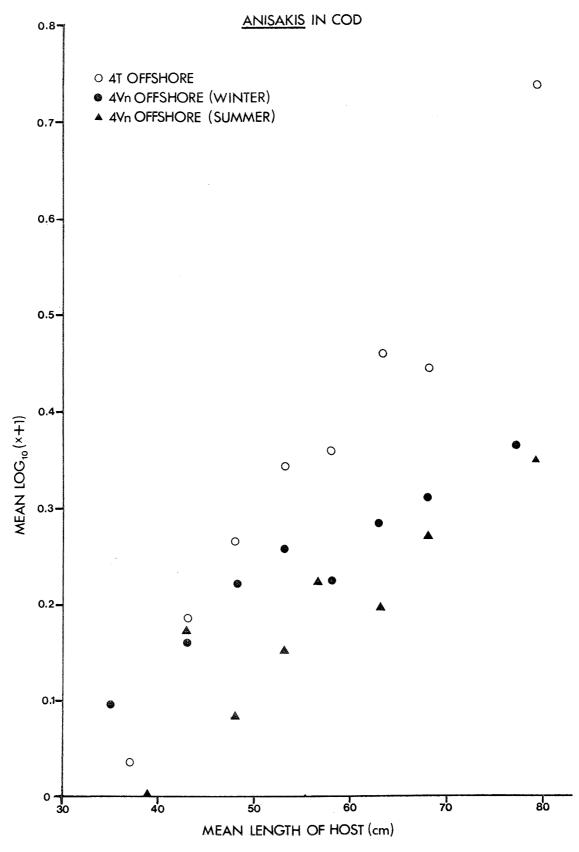


FIG. 6. Continued.

0 4T, 4Vn OFFSHORE 0.7 4T,4Vn INSHORE ¢\$ 0.6-0.5-0.4-MEAN LOG₁₀ (x+1) 0 0.3-0 0 0.2-0 0.1-@ O 0 0 | 30 60 70 80 50 40

CONTRACAECUM IN COD

MEAN LENGTH OF HOST (cm)

FIG. 7. Mean transformed counts of <u>Contracaecum</u> (<u>Phocascaris</u>) sp. versus mean host length in 5-cm length groups of 4T and 4Vn cod.

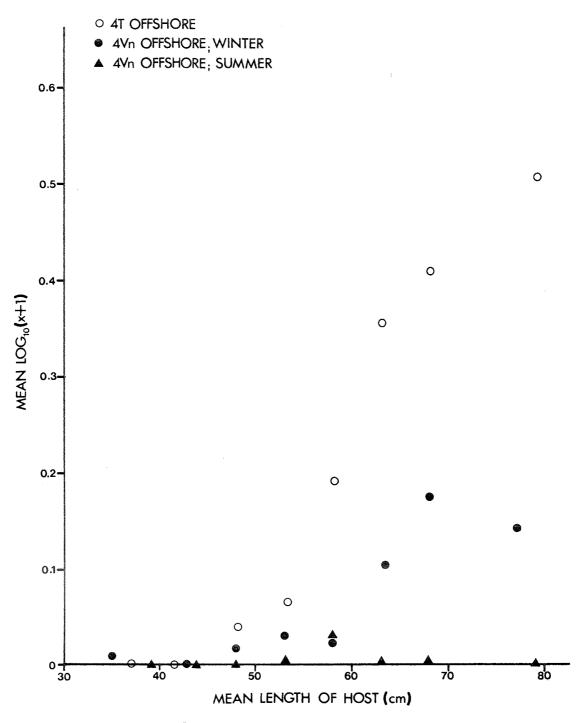
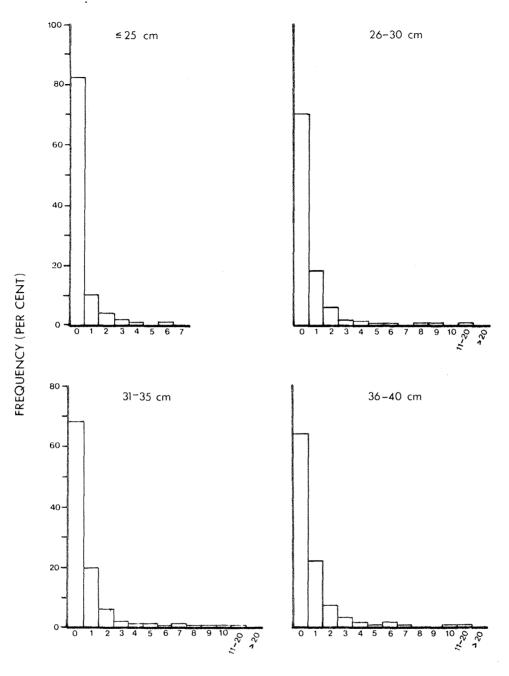
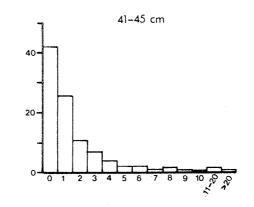


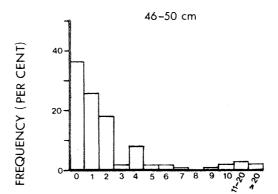
FIG. 7. Continued.

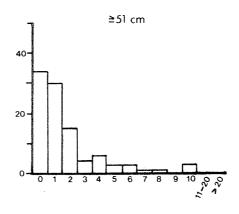


NO. OF PHOCANEMA IN PLAICE

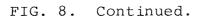
FIG. 8. Frequency distributions of worm counts for <u>Phocanema</u> decipiens in 4T and 4Vn plaice: plaice stratified into 5-cm length groups.







NO. OF PHOCANEMA IN PLAICE



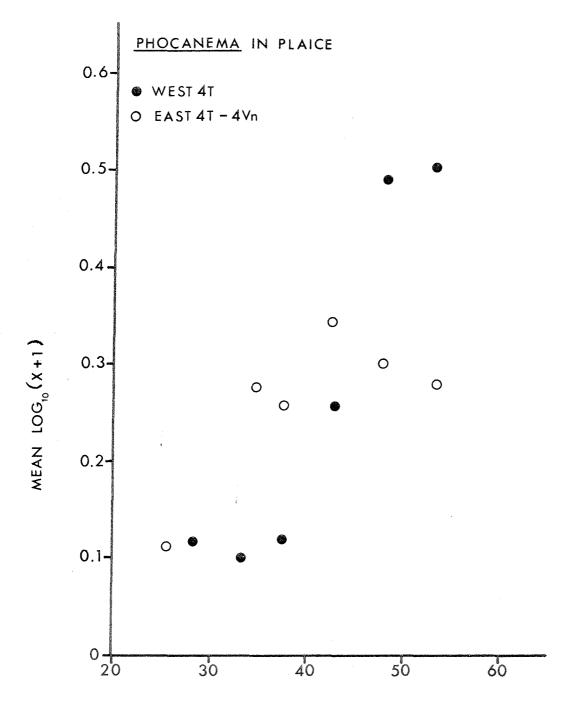
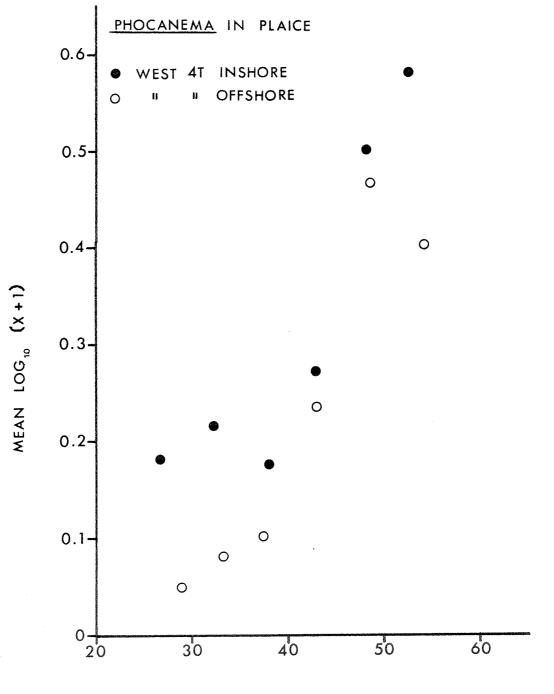
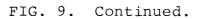




FIG. 9. Mean transformed counts of <u>Phocanema decipiens</u> versus mean host length in 5-cm length groups of 4T and 4Vn plaice.







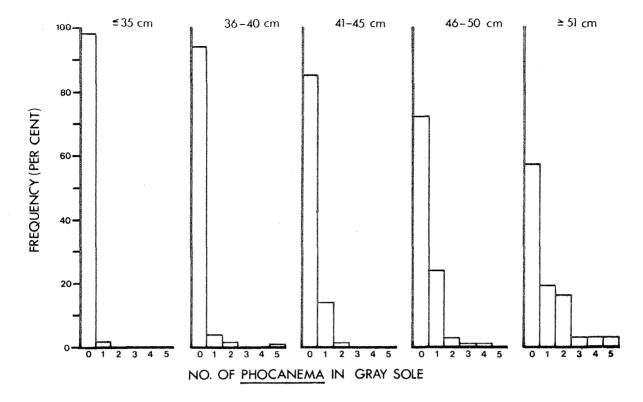
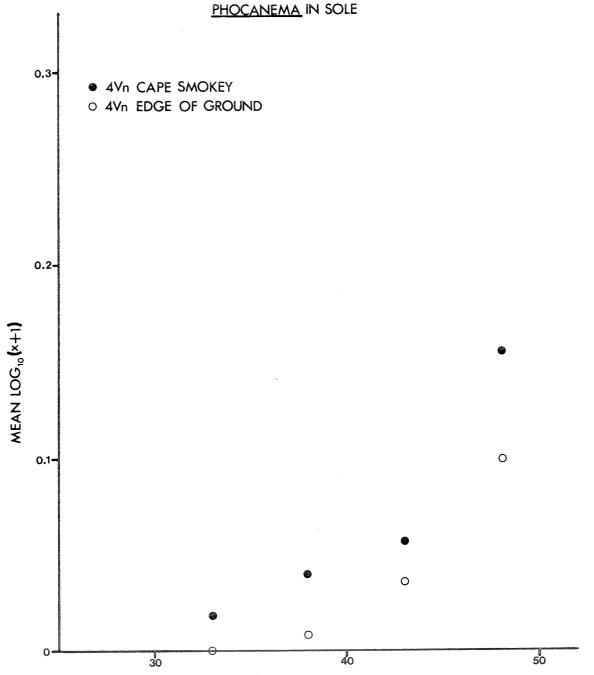


FIG. 10. Frequency distributions of worm counts for Phocanema decipiens in 4Vn witch (gray sole): witch stratified into 5- $\frac{1}{2}$ cm length groups.



MEAN LENGTH OF HOST (cm)

FIG. 11. Mean transformed counts of <u>Phocanema decipiens</u> versus mean host length in 5-cm length groups of 4Vn witch (gray sole).

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