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The Bulletin is a membership journal of the Scandinavian Society for Parasitology. Besides membership information, it also presents articles on all aspects of parasitology, with priority given to contributors from the Nordic countries and other members of the Society. It will include review articles, short articles/communications. Comments on any topic within the field of parasitology may be presented as Letters to the Editor. The Bulletin is also open for a short presentation of new projects. All contributions should be written in English. Review articles are commissioned by the editor, however, suggestions for reviews are welcomed.

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Cover: In Norse mythology, the giant ash tree - Yggdrasill - spreads its limbs over the entire mankind. The ash has three roots, each of them sucking water from its own spring.

The first spring- Hvergelmir - is found in the ice cold North; next to the spring, the serpent Níðhoggr is ceaselessly gnawing at the roots of the ash. The second spring - Mímisbrunnr - is the source of wisdom and is guarded by Mímir. The third spring - Urðarbrunnr - is guarded by three women, the Norns, which mete out man's thread of life.

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PROCEEDINGS

of the

symposium on

PARASITES OF BIOLOGICAL AND ECONOMIC SIGNIFICANCE IN THE AQUATIC ENVIRONMENT

-Thirty years of research and future trends-

SSP PARAQUA '94; Scandinavian Society for Parasitology
Heimaey * Westman Islands * Iceland

July 2 - July 6, 1994

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- **The Committee of Collaboration between the University of Iceland and Westman Islands**

PREFACE

The present proceedings are based on the contributions presented at the SSP PARAQUA '94 symposium on the Westman Islands, Iceland, 02.-06. July 1994. The biannual symposia of the Scandinavian Society for Parasitology (SSP) have traditionally been arranged in Denmark, Finland, Norway and Sweden. It has long been a wish to include Iceland in the activities of SSP. The PARAQUA '94 is the first, but very likely not the last special SSP symposium to be held in Iceland. The importance of fisheries to Scandinavian countries, and to Iceland in particular, the economic importance of parasites to the fishing industry, and the intriguing biology of the parasites themselves have aroused a great research interest in parasites of aquatic origin among the biologists in Scandinavia. The themes of the symposium were chosen to mirror some of the work which has been carried out in this field in Scandinavia during the last few decades and to analyse new results, especially in relation to the taxonomy and biology of the anisakid nematodes. The intention was to demonstrate the basic importance of establishing the identity and range of parasite populations as a basis for studies relating to other aspects, such as epidemiology and ecology, of parasitic infections. Extensive reviews of the main themes dealt with by the guest lecturers are appended to these proceedings.

One problem which was discussed during the symposium was how to find some practical taxonomic solution when dealing with populations of aquatic anisakid nematodes, most of which have recently been shown to consist of genetically isolated sympatric or allopatric siblings with no or only small morphological differences. This problem has previously been analysed in general terms by Sturham (1983) during the meeting on "Concepts in Nematode Systematics", but really needs to be addressed by the International Code of Zoological Nomenclature.

All participants are thanked for their valuable contribution to the success of this symposium. We are all grateful for the chance of attending this symposium on the Westman Islands, with its astounding nature, sunny and calm weather, and friendly people; a community which, after the threatening volcano eruption in the early 1970s is again flourishing. We all wish to extend our thanks to the sponsors of the PARAQUA '94, who helped make the participation of the guest lecturers and the numerous post-graduate students possible.

Autumn 1994

H-P Fagerholm
E Hauksson

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WELCOME BY THE LOCAL ORGANIZING COMMITTEE

E. Hauksson
Icelandic Fisheries Laboratories, Reykjavík, Iceland

Together with the other members of the local organizing committee (Droplaug Ólafsdóttir, Gísli Jónsson, Jónbjörn Pálsson, Karl Skírnisson, Matthías Eydal, Sigurdur H. Richter), I would like to welcome you all to this symposium here at Ásgardur Heimaey, Westman Islands. When the question was raised as to where we should hold this SSP symposium here in Iceland, we had several options to consider. We in the local organising committee evaluated these options and the Westman Islands was chosen as the most appropriate site. I hope you all will agree with us, after you have been here for a few days, that the flora and fauna is superb here in Heimaey and that there are also many interesting places to see. The islands also have quite an interesting history, as was mentioned in the welcoming speech given by Ólafur Lárusson of the town-council.

Here in Heimaey are branch offices of our largest governmental research institutes, the Marine Research Institute and Icelandic Fisheries Laboratories: their directors, Hafsteinn Gudfinnsson and Gísli Gíslason, are with us here on the symposium. I know that they will help us to make our stay here as interesting as possible. If you want to do or see something here in the Westman Islands, which is not included in the programme, you should ask them for help. They know their way around, and they will surely assist you.

This symposium would not have been possible without financial backing. We are proud to acknowledge the sponsors of this SSP PARAQUA '94 symposium. These are: the Nordic Academy for Advanced Studies (NorFA), the Ministry of Fisheries, the Research Committee for Biological Seafood Quality and the Scandinavian Society for Parasitology. The Committee for Collaboration between the Westman Islands and the University of Iceland is also going to sponsor us in a wonderful way, by inviting the participants of the symposium to the symposium dinner on Thursday, enabling us taste some of the local delicacies. During our stay here we will also have the opportunity to explore the islands both on land and from the sea.

Finally, I will thank you all for taking part in this symposium, especially those who come far away, i.e. from Russia, Italy and Canada. We here in Iceland very much welcome visits such as this by scholars to break our professional isolation. Icelandic scientists feel a strong desire to discuss professional problems with colleagues from abroad, and we find the opportunities to do so to be altogether too few. As you have surely noticed, it is not the easiest matter in the world to travel to and from Iceland. This makes all of you who made it here so 4especially welcome. ...Thank you.

INVITED PAPERS

CURRENT LABORATORY AND FIELD RESEARCH ON THE LIFE CYCLES AND POPULATION DYNAMICS OF ECONOMICALLY IMPORTANT ANISAKID NEMATODES

G. McClelland
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Much has been learned about the life cycles and distributions of anisakid species such as the economically and medically significant marine mammal parasites, *Anisakis simplex* and *Pseudoterranova decipiens*, various *Contracaecum* spp. of seals and piscivorous birds, *Sulcascaris sulcata* of marine turtles and *Hysterothylacium* spp. which reach maturity in fish hosts. Nevertheless, some fundamental aspects of the development of these nematodes, e.g. the timing of moults, have not been resolved. Moreover, recent research has shown that anisakid phylogeny and transmission patterns are more complex than previously assumed and, as a consequence, has confounded interpretation of survey data and efforts to model the population dynamics of important species. While continuing to elucidate the life cycles of the parasites and to document their spatial and temporal distributions in natural invertebrate and vertebrate hosts, current research also addresses factors which influence anisakid distribution and abundance. These factors include: ecological and behavioural barriers to transmission; variation in host susceptibility and response to infection; variation in nematode pathogenicity with host species and parasite density; selective predation on infected invertebrate or fish intermediaries owing to parasite induced behavioural modification; effects of parasite densities on nematode survival, maturation, and reproduction, and, temporal changes in host abundance or distribution.

MOLECULAR TAXONOMY IN ANISAKIDS

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Data are reported on some significant results obtained by the use of molecular markers on anisakid systematics, ecology and evolution. The analysis of genetic variation in 27 ascaridoid species has shown a highly significant correlation between mean heterozygosity per locus and the degree of environmental heterogeneity experienced by the parasite. The variability found in ascaridoid nematodes parasitizing only homeothermic vertebrates is similar to that of their hosts (H_e=0.04 in both), while that of ascaridoids parasitizing both poikilothermic and homeothermic species is similar to that found in their poikilothermic hosts (H_e=0.16 and 0.12 in the parasites and hosts, respectively).

A number of morphologically undifferentiated, but reproductively isolated species (sibling species), were detected within morphospecies belonging to different anisakid genera by the use of genetic markers (e.g. the complexes *Anisakis simplex, Pseudoterranova decipiens, Contracaecum osculatum, C. rudolphii*).

Interspecific hybridization is a very rare event, indicating that efficient barriers to gene flow are operating within the different sibling species complexes studied till now. A single case of introgressive hybridization has so far been detected between *Phocascaris phocae* and *P. cystophorae*. Recombinant specimens were found at relatively low frequencies in the harp seal (where "pure" *P. phocae* prevails), in the hooded seal and the grey seal (where "pure" *P. cystophorae* prevails), while they are largely prevailing over both parental species in the ringed seal. A scenario for the origin and evolution of this hybrid zone is proposed.

Various anisakid morphospecies, often considered cosmopolitan and euriecious, were found by molecular taxonomy to include a number of distinct biological species, ecologically differentiated with respect to their geographic distribution and host preferences. The latter appears to be due to differential host parasite coadaptation and coevolution as well as to interspecific competition. This acts either by reducing the range of potential hosts, or promoting a differential use of resources from a single individual host in sympatric conditions. Examples of niche subdivision are given, involving the *P. decipiens*, *C. osculatum*, and *P. phocae* complexes.

Some final remarks are drawn from the reported data both at the theoretical level and the applied level.

THIRTY YEARS OF ANISAKID RESEARCH AND FUTURE TRENDS

B. Berland[1] and H.-P. Fagerholm[2]
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Nematode larvae in fishes, and their adult stages in fish-eating predators, were largely of academic interest until the 1950s. The turning point was a newly recogniced human affliction - anisakinosis/ anisakiasis - caused by the consumption of raw fish infected with live *Anisakis* larvae; this led to many studies of nematode parasites in fishes whales, seals and fish-eating birds.

The genera involved in these studies are *Anisakis, Pseudoterranova, Contracaecum, Phocascaris* and *Hysterothylacium*, the latter maturing in fishes. In the 1980s, their taxonomy seemingly settled, workers in Rome, Italy applied enzyme electrophoresis to study their genetics. In recent papers they showed that *Anisakis simplex* is in fact composed of two sibling species, A and B, *Pseudoterranova decipiens* in seals in the north Atlantic of three sibling species, A, B and C, and *Contracaeceum osculatum* in the North Atlantic of three sibling species, A, B and C.

The second-stage (L2) larva, surrounded by the cast cuticle of the first stage, was believed to hatch from the egg. Recent work has shown that two moults occur in the egg; the L3 larva leaves the egg. This is important for our understanding of their life cycles.

We believe that the future trends in anisakid research are to: 1) identify the species worldwide using the new biochemical methods; 2) try to find morphological characters which permit species identification; 3) establish the genetic distance between *Contracaceum*-like species in seals and those in fish-eating birds, and classify them accordingly; and 4) study the *Hysterothylacium* species in the North Atlantic.

THIRTY YEARS OF *DIPHYLLOBOTHRIUM* RESEARCH, AND FUTURE TRENDS

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Until the fundamental works of Kuhlow, a German parasitologist, were published in the middle of the fifties, recognition of *Diphyllobothrium* species were based almost exclusively on structural features of the adult worms. For the next ten years several taxonomical reports were published, still only of a descriptive nature, but utilizing specific criteria of larvae as well as of adult worms. Only from the middle of the sixties it was recognized that the problems encountered in the *Diphyllobothrium*-taxonomy could not be tackled by merely descriptive studies but had to include experimental works and a critical re-evaluation of the taxonomical criteria so far used.

For the next ten years (1965 - 1975) experimental studies carried out mainly by Scandinavian workers ascertained details of the life cycles, host ranges, morphological variation etc. of European *Diphyllobothrium*-species, and delimited three well defined species occurring in freshwater fish, *D. latum*, *D. dendriticum* and *D. ditremum* and a fourth species, *D. vogeli*, the validity of which is still sometimes subject to discussion. More recent studies have shown that these species are widespread in most parts of the northern hemisphere (and South America) and that numerous species previously described under separate names are identical with either of these species.

The progress in studies on *Diphyllobothrium* species occurring in marine fish has been slow.

Epidemiological research laid the basis for a rapid drop in the prevalence of human diphyllobothriasis in many endemic areas during the past three decades. It is evident that in addition to *D. latum*, several other species are implicated in human infections in circumpolar regions and in the Pacific area.

THIRTY YEARS OF FISH TREMATODE (DIGENEA) RESEARCH IN THE NORDIC ENVIRONMENT AND FUTURE TRENDS

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Thanks to Müller, Fabricius, Rudolphi, Olsson, Levinsen, Odhner and Nybelin and non-Scandinavian researchers such as Creplin, Looss, Lebour and Nicoll, most digeneans in the Nordic environment were already described by the beginning of this century. During the last thirty years the digenean research in the Nordic countries has primarily focused on the morphology and biology of species already described. The present review deals with research on digeneans which have fish as intermediate and/or definitive hosts and includes new species described within the last 30 years from Nordic areas. Emphasis is set upon distribution, life-cycles and ecological aspects done by mainly Nordic researchers. The review includes nearly one hundred references. Based on accumulated knowledge on digenean life-cycles further studies on fish stocks and biological tagging using diegenean markers should be implemented. Likewise the accumulated results regarding interaction between pollution and parasites, including digeneans, indicate the possibility to use fish parasites as indicators of pollution.

THIRTY YEARS OF *GYRODACTYLUS* RESEARCH, AND FUTURE TRENDS

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In 1932 the first *Gyrodactylus* species, *G. elegans* was described by Alexander von Nordmann, i.a. holding a professorship in Finland. During the 162 years since then, in broad outlines, investigations on *Gyrodactylus* embrace five periods:

1. The period before a sufficient development of light microscopy (microscope and sectioning technique), when frequently, new *Gyrodactylus* species were incorrectly named *G. elegans*.

2. The period from about 1860 to 1905 rendering a number of important publications on the anatomy and embryology of *Gyrodactylus*.

3.The period from about 1905 to about 1962, when many new *Gyrodactylus* species were described and important faunistic investigations were performed. The species discrimination, however, was still a problem.

4. The period from about 1962 to 1975 rendering important publications on the relevant species character and redescriptions of a number of insufficiently known *Gyrodactylus* species.

5. The period since 1975, when the *Gyrodactylus* research has been much influenced by the gyrodactylosis caused

by *G. salaris* on the Norwegian Atlantic salmon in natural waters.

Fear for a further spreading of the parasite to other Atlantic salmon stocks - not only in Norway but also in e.g. Finland, Sweden, England, Scotland and Canada - has resulted in important experimental studies regarding host specificity, resistance and susceptibility to *G. salaris*, and on investigations on macroenvironmental influences and reproducing capacity of the parasite. The present review mainly concerns the time after 1962, recent research fields and views on future investigations on *Gyrodactylus*.

PARASITES OF FISHES OF ECONOMIC INTEREST IN NORTHERN EUROPE, A LITERATURE ANALYSIS

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Investigations on fish parasites in European fresh, brackish and marine waters date back several centuries. In other words, fish parasitological research has a history comparable to that of parasitological science as a whole. It goes back to the times of the first naturalists. Thus, when analysing the literature relating to the parasites of fishes of economic value in Northern Europe, it is appropriate to review the situation during the last three centuries.

The number of publications increased constantly from 1700 to 1940 with a increase 1850-1900. The effects of the Second World War are evident, resulting in a decrease to the level of the period 1800-1850. The number of published papers increased rapidly during the period 1950-1980, but even more so in the 1970s. In the last decade (1980-1990) the number of papers published was similar to that of the previous one. Fishes of the orders Salmoniformes and Gadiformes have been extensively studied, while the order Clupeiformes and the large order, the Perciformes, did not attract the same level of attention. The Cestoda, Trematoda, Monogenea and the parasitic Crustacea were extensively studied. The Myxosporidia was the most studied group of the Protozoa. A major portion of the publications has dealt with the parasite fauna of fishes of the Baltic Sea, whereas the number of studies from the White Sea, Barents Sea and Arctic Ocean is low. Freshwater fish parasites were extensively studied in Russia, Norway and Finland while in Denmark and Sweden more attention has been paid to the study of marine fish parasites. The number of books and monographs published during the periods reviewed tended to decrease in recent years and is now at the same level as at the beginning of the century.

During the last few decades much new primary data have been made available, but we have apparently not made enough general summaries and reviews. Based on the present analysis it is possible to predict a decrease in the number of publications dealing with parasites of fish during the next decade. It seems reasonable to assume that in the future the use of computer based technology will increase markedly as one means of publishing scientific information.

SUBMITTED PAPERS - ORAL PRESENTATION

LARVAL ANISAKID NEMATODES IN VARIOUS FISH SPECIES FROM THE COAST OF ICELAND

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The occurrence of larval anisakine nematodes was surveyed in several fish species from Icelandic coastal waters. The sea-scorpion (*Myoxocephalus scorpius*) is the most heavily infected fish species with seal worm (*Pseudoterranova decipiens*). In demersal fish species, such as cod (*Gadus morhua*), seal worm larvae are also abundant while in pelagic species, such as herring (*Clupea harengus*) and saithe (*Pollachius virens*), *Anisakis simplex* is dominant. In flatfishes and sand-eels, (*Ammodytes sp.*), larvae of *Contracaecum* and *Hysterothylacium* are most abundant.

Sealworms are primarily located in the flaps and fillets of the fish. *Anisakis* occurs most frequently in and on organs of the body cavity, but also in the flaps of the fish. *Contracaecum* and *Hysterothylacium* are nearly always found in the body cavity.

The nematode fauna of each fish species is discussed in relation to the feeding habits of the fish.

INTROGRESSIVE HYBRIDIZATION BETWEEN *PHOCASCARIS PHOCAE* HOST, 1932 AND *P. CYSTOPHORAE* BERLAND, 1964 (NEMATODA, ASCARIDOIDEA)

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Genetic studies were carried out at 21 enzyme loci on specimens of the genus *Phocascaris* collected in various locations in North-East and North-West Atlantic, from the hooded seal (*Cystophora cristata*), the harp seal (*Pagophilus groenlandicus*), the grey seal (*Halichoerus grypus*) and the ringed seal (*Phoca hispida*). The samples showed a marked heterogeneity, especially at the loci *Sod-1, Est-1*, and *Mpi*. Two differentiated gene pools could be evidenced, corresponding respectively to *Ph. phocae* Host, 1932 ($Sod\text{-}1^{100}$, $Est\text{-}1^{100}$, Mpi^{103}, Mpi^{110}), and *Ph. cystophorae* Berland, 1964 ($Sod\text{-}1^{122}$, $Est\text{-}1^{96}$, Mpi^{94}), as well as an array of genotypes, more or less intermediate between the two, including F_1 and F_n hybrids, backcrosses and introgressed individuals. The various genotypes were assigned to parental and recombinant classes according to compound probabilities at the three diagnostic loci. Different proportions of the various classes were observed in the different definitive hosts and locations. In the hooded seal, "pure" *Ph. cystophorae* predominate, followed by recombinants, while "pure" *Ph. phocae* are rare. In the grey seal, *Ph. cystophorae* also prevails, but with a higher proportion of recombinants and of "pure" *Ph. phocae;* the latter are generally rarer in the North-West than in the North-East Atlantic. In the harp seal, *Ph. phocae* prevails in NE Atlantic, whereas *Ph. cystophorae* predominates in the NW Atlantic; recombinants are relatively abundant in both areas. Last, in the ringed seals from NW Atlantic, recombinant genotypes largely prevail over both parental species, and their gene pool proves to be in Hardy-Weinberg equilibrium. The available data suggest that *Ph. phocae* and *Ph. cystophorae* started to diverge allopatrically about half million years ago as estimated from their genetic distance (Nei's D=0.11), adapting to and coevolving with different definitive host: the harp and the hooded seal, respectively. Secondary contacts between these two seals and their *Phocascaris* parasites in the interglacial led to hybridization and introgression between *Ph. phocae* and *Ph. cystophorae*. Hybrids and recombinants were presumably selected against over parental species in their respective definitive hosts, while they appear to have been favoured in the ringed seal. Evolution of assortative mating in *Ph. phocae* and *Ph. cystophorae* seemingly took place, as indicated by the very rare occurrence of F_1 hybrids. Two possible evolutionary outcomes seem to be the most plausible for this hybrid zone: (a) maintenance of a dynamic equilibrium between *Ph. phocae*, *Ph. cystophorae* and their recombinants, gene flow being counterbalanced by differential selective pressures; (b) completion of reproductive isolation, possibly giving origin to a stabilized recombinant species, adapted to the ringed seal.

THE SPECIES COMPOSITION OF ANISAKID NEMATODES IN THE GASTRIC ULCERS IN HARBOUR SEALS (*PHOCA VITULINA*) FROM THE OUTER OSLOFJORD

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A total of 45 harbour seals (*Phoca vitulina*) from the outer Oslofjord were examined for gastric ulcers. The attached nematodes were identified to species, stage and sex. Gastric ulcers were found in 28 seals, 20 of these seals had ulcers containing nematodes. The site of the ulcers were registered.

About 70 % of the ulcers were located in the ventral and left lateral section in the fundus and in the pylorus section of the stomach. The highest number of ulcers was found in males. The nematodes present in ulcers were totally dominated (99.8 %) by *Anisakis simplex*, with an average intensity of 10 worms per ulcer. The average number of attached *A. simplex* specimens per male was 60, and 15 per female. The portion of attached *A. simplex* were about 20 % of the total infection in harbour seals. More than 75 % of the attached. *A. simplex* were in their third stage, and less than 1 % had developed into fifth stage. *Contracaecum osculatum* and *Pseudoterranova decipiens* occurred rarely in the gastric ulcers of the harbour seals from the outer Oslofjord. Less than 1 % of the total infection of *C. osculatum* and *P. decipiens* were found in ulcers.

DISTRIBUTIONS OF ANISAKID NEMATODES IN THE DIGESTIVE TRACT OF COMMON SEAL (*PHOCA VITULINA* L.) AND GREY SEAL (*HALICHOERUS GRYPUS* (Fabr.)).

D. Olafsdóttir and E. Hauksson
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Infections of intestinal nematodes were investigated in 74 common and 44 grey seals. Most nematodes were located in the stomachs of the seals: 96% and 99% of *Pseudoterranova decipiens*, 98% and 98% of *Contracaecum osculatum*, 55% and 36% of *Phocascaris cystophorae* and 79% and 83% of *Anisakis simplex* respectively.

Correlation analyses on the number of worms in the stomachs and seal weight gave significant positive results for the common seal but not for the grey seal.

Correlation analyses on the proportions of each nematode species found in the seal stomach and seal weight gave no significant positive results, but significant negative results were found in the case of *A. simplex*. This may indicate increased host reactions against the parasites with repeated infections of *A. simplex*.

Correlation analyses on the proportions of each nematode species found in the stomachs and the worm burden, performed after controlling for any effects of the seal weight, gave no significant negative correlations and hence no sign of intensity-dependent infections in the seal stomachs.

SEASONAL AND REGIONAL VARIATIONS OF ANISAKIDS (NEMATODA) IN COMMON SEAL (*PHOCA VITULINA* L.) AND GREY SEAL (*HALICHOERUS GRYPUS* (Fabr.)) IN ICELANDIC WATERS

D. Olafsdóttir and E. Hauksson
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The occurrence of anisakid nematodes were investigated in the stomachs of 95 common seals in the years 1979-82, and in 74 and 179 grey seals in the years 1979-82 and 1989-1993 respectively. *Pseudoterranova decipiens, Contracaecum osculatum, Phocascaris cystophorae* and *Anisakis simplex* were found in stomach of both seal species in all coastal regions and seasons.

The prevalence and intensity of infection were higher in the grey seal than in the common seal. *P. decipiens* and *C. osculatum* were the most abundant species. The abundances of *P. decipiens* and *C. osculatum* showed no seasonal fluctuations in common seals but were higher in seals from the West-coast than in other coastal areas.

A clear regional and seasonal pattern was observed in grey seals. *P. decipiens* was common in grey seals in all areas but it was most abundant in seals from the West-coast and the abundance was highest in the autumn. *C. osculatum* was common in grey seals from the West- and South-coasts and the abundance was highest in the spring.

Proportions of mature *P. decipiens* and *C. osculatum* decrease gradually with increasing intensity of infection. An increase in the proportion of mature worms when the intensity of infection declines is not as obvious.

FECUNDITY OF *ANISAKIS SIMPLEX* FROM MINKE WHALES (*BALAENOPTERA ACUTOROSTRATA*)

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Anisakis simplex is the most common nematode in the stomach of minke whale, with a mean intensity of 40.000 specimens. A varying portion of the nematodes, 5% at an average, are mature females. In the present study the fecundity of *A. simplex* is established and related to the length of the nematodes.

Frozen females in different length groups (in the range 70-160 mm) were examined. The nematodes were cut longitudinally and their gonoducts were removed. The gonoduct was divided in an uterus and oviduct section, and thereafter treated separately. A magnetic stirrer was used for releasing the eggs from the uterus. The eggs were counted in a Fuchs-Rosenthal chamber under a microscope. The oviducts were placed on a slide, length measured, and the number of egg counted in sections of the oviducts. The counting was done in 4 egg wide bands, each 10 mm. Then the total number of eggs was estimated. Nematodes of about 130 mm length contained some 1.000.000 eggs in their uterus.

The following questions are discussed: How is the number of eggs in *A. simplex* related to worm length (average number of eggs in respective length group)? What is the relative number of eggs in uterus versus oviducts?

INFECTION OF THE BARENTS SEA COD, *GADUS MORHUA*, AND REDFISH, *SEBASTES MENTELLA*, WITH LARVAL ANISAKID NEMATODES: LONG-TERM DATA

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The main objective of this study was to compare fish infection by larval *Anisakis simplex* and *Pseudoterranova decipiens* with changes in the Barents Sea ecosystem for a longterm period.

Prevalence, intensity of infection and abundance index were used for the estimation of numbers of larval nematodes in cod and redfish.

There are essential change in the infection of cod by both nematode species. In our opinion, these changes might be caused by disbalanced trophic relations in the sea ecosystem. For example, in mid 1980s the occurrence of capelin and its role as food for cod declined. At the same time feeding of cod on crustaceans - intermediate hosts of nematodes, increased. As a result, in this period the abundance index of *Anisakis simplex* for cod was eight times larger than in periods more favourable for capelin.

ABUNDANCE OF *PSEUDOTERRANOVA (=PHOCANEMA) DECIPIENS* KRABBE LARVAE IN ICELANDIC COD. COMPARISONS BETWEEN THREE SURVEYS DURING 1980-81, 1985-88 AND 1990-91

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Total of 600, 944 and 823 cod of different lengths were collected from seven different coastal areas in surveys during 1980-81, 1985-88 and 1990-91, respectively. At least 10 cod of each 10 cm length-group were collected in each coastal area, while cod larger than 90 cm formed one length-group.

The prevalence and abundance of *P. decipiens* in gutted cod usually increases with length and age of fish. There is also some correlation between the coastal areas, ranked according to relative abundance of seals in the areas where the cod were collected, and the infection of cod with *P. decipiens* larvae. Environmental factors play a role in this too, as the infection of *P. decipiens* larvae in cod is higher where the coast is indented, with shallow waters and numerous islands and skerries, than where the coastline is straight and sandy beaches dominate.

The prevalence and abundance of *P. decipiens* larvae in cod has not changed significantly between 1980 and 1991.

SURVIVAL OF LARVAE OF *PSEUDOTERRANOVA DECIPIENS* IN COD (*GADUS MORHUA*)

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A study of the survival of *Pseudoterranova decipiens* larvae in cod (*Gadus morhua*) was carried out in a 4700 litre sea-water tank, with a closed self-cleaning circulation system, and with the fish being fed on a worm-free diet. The study lasted for 80 days.

During that time no change in the mean worm-burden in the flesh of cod was observed, despite the declining condition of the fish, due to self-induced starvation.

From these results, it can be concluded that no rapid changes due to the death of *P. decipiens* larvae in cod occurs. Survival of the larvae in cod is more likely to be counted in years than in months.

SPECIES DESIGNATION IN ANISAKID PARASITES

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The species diagnosis of ascaridoid nematodes is based on only a rather small number of structural features. These include the oesophagus and intestine, the excretory system, lip morphology and the number and distribution of caudal papillae in the adult male worm.

Traditional diagnostic criteria have proved inadequate since it was shown that there are reproductively isolated sympatric and allopatric sibling species in all of the major anisakid genera. There is therefore an urgent need for refining the methods used in the structural analysis of these parasites.

The specific distribution of sensory structures has proved useful in species diagnosis. Known papillary structures present in both sexes include labial papillae, amphids, deirids and phasmids. Recently the presence of a single pair of lateral papillae in the mid-body region in both sexes of ascaridoid nematodes has been observed (Fagerholm *et al.* subm.). These structures were named centrids. Further studies are intended to demonstrate the significance of the centrid in systematic and taxonomic analyses.

The designations of the caudal papillae in the male as proximal, median (n=1), paracloacal (2 pairs) and distal (4 pairs) (plus phasmids), recognizing them as isolated groups, and the analysis of their relative positions and dimensions can be of diagnostic value. The results by Paggi *et al.* on *Pseudoterranova,* who used multivariate analysis of morphometric data, including that relating to the caudal papillae, suggest that detailed analysis can distinguish siblings.

The specific distribution of ganglia and nerves in relation to different sensory structures, using immunocytochemical methods, has also been studied in order to evaluate the taxonomic and systematic significance in the nervous system of ascaridoid parasites.

DISPERSION PATTERNS AND HOST-PARASITE INTERACTIONS OF *DIPHYLLOBOTHRIUM DITREMUM* PLEROCERCOIDS IN BENTHIC WHITEFISH, *COREGONUS LAVARETUS* L. IN FINNISH LAPLAND

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The material consisted of 300 benthic whitefish from lake Kilbbesjavri and of 185 specimens from 8 other smaller lakes of subarctic birch forest zone in the border areas between Finland, Norway and Sweden (Lapland). Lake Kilbbesjavri was stocked with 440 000 larvae of migratory whitefish (*Coregonus lavaretus* L.) during 1959-64. Thereafter the hybrid stock has been in poor condition and almost unexploited by fishermen.

The objectives of the present study are 1) to compare the frequency distribution and dispersion patterns of *Diphyllobothrium* ditremum plerocercoids in different age groups of benthic whitefish in different arctic lakes and 2) to study possible regulation effects by the parasite on the fish intermediate host.

A total of 485 whitefish were caught by experimental gillnet series during the period July 1992 - September 1993; the cysts with *D. ditremum* plerocercoids in each fish were counted.
The prevalence and intensity was estimated and the negative binomial distribution was fitted to the observed frequency distribution within each age group of the fish in Lake Kilbbesjavri and in the pooled material of the other lakes.

In Lake Kilbbesjavri the intensity ranged between 85 and 130, while in the pooled material between 40 and 65 plerocercoids per host. The mean intensity and the variance to mean ratio were descending in former case after age group 7, but in the pooled one the opposite was true. The fit to the model was significant for Kilbbesjavri, but not for the pooled material. The parasite burden in former case is extremely high, but still non-aggregated. Assuming, that the host is regulated by the parasite, increasing parasite aggregation has a stabilizing effect on the host parasite interaction (Anderson & May 1978). The results are discussed in regard to possible indications of parasite-induced host mortality in the poorly adapted hybrid stock.

When Fulton's condition coefficient was plotted against number of plerocercoids, no negative correlation was found (r=-0,005; p=0,92). Other plerocercoids (*D. dentriticum* and *Triaenophorus spp.*) were not found in whitefish of any lake studied, while ectoparasites (*Acanthopdella peledina*) occurs frequently.

VENDACE AND WHITEFISH AS SECOND INTERMEDIATE HOSTS OF *TRIAENOPHORUS CRASSUS* IN LAKE SAIMAA SE FINLAND

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Triaenophorus crassus was found to have nearly a 100 % prevalence in whitefish, *Coregonus lavaretus*, in Lake Saimaa, SE Finland throughout the years 1991-1993. At the same time vendace, *C. albula*, were found to be infected only accidentally. However, vendace from other areas have been found infected with *T. crassus*. For example, Valtonen *et al.* (1) found an 11% prevalence in the Bothnian Bay, Baltic Sea.

The transmission of *T. crassus* from copepods to whitefish and vendace was studied experimentally. Possible hypotheses for the differences between the levels of infection on these fishes are: (1) whitefish and vendace populations are segregated by their feeding areas; (2) they have different feeding habits; (3) copepod behaviour is affected by the procercoids of *T. crassus* such that vendace are not exposed to the infected copepeods; or (4) the specificity of the *T. crassus* strain and/or the susceptibility of vendace in Lake Saimaa may have been altered as compared to the situation in, for example, the Bothnian Bay.

DENSITY-DEPENDENT HOST RESPONSES IN COD INFECTED BY *CRYPTOCOTYLE* SPP. METACERCARIAE

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In natural situations macroparasites are usually aggregated in a small number of host individuals. If overdispersion changes in wild populations, it is difficult to distinguish between factors related to transmission and to host susceptibility. Decreasing overdispersion in wild parasite populations has usually been explained by parasite induced host mortality. A density-dependent increase in host resistance to parasites can, however, cause the same pattern.

In the present study overdispersion of *Cryptocotyle* spp. metacercariae increased during the first months after caging of 1000 Atlantic cod (*Gadus morhua*), but thereafter showed a significant decline over the next nine months. Host deaths were negligible during this period.

Cod usually respond to cercariae attack by surrounding the parasite by pigmented cells. In the present study a marked difference between the number of spots and metacercariae appeared at low infections. Some fish had few spots either because they evolved pigmentation slowly or they did not respond to all the metacercariae. Others had many spots but revealed few metacercariae because they may have killed some of the parasites. In fish with higher levels of infection there is a better agreement between counts of spots and metacercariae. This may reflect a more extensive response to the parasite.

We conclude that resistance to the parasite probably increased in the most infected hosts with increasing parasite intensity.

THE OCCURRENCE OF THE DIGENEAN *CRYPTOCOTYLE LINGUA* IN THE COASTAL ENVIRONMENT OF ICELAND

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The adult stage of the digenean trematode *Cryptocotyle lingua* was recently found in several arctic foxes inhabiting coastal areas of western Iceland (Skirnisson K, Eydal M, Gunnarsson E, Hersteinsson P. Parasites of the arctic fox (*Alopex lagopus*) in Iceland. J Wildl Dis 1993; 29: 440-446). This was an unexpected finding since the larval stages of this digenean had never been recorded in Iceland. The primary first intermediate snail host, *Littorina littorea*, does not occur in Iceland and metacercariae ("black spots") had not been reported in fish (second intermediate hosts) caught in Icelandic waters.

The aim of the present study, which started in 1993, is to find the larval stages of *C. lingua* in Icelandic *Littorina* snails living in the intertidal zone, and in fish from shallow waters. Furthermore to investigate whether gulls in Iceland are also final hosts of this parasite.

Our first results have revealed that at least one snail species, *Littorina obtusata*, is infected with rediae and cercariae of *C. lingua*. The intensity of infection was high but the prevalence of infected snails was low (2%). No infected *Littorina saxatilis* were found. The metacercarial stage, which is usually easily recognized as black spots in the skin of fish, has so far only been found in fish caught inshore (*Myoxocephalus scorpius*, *Limanda limanda*, *Pollachius virens* and *Salvelinus alpinus*). Prevalence ranged from 12% to 44% but the intensity of infection was low. Black spots have not been detected in fish (mainly cod) caught offshore.

Adult *C. lingua* have been found in five gull species examined, *Larus marinus* (prevalence 71%), *L. hyperboreus* (36%), *L. argentatus* (67%), *L. glaucoides* (25%) and *L. fuscus* (10%).

The infectivity of *C. lingua* cercariae shed from Icelandic *Littorina obtusata* snails was tested experimentally by exposing sticklebacks (*Gasterosteus aculeatus*) to the cercariae. Infection was easily established in the skin and fins of the sticklebacks followed by the development of encysted metacercariae.

Our studies indicate that the digenean *C. lingua* can complete its life-cycle in the coastal environment of Iceland. However, the infection is not readily recognised in fish.

BRACKISH WATER DISPERSAL OF THE FRESHWATER MONOGENEAN GYRODACTYLUS SALARIS MALMBERG, 1957

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A possible route of dissemination of the freshwater monogenean *Gyrodactylus salaris* Malmberg, 1957 is through brackish water migration of infected fish. A prerequisite for this dispersal route is a relatively high salinity tolerance of the parasite, and migration of infected fish between rivers. The salinity tolerance of the parasite has previously been examined in laboratory experiments (Soleng & Bakke, 1993). A review of both susceptibility of the most important potential hosts to *G. salaris* and their migration in estuaries and fjord areas will be presented.

Potential hosts for transportation include: Atlantic salmon (*Salmo salar*), sea trout (*S. trutta*), sea charr (*Salvelinus alpinus*), brook trout (*S. fontinalis*), rainbow trout (*Oncorhynchus mykiss*) and European eel (*Anguilla anguilla*). The susceptibility for flounder (*Platichthys flesus*), three-spined stickleback (*Gasterosteus aculeatus*), nine-spined stickleback (*Pungitius pungitius*) and northern pike (*Esox lucius*) to *G. salaris* have not been experimentally examined.

Migrating infected salmon smolts transport parasites into brackish and marine environments where transmission to other host-species is possible, for example by predation. In addition, migration of salmon parr in fjord areas has been reported. Adult salmon migrate in the upper brackish layer and may come in contact with many estuaries and freshwater systems when returning to their home river. The migration pattern of sea trout, rainbow trout and sea charr indicates that the fishes can come in contact with many rivers in relatively short time. Both sea charr and sea trout spend about two months in marine environments with possibly short migrations between different estuaries, freshwater systems and the sea. They also make frequent use of different river systems for overwintering and spawning. A great number of escaped farmed rainbow trout are found along the Norwegian coast and in rivers. Brook trout are being intensively stocked in many Norwegian lakes because of its tolerance to low pH. However, the brook trout has only a limited migration into brackish water. Many eels grow and maturate in estuaries. The survival time of *G. salaris* on eels is short, restricting their potential as transport hosts.

USE OF PARASITES AS BIOLOGICAL TAGS WHEN STUDYING INTRASPECIES STRUCTURE OF COD IN THE COASTAL AREAS OF RUSSIA AND NORWAY

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The primary aim of this investigation was to study the cod parasitic fauna in the Kola Peninsula fiords. Secondary objectives were to summarize data on the cod parasites in the Barents Sea and to evaluate the possibility for the application of parasites as biological tags.

A total of 165 cods from the Kislaya and Ura fiords (western coast of the Kola Peninsula) were examined using the Dogiel's method of complete parasitological dissection. The age of sampled fish varied from 1 to 7 years. More than 20 scientific papers on parasites of the Barents Sea cod were analysed.

At present, 45 species are known to occur on cod from off-shore and coastal areas of the Barents Sea and adjacent waters: Microsporidia - 1, Myxosporea - 4, Peritricha - 2, Monogena - 6, Cestoda - 8, Trematoda - 11, Nematoda - 6, Acanthocephala - 3, Crustacea - 4. Variations in the parasite fauna of hosts from different areas are discussed and zoogeographical characteristic are given. Parasite species which could be used as biological tags are identified.

THE PARASITES OF EPIPELAGIC LUMPFISH (*CYCLOPTERUS LUMPUS* L.) IN THE NORWEGIAN SEA. INDICATORS OF BIOLOGY?

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Close to 300 *Cyclopterus lumpus* L. were caught by epipelagic trawling in the Norwegian Sea during a three week research cruise in July-August, 1993. A total of 260 lumpfish were frozen; of these 127 were examined fresh for flagellates, ciliates and gyrodactylids (for the results presented in this abstract, N=127 for the groups listed, and N=51 for the other parasites).

Preliminary results are: The fungus *Cycloptericola marina* occurred in the stomach mucosa of 68 %. Additional 23 species of parasites, representing 7 protozoans and 16 metazoans, are recorded. The core species in addition to *C. marina* are, *Cryptobia dahlii* (stomach, 91 %) *Trichodina cottidarum* f. *cyclopteri* (gills, 53 %), *Gyrodactylus cyclopteri* (gills, 38 %), *Myxidium inflatum* (gall bladder, 33 %), *Opechona bacillaris* (intestine, 20 %), *Podocotyle reflexa* (intestine, 20 %), *Scolex pleuronectis* (intestine, 18 %), *Hysterothylacium aduncum* III (encapsulated on viscera, 47 %), *H. aduncum* III, IV, V (lumen of stomach and intestine, 33 %), *Anisakis simplex* III (encapsulated on stomach and liver, 26 %), and *Caligus elongatus* (mainly juvenile stages, skin and fins, 24 %). Two species, *Opechona bacillaris* and particularly *Lernaeocera branchialis* (gills, 7 %), appear to be restricted to lumpfish caught on or close to the Norwegian shelf. Infections with *P. reflexa*, present also in the western Norwegian Sea, consist only of large oviferous worms, suggesting they represent old infections. Both juvenile and larger lumpfish was infected. This trematode, with the possible addition of three myxosporeans, is the only parasites of undoubted littoral origin. In general, lumpfish parasites are either monogenetic, or they represent species commonly acquired from planktonic intermediate hosts. The implications drawn from the species composition and distribution patterns of the lumpfish parasites are discussed in relation to lumpfish biology and origin.

INFECTION BY *SPYRION LUMPI* ON THE OCEANIC *SEBASTES MENTELLA* IN THE IRMINGER SEA

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The existence of a huge pelagic stock of *Sebastes mentella* inhabitating mainly the Irminger Sea has been known of since over 20 years. It is commercially utilized since the beginning of the eighties and it was decided to call it oceanic redfish. One of the characteristics of this stock is the infection by the parasite copepod *Sphyrion lumpi*. It had a noticeable influence on the discard rate of the commercial catch. The established prevalence besides of ulcers caused by the remnants of this parasite has been one of the subjects of research on the oceanic redfish by the Icelandic Marine Research institute emphasized since 1989 and is continuing both on commercial catches and on research cruises. Preliminary results have shown that the prevalence varies between the eastern and western part of Irminger Sea and that it is higher in females than in males. Generally, a decline has been observed in the occurrence of *Sphyrion lumpi* remnants since 1989.

SUBMITTED PAPERS - POSTERS

ISOLATION OF NEMATODE EGGS

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Isolation of eggs from adult *Anisakis simplex* nematodes has been made by a blending method. The method, apparently not used before in fish parasitology, gives clean and viable eggs, which hatched in the same proportions and within the same time intervals as eggs isolated by dissection, like in. The principles of the method are: blending-filtration-centrifugation. Some examples of results from hatching experiments are given.

OCCURRENCE OF *GYRODACTYLUS* ON THE ATLANTIC GROUP OF SALMON ON THE SWEDISH WEST-COAST

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Since 1975, gyrodactylosis caused by *Gyrodactylus salaris* Malmberg, 1957 has badly influenced the natural reproduction of salmon in some 35 Norwegian rivers. The salmon in all Norwegian rivers and in the rivers on the Swedish West Coast belongs to the Atlantic group of salmon, which is less resistant than the Baltic salmon to infection by *G. salaris*.

In 1989, investigations were initiated on the occurrence of *Gyrodactylus* in Swedish West Coast rivers which have a natural reproduction of salmon. Of four distantly separated rivers: Örekilsälven, Säveån, Fylleån and Stensån, only the River Säveån had *G. salaris*. In 1991, The River Högvadsån was also investigated and found to be the most *G. salaris* infected river in Sweden. Since then, the infection in the river has been monitored. The presence of *G. salaris* correlates with a decreasing salmon reproduction. The presentation deals with the question whether or not the lowered reproduction in the River Högvadsån depends on the comparatively high *G. salaris* infection. Results of repeated investigations in the River Säveån and the infection course in badly infected Norwegian rivers are of special interest to our discussion.

EFFECTS OF EYEFLUKE INFECTIONS ON GROWTH OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) IN A MARICULTURE SYSTEM

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Infections with *Diplostomum* spp. metacercariae are known to cause parasitic cataract in rainbow trout and is often stated to reduce growth of freshwater fishes. However, only few studies have actually provided clear evidence of effect of *Diplostomum* infection on rainbow trout growth under freshwater conditions. In addition, no reports are available on the association between eyefluke infection and growth of rainbow trout after their transfer to mariculture systems. We here report on the observed association between eyefluke infections and growth of fishes in such a aquaculture plant. Rainbow trout infected with *Diplostomum* sp. metacercariae in their lenses during a freshwater period and later transferred to marine conditions (20 ppt salinity, 10°C) exhibited notable variations in growth rate. Correlation analysis revealed significant negative correlations between the number of metacercariae in the least infected eye of the fishes and their total body weight, gutted weight and condition factor. Nonsignificant negative correlations between the total parasite number and the weight parameters were found. It is suggested that the feeding ability of trout in the studied mariculture system (with easily accessible food resources) is determined by the vision of the least infected eye.

A CHECKLIST OF METAZOAN PARASITES FROM RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) WITH NEW DANISH RECORDS

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A literature survey has been conducted to produce a checklist of metazoan parasites from rainbow trout (*Oncorhynchus mykiss*). More than 100 species from various systematic groups including Monogenea, Cestoda, Digenea, Nematoda, Acanthocephala, Hirudinea, Crustacea and Mollusca are listed. In addition, the preliminary parasitological results from a program supported by the Danish Agricultural and Veterinary Research Council on disease prevention in Danish rainbow trout farming are presented. Several of these reported metazoan parasites are new geographical records for Denmark.

WORKSHOPS

NEMATODA

Chairman Dr. Gary McClelland, rapporteur Jónbjörn Pálsson

Taxonomy

Dr. S. D'Amelio presented some preliminary results on a genetic study of the anisakid *Contracaecum ogmorhini* in Otariidae from the west coast of Canada and from Australia. The genetic distance between nematodes from these two areas was relatively high, indicating low gene flow and a high probability of reproductive isolation between the Canadian and Australian populations. It was noted that *C. ogmorhini* seems to be more closely related to *Contracaecum* species from birds than to other *Contracaecum* species from pinnipeds; this corresponds with observations of Fagerholm & Gibson (1987).

It was stated that a revision of the genera *Contracaecum* and *Phocascaris* was needed. Morphological differences between species of the two genera contrasting with a genetic affinity were discussed.

Life history

There have been contradictory ideas on the number of moults which occur within the egg of anisakid nematodes. It was believed that only the first moult of the larvae occurred within the egg, but it has recently been suggested that the second moult also takes place in the egg and that it is the third stage larva that hatches from the egg. Dr. Køie presented photographs showing larvae just removed from the eggs, and she pointed out the presence of two layers of cuticle, indicating that two moults take place within the egg.

It has been shown that in these nematodes it is the third stage larva that infects the final host. In the final host the worms moult twice to reach the fifth or adult stage.

Regarding the lifespan of anisakid nematodes in the final host, Dr. McClelland has found that in seals it takes the larvae approximately three weeks to mature followed by four months of egg production. In the case of *Anisakis* in minke whales Mr. Aspholm has found evidences that the approximate lifespan was about three months, and during this time the worms grow about two millimetres. per day. He also found increasing proportions of mature females in minke whales move from the first to the fourth (posteriormost) stomach.

It is not known whether these nematode larvae affect the behaviour of the fish intermediate hosts. Larval infection has at times been claimed to have deleterious effects on the condition of the host, but some of these claims have been doubted. It was pointed out that figures supposedly showing the effects of nematode larvae (*Contracaecum osculatum)* on the liver of Baltic cod might only show a difference in the visibility of the larvae, depending on the nutritional state of the fish. These larvae are more visible in fish with a small liver than in fish with large liver. It seems that in the case of *Pseudoterranova*-larvae in cod the larvae are rather quickly encapsulated by the host; however, in American plaice Dr. McClelland has found that *Pseudoterranova decipiens* larvae roam free in the muscles.

It was also mentioned by the chairman that an unexplored field of research for the future would be to investigate chemical factors involved in host-nematode relationships. It has been shown that, in fish, *P. decipiens* larvae release ketones into the muscles, probably anaesthetizing the fish and making them slower and thus more vulnerable to being caught by predators or fishing gear.

CESTODA

Chairman doc. Göran Bylund, rapporteur Dr. Hans-Peter Fagerholm

It was stated that the occurrence of *Diphyllobothrium latum* in the Finnish human population has decreased drastically since the 1950s and 1960s, while at the same time in certain lakes in E. and N. Finland the prevalence of plerocercoids in fish may be very high. This reflects a situation where only a few infected individuals can cause a high prevalence in the fish due to local problems in the handling of sewage. At the same time the occurrence of new infections are rather rare because of the high standards of hygien and readily available information on how the parasite is transferred to man.

It was pointed out that because of the new political situation it is today important to get information on the prevalence of diphyllobothriasis in the eastern countries and in Russia.

The need for taxonomic studies using modern methods in certain cestode genera other than *Diphyllobothrium* was stressed. The genera mentioned included *Proteocephalus* from freshwater fishes and the frequently occurring trypanorhynch larvae in marine fishes.

TREMATODA

Chairman Dr. Marianne Køie, rapporteur Dr. Kurt Buchmann

During the discussions the economic and ecological importance of eye-fluke infections (*Diplostomum* spp.) in fish were stressed. Mass mortality, morbidity and reduced growth in fish farms (rearing trout, cyprinids and sturgeons) due to these infections were reported by many members of the group. Several prophylactic measures including sonication or filtration of inlet water to fish farms, the use of uninfected well water and treatment with anthelmintics (praziquantel) were suggested.

The feral populations of many fish species in the Baltic Sea are known to harbour infections of *Diplostomum* with, in some cases, fatal consequences.

The zoonotic importance of several trematodes infecting fish and inverte-brates in the metacercarial stage (*Metagonimus yokogawai, Clonorchis sinensis, Opisthorchis felineus, Paragonimus westermani*) were stressed. Several millions of humans throughout the world are seriously infected with the adult stage of these trematodes in. Our Russian colleagues confirmed the in-creasing significance of *Metagonimus* in the former Soviet territory. The problem can be solved by avoiding the consumption of raw fish, raw shellfish and their uncooked products.

Although the ecology and life cycles of digeneans in Danish waters have been well studied a few problems still remain to be elucidated. For example two totally different cercariae are known to develop into indistinguishable adult forms of the digenean *Derogenes varicus*. This indicates that *D. varicus* actually comprises two species. Further research should elucidate these and other problems.

MONOGENEA

Chairman Doc. Göran Malmberg, rapporteur Dr. Kurt Buchmann

Gyrodactylus salaris has now been reported from a Russian river draining into waters connected to the Barents Sea. It has been suggested that stocking with infected *Salmo salar* from hatcheries should explain this new finding, but the discussions at the meeting revealed controversies about the original distribution of this gyrodactylid. Recent investigations have not detected *G. salaris* in rivers of the Kola peninsula, but according to Russian reports from the early 1970s *G. salaris* was already present in this zoogeographical area before the first reports of the parasite in Norway. Hopefully, future cooperation between the parasitologists in the countries involved will clarify these questions.

Resulting from several experiments it is now clear that the susceptibility of some Scottish and Norwegian strains of *Salmo salar* to infection with *G. salaris* is higher compared to that of salmon strains from the Baltic area. Recent findings of a rather high infection in salmon from rivers in western Sweden call for similar investigations involving these salmon strains.

In addition, the lack of information on the influence of macroenvironmental factors on the bionomics of *G. salaris* was stressed. Future work should focus on these parameters.

The group agreed that monogeneans are in many cases able to produce dangerous levels of infections in feral and reared fish stocks. Due to their simple life cycles these parasites can exhibit impressive population increases within a few weeks, as epizootic in the host population. Recent reports have documented mortalities of endangered fish species (*Arapaima gigas*) in a public aquarium due to heavy infections with gill monogeneans (*Dawestrema cycloancistrium*). Likewise, the gill monogeneans of the genus *Pseudodactylogyrus* are still causing problems in some eel farms. Mebendazole was previously shown to be totally effective against this monogenosis. However, in the laboratory some researchers have demonstrated the experimental selection of mebendazole resistant gill parasites. Such a resistance may explain recent reports of failing efficacy in commercial eel farms after several years of successful mebendazole use. Such experiences accentuate the need for further research on the biology and control of monogeneans.

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MOLECULAR TAXONOMY IN ANISAKIDS

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Introduction

Anisakids are ascaridoid nematodes dependent upon aquatic hosts for the completion of their life history, which generally involves an array of invertebrates and fish as intermediate or paratenic hosts, and marine mammals or fish-eating birds as definitive hosts. Morphological characters of taxonomic significance so far available in this group of parasites are very few (i.e. morphology of excretory system, number and distribution of caudal papillae), and often applicable only to adults.

Significant contributions to the study of parasite systematics, evolution and ecology have been provided by molecular methods, such as isozyme analysis, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA analysis (RAPDs), mini- and micro-satellite DNA polymorphisms, etc. Among these approaches, electrophoretic analysis of gene-enzyme systems, that came into large use since the late sixties, has so far yielded the major bulk of data, allowing: (a) to analyse the patterns of genetic variation in populations and species, and to relate them to different life history and ecological variables; (b) to estimate the amount of genetic divergence between conspecific and heterospecific populations; (c) to detect sibling species (that are biological species virtually identical at the morphological level) in both sexes and at any life stage; (d) to quantify gene flow within species; (e) to detect reproductive isolation both between sympatric and allopatric populations; (f) to analyse hybridization phenomena, by recognizing F1 hybrids, backcrosses, recombinants, and introgressed genotypes; (g) to evaluate by means of an alternative method phylogenetic relationships among taxa. The main systematic and evolutionary findings obtained for anisakid nematodes by isozyme studies are reviewed in the following paragraphs.

Genetic variation and environmental heterogeneity

Allozyme studies carried out on 17 species of ascaridoid nematodes having different life-cycles have shown that their genetic variability is positively related to the degree of environmental heterogeneity they experience (Bullini *et al.*, 1986). It was found that species whose life-cycle is carried out on homeothermic hosts (genera *Parascaris, Ascaris, Baylisascaris, Toxascaris, Toxocara*) show a significantly lower genetic variability (average expected mean heterozygosity, He=0.04) than do those needing both poikilothermic and homeothermic hosts (genera *Anisakis, Pseudoterranova, Contracaecum, Phocascaris, Hysterothylacium*) (average He=0.15). A major role of natural selection in this phenomenon was suggested (Bullini *et al.*, 1986). These and other data on helminth genetic variability were critically reviewed by Nadler (1990).

The study of further populations and of ten more ascaridoid species has confirmed the general trend previously observed: mean H_e=0.04 in the first, single-host group, versus 0.16 in the multiple-host group (Bullini *et al.*, 1991, 1994). However, some species of the second group showed lower values of genetic variability than found in the previous study. Differences in population size, degree of inbreeding, bottleneck events, time and mode of speciation can account for the discrepancies found between some multiple-host ascaridoid species. Nevertheless, the overall picture confirms a major role of natural selection in determining different levels of genetic variability in these parasites. Interestingly, the average values of variability found in ascaridoid worms parasitizing only homeothermic vertebrates is similar to that of their hosts (H_e=0.04, from Nevo *et al.*, 1984). On the other hand, the variability of ascaridoids parasitizing both poikilothermic and homeothermic species is similar to that found in their poikilothermic hosts (H_e=0.12, from Nevo *et al.*, 1984).

Among the various hypotheses proposed (for a review see Nevo *et al.*, 1984), our data appear to be in good agreement with the "niche-width variation" hypothesis (Van Valen, 1965), according to which a great genetic variability is an adaptive strategy conferring higher fitness in spatio-temporally variable conditions.

Antarctic species of both *Contracaecum* and *Pseudoterranova* genera show a higher genetic variability (average H_e=0.23) than the Boreal ones (H_e=0.13). This finding can be related to a lower degree of habitat disturbance in the Antarctic region, allowing species to reach higher population sizes, with reduced probability of genetic drift phenomena. Accordingly, higher degrees of seal infestation by *Contracaecum* species have been found in Antarctic waters than in the northern hemisphere (Klöser *et al.*, 1992).

Speciation without morphological differentiation: sibling species

The most important application of multilocus electrophoresis to parasite taxonomy concerns the detection of biological species, especially when speciation processes occurred without morphological differentiation (sibling species), a case particularly frequent in endoparasites (Bullini, 1985). Sibling species are taxa morphologically very similar (or even identical), differentiated at the genetic, ecological and behavioural level, and reproductively isolated in nature. Until recently, sibling species were frequently designated as "ecotypes" or "biological races'; these two terms, however, cover different phenomena, often unrelated (Mayr, 1970).

Speciation without morphological differentiation includes different cases, among which the following: (a) recent completion of reproductive isolation, with not enough time for morphological differentiation to be attained; (b) pre-mating reproductive isolation mechanisms not involving visual cues, that are easily recognized by man (as e.g. for butterflies and birds, in which sibling species are very few), but acoustical or chemical signals; the last ones seem to be the rule in endoparasite groups; (c) selection against phenotypic changes, as the realised phenotype represents an optimum in certain environmental conditions, such as endoparasitic life in a stable environment (e.g. a digestive trait in a given group of vertebrates, as in the case of sibling species in equid parasites of the genus *Parascaris*); (d) species whose taxonomy is based on a small number of characters, often reflecting phenomena of adaptive convergence or parallelism (Bullini, 1983; 1985).

In the case of sympatric or partially sympatric sibling species, a significant deficiency or complete lack of some heterozygote classes at polymorphic loci strongly suggests that the sample we are dealing with comprises distinct gene pools. Often, "diagnostic" loci occur, with alternative allozymes in the different sympatric forms.

By the use of genetic markers, it has been shown that many anisakid morphospecies considered cosmopolitan, include a number of sibling species, reproductively isolated and with distinct ecological niches. This is, for example, the case of *Anisakis simplex* (Mattiuccci *et al.*, 1986, 1990; Nascetti *et al.*, 1981, 1983, 1986, and unpublished), *Pseudoterranova decipiens* (Orecchia *et al.*, 1988; Paggi *et al.*, 1988, 1990, 1991; D'Amelio *et al.*, 1992), *Contracaecum osculatum* (Bullini *et al.*, 1992; Nascetti *et al.*, 1986, 1990a, 1993; Orecchia *et al.*, 1994) and *C. rudolphii* (D'Amelio *et al.*, 1990, 1991; Cianchi *et al.*, 1992), each of which have been shown to comprise a complex of several (at least three to five) sibling species.

In the case of allopatric populations multilocus electrophoresis may provide reliable information on specific status. As the most common mode of speciation in animals, including endo-parasites, has been shown to be the allopatric one (and in particular the "peripatric" model, Mayr, 1963, 1970), the buildup of genetic divergence has proved to be a better predictor than conventional morphology of whether two allopatric populations will interbreed upon recontact. As far as anisakids are concerned, available data show that when Nei's genetic distance (see below) reaches values of about 0.2, gene exchange is interrupted and reproductive isolation takes place.

Genetic divergence among populations and species

The amount of genetic divergence between populations and species is calculated from allele frequencies at a sufficiently large and representative sample of loci with appropriate statistics. The most commonly used is Nei's (1972) index of standard genetic identity (I) and distance (D). The latter estimates the average number of electrophoretically detectable allele substitutions accumulated in the genomes since the two compared populations began to diverge. Nei's I ranges from 0 to 1, while D (=-$\log_e$ I) from 0 to infinity; the latter value is obtained when the compared populations share non-common alleles at all of the loci studied.

In spite of few exceptions, the values of Nei's D found between local populations, subspecies and related species in various animal groups can be summarized as follows (Ayala, 1975; Bullini & Sbordoni, 1980; Ferguson, 1980; Bullini, 1985): between local populations of the same species D generally ranges from 0.001 to 0.05; between subspecies the values observed are from 0.05 to 0.2; genetic distance between closely related species often ranges from 0.2 to 1. Higher values of D are generally found between subgenera and related genera. As to sibling species, a large variance exists, from 0.01 to ∞, indicating that genetic and morphological differentiation can be largely independent. An example is represented by the ascarid worm *Parascaris equorum*, with its two chromosome forms univalens and bivalens; they are morphologically almost identical and live in the same specialized and highly constant environment: the equine intestine. Univalens and bivalens were considered conspecific, until multilocus electrophoresis has revealed that they are distinct species, genetically differentiated virtually at all the loci studied (Bullini *et al.*, 1979, 1981; Biocca *et al.*, 1979).

As to the anisakid species genetically studied so far, values of genetic distance for intra- and interspecific comparisons generally fall within the ranges mentioned above; however, some peculiar features must be considered. Conspecific populations generally show similar allele frequencies even when located thousands of kilometers apart; accordingly, their values of D are quite low (0.001-0.02). For example, D≈0.005 was found between *Anisakis pegreffii* from the Mediterranean Sea and the Falklands (=Malvine) Islands (D'Amelio*et al.*, 1993, and unpublished). These findings indicate a general lack of spatial structuring of genetic variation in geographical races and subspecies as supported by indirect estimates of gene flow from allele frequencies (Nm more than 3.5, Bullini *et al.*, in press). The high agility of a number of hosts (fish, seals, dolphins, aquatic birds) allows high levels of gene flow in anisakids, which therefore are, generally, monotypic species. Some controversial taxa recorded in this group have proved to be, when genetically tested, either synonym or good species, as in the case of *Contracaecum radiatum* and *C. septentrionale* (Cianchi *et al.*, 1992; Arduino *et al.*, 1994). As mentioned above, when genetic distance between two allopatric anisakid populations reaches values of about 0.2, gene flow is interrupted by intrinsic reproductive isolating mechanisms (RIMs).

A second point concerns the very high values of genetic distance (Nei's D from

2 to infinity) found between species considered as congeneric. A striking example is that of the genetic heterogeneity found within the genus *Contracaecum* (Orecchia *et al.*, 1986; Nascetti *et al.*, 1990b). The *Contracaecum* species having phocid seals as definitive hosts appear to be genetically closely related to each other (D from 0.25 to 0.69), while they are differentiated at all the loci tested from the congeneric species parasitizing birds (D=∞). On the other hand, they are genetically closely related to *Phocascaris* species, which also parasitize phocids (D from 0.27 to 0.53). These data strongly support Berland's proposal (Berland, 1964), according to which the *Contracaecum* species that have seals as definitive host should be included in a same genus with *Phocascaris* species. As *Phocascaris* was erected to include species devoid of interlabia (Høst, 1932), a character that has proved of no taxonomic value, a revision of the two genera appears needed. Another case is that of the genus *Anisakis*: *A. simplex* sensu lato shows a very high genetic distance from *A. physeteris*, as well as from other *Anisakis* species recently detected (Paggi *et al.*, 1985, and unpublished). *Anisakis* appears to be a polyphyletic group, to be split in distinct genera. A possible genus name for *A. physeteris* is *Skrjabinisakis* Mozgovoi 1953, a name originally proposed as subgenus for this species. *Skrjabinisakis* was later synonymized with *Anisakis* (Davey, 1971) but the validity of this taxon was recently conformed by both genetic and morphological data (Mattiucci *et al.*, 1986).

Also for other ascaridoid species, genetic distance data have proved useful in order to assess the generic ranking of a number of taxa. For instance, no alleles are shared (D=∞) between *Toxascaris* and *Baylisascaris* (a genus created by Sprent, 1968), as well as between *Toxocara* and *Neoascaris* (considered as a synonym of the former by Warren, 1970). Both *Baylisascaris* and *Neoascaris* appear to be valid genera, being the result of very ancient processes of evolutionary divergence (Paggi *et al.*, 1985).

Interspecific hybridization in the field

Multilocus electrophoresis carried out on various sympatric anisakid species has revealed that very efficient barriers to gene flow are operating within the different sibling species complexes studied so far. These isolating mechanisms seem to be essentially pre-mating. Indeed, a single F_1 hybrid was detected between *P. decipiens* A and B from Norwegian Sea; no recombinant or backcross genotypes were recovered among the 275 specimens of the sympatric sample, indicating lack of gene exchange, presumably due to hybrid sterility (Paggi *et al.*, 1991). No hybrids were until now detected in the other complexes investigated (e.g. *C. osculatum*, *C. rudolphii*, *A. simplex*), despite multiple infections found in a single host.

Hybrid zones and introgression

The occurrence of introgressive hybridization and its evolutionary significance both in animals and plants has been investigated in a number of recent papers (Barton & Hewitt, 1985; Bullini, 1985; Hewitt, 1988; Bullini & Nascetti, 1990; Abbott, 1992). Particularly relevant and widespread appear to be the so called "hybrid zones", i.e. areas of different width (from a few hundred meters to hundreds of kilometers) where populations, which genetically diverged allopatrically, come into contact and interbreed, giving origin to new gene combinations. The role in these phenomena of selective pressures in favour or against hybrid or introgressed individuals, of differential dispersal, and of environmental heterogeneity has been theoretically modeled and experimentally tested (Key, 1968; Moore, 1977; Szymura & Barton, 1991). The evolutionary consequences of hybrid zones include: (i) a complete mixing up of the interbreeding gene pools; (ii) more or less stable dynamic equilibria; (iii) the evolution of reproductive isolating barriers between the interacting taxa. Morphological analysis, although often revealing the existence of hybridization phenomena, fails to discriminate between F_1 or F_n hybrids, backcrosses or introgressed individuals. This is made possible by genetic markers, that differentiate "pure" interacting populations and their hybrid derivatives.

The only case of "hybrid zone" so far detected in anisakid nematodes involves *Phocascaris phocae* and *Ph. cystophorae*, two taxa differentiated at the genetic and ecological level, but not having reached so far complete reproductive isolation (unpublished data). Besides individuals genetically corresponding to "pure" *Ph. phocae* and *Ph. cystophorae*, a number of F_1, recombinant and introgressed genotypes were recovered both in the East and West Atlantic, from different seal species. Only very few F_1 genotypes were found over various hundreds of specimens tested, showing that hybridization between *Ph. phocae* and *Ph. cystophorae* is presently a rare event. Recombinant specimens were found at relatively low frequency in the harp seal, *Pagophilus groenlandicus* (where "pure" *Ph. phocae* prevails, at least in North East Atlantic), in the hooded seal, *Cystophora cristata* and the grey seal, *Halichoerus grypus* (where "pure" *Ph. cystophorae* prevails), while they are largely prevailing over both parental species in the ringed seal, *Phoca hispida*. A possible scenario suggested by the available data is the following: *Ph. phocae* and *Ph. cystophorae* started to diverge allopatrically about half a million years ago (as estimated from their genetic distance, D=0.11), adapting to (and coevolving with) different definitive hosts: the harp and hooded seals, respectively. Secondary contacts between these two seals and their *Phocascaris* parasites took place during the last interglacial, resulting in hybridization and introgression between *Ph. phocae* and *Ph. cystophorae*. Hybrids and recombinants were presumably selected against over parental species in their respective definitive hosts; on the contrary, they appear to have been favoured in the ringed seal. Accordingly, matings involving both hybrids and recombinants became a very frequent event and their progeny successfully colonized *Phoca hispida*. At the same time, hybridization between recombinants and both parental species became less frequent and less successful, as indicated by the lack of mixing up of *phocae* and *cystophorae* genotypes, still dominant in their respective hosts (Fig. 1). Evolution of assortative mating in *Ph. phocae* and *Ph. cystophorae* seemingly took place, as indicated by the very rare occurrence of F_1 hybrids. Gene flow between them is now very limited, and occurs only through their recombinants. The "hybrid taxon" presumably gains selective advantage over parental forms as the result of interspecific gene flow, involved in its hybrid origin, that strongly increases genetic variability, thereby allowing rapid responses to new or changing environments, as represented by the "new" host, *P. hispida.* At the same time, the adaptation to ringed seals and the development of a certain degree of host specificity may allow recombinants to reach some level of reproductive isolation from parental species, through pre-mating ecological mechanisms. Two possible evolutionary outcomes are therefore the most plausible: (1) maintenance of a dynamic equilibrium between *Ph. phocae, Ph. cystophorae* and their recombinants, gene flow being counter

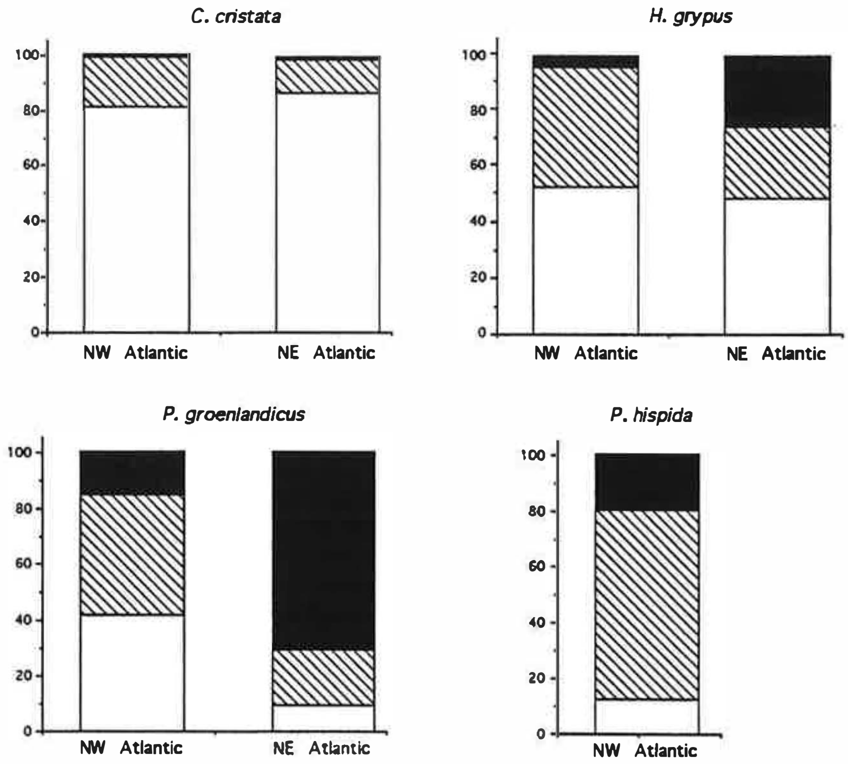

Fig. 1 - Relative proportion of specimens recovered from different definitive host species, assigned to *Phocascaris phocae* (■), *Ph. cystophorae* (□), and their recombinants (▧), on the basis of their genotypes at the diagnostic loci *Sod-1*, *Est-1*, and *Mpi*.

balanced by differential selective pressures; (2) completion of reproductive isolation, possibly giving origin to a stabilized recombinant species, adapted to the ringed seal. This kind of speciation, rather common in plants (Templeton, 1981; Grant, 1981; Abbott, 1992) is so far represented in animals by a single case: the cyprinid fish *Gila seminuda* (De Marais *et al.*, 1992). Further research is expected to allow to evaluate the frequency and evolutionary importance of this way of hybrid speciation in parasites.

Ecological niche and competition

As mentioned above, one of the most important outcomes of the use of genetic methods, such as multilocus electrophoresis, in anisakid taxonomy has been the finding that various morphospecies, often considered cosmopolitan and euriecious, include a number of distinct biological species reproductively isolated and differentiated both genetically and ecologically, e.g. with respect to geographic distribution and host preferences. Differences in life history between related species can be due both to differential host-parasite coadaptation and coevolution, and to interspecific competition, that can reduce the range of potential hosts in sympatric conditions (i.e. the realized niche is more restricted than the fundamental one). An example of niche subdivision was found between *P. decipiens* A and B as to their definitive hosts. Both species parasitize the common seal, *Phoca vitulina* and the grey seal, *Halichoerus grypus*. In North-East Atlantic, *P. decipiens* A outnumbers *P. decipiens* B about tenfold in the grey seal, while the opposite is true for the common seal, where *P. decipiens* B prevails about tenfold (Fig. 2a). This could reflect differential host

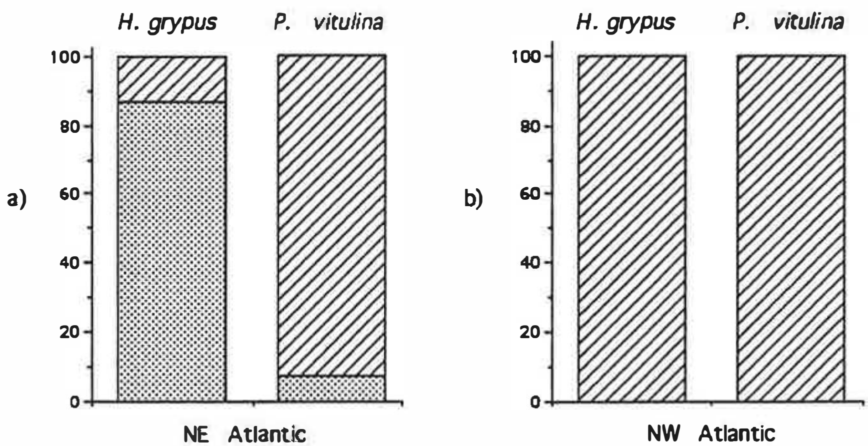

Fig. 2 - Relative proportion of specimens of *Pseudoterranova decipiens* A (▦) and B (▨), recovered from the definitive host species *Halichoerus grypus* and *Phoca vitulina* from: a) NE Atlantic; b) NW Atlantic. Data from Paggi *et al.*, 1991.

adaptation of the two parasites, evolved in allopatry. However, in Canadian Atlantic waters, where *P. decipiens* A is lacking, *P. decipiens* B is found to equally parasitize both grey and common seals (Fig. 2b). Niche subdivision found in the East Atlantic appears therefore a consequence of the sympatric occurrence of the two *Pseudoterranova* species, due to secondary contact, which has promoted character displacement (Paggi *et al.*, 1991).

Niche subdivision may also occur within the same host individual. For example, when members of the *P. decipiens* and *C. osculatum* complexes infest the same seal, a differential use of resources takes place, as the former invade the seal intestine if the stomach (the elective target for both) is highly infested by *Contracaecum* (Berland, 1964; McClelland, 1980; Klöser *et al.*, 1992). *C. osculatum* sensu lato appears to be the better competitor also when co-occurring with *Phocascaris phocae* and/or *Ph. cystophorae* in a same seal. Also in this case, *C. osculatum* is found almost exclusively in the stomach, while *Phocascaris* prevails in the upper section of the intestine; when *Phocascaris* is alone, it mostly occupies the stomach (Nascetti, 1993, and unpublished). These phenomena of niche subdivision, evolving by character displacement, reduce the level of interspecific competition for trophic and spatial resources.

Concluding remarks

It is expected that field studies using genetic markers will provide a dramatic increase of our knowledge on taxonomy, life history and evolution of these important parasites, with both theoretical and practical impact. A first point concerns speciation mechanisms in endoparasites. Up to recently, the favourite mode of speciation for many parasitologists was a sympatric model, involving the parasite's shift to a new host and its subsequent coadaptation, up to the rise of a new species. However, this hypothesis does not take into account that in sympatric conditions even limited levels of gene flow would homogenize population gene pools on distinct hosts, either preventing or disrupting ongoing adaptive processes. The need for geographic isolation in the buildup of adaptation and speciation was for the first time clearly stated by Mayr (1942, 1959). The same author later proposed a particular mode of allopatric speciation - the peripatric one - as the most widespread and efficient (Mayr, 1963, 1970). This model, which has been proved to apply to a wide array of animals and plants, involves geographical isolation of small populations whose genetic structure differs from that of the ancestral population since the beginning of the process, by genetic drift phenomena.

The peripatric model apparently fits well for endoparasites; the anisakid nematodes genetically studied so far strongly suggest that adaptation to different hosts and speciation is strictly related to geographic isolation of the hosts. In the case of the *A. simplex*, *C. osculatum*, and *P. decipiens* complexes, such processes apparently occurred in different times, from lower Miocene to Plio-Pleistocene, when extreme climatic variations took place. During glacial maxima (periods of highest reduction of sea level) reduced-sized populations of hosts and their parasites would have remained isolated in marine refuge areas, promoting genetic divergence and coadaptation; during interglacial periods, geographic ranges might expand, favouring host shift (Bullini *et al.*, 1993). Similar coevolutionary processes have been proposed, on morphological basis, for other host-parasite interactions, involving holartic cestodes (Dilepidids and Tetrabothriids) and their definitive hosts, fish-eating birds (Hoberg, 1986, 1991, 1992) and pinnipeds (Hoberg, 1992; Hoberg & Adams, 1992).

A second relevant point of great practical importance concerns specific identification of parasites at the larval stages: they are often very difficult to rear and offer poor morphological characters of taxonomic use. An example is represented by the recognition of the anisakid larvae indicated as type I and type II by Berland (1961): they have been found to correspond, respectively, the former to the various members of the *A. simplex* complex and the latter to *A. physeteris* sensu lato (Paggi *et al.*, 1983; Bullini, 1985; Orecchia *et al.*, 1986; and unpublished). The genetic identification of anisakid larvae will allow to relate the appropriate species to the different pathologies they cause to man (e.g. gastric and intestinal anisakiosis, pseudoterranovosis, contracaecosis and other anisakiosis-like anthropozoonoses); moreover, it will be possible to detect the geographic areas and period of the year with higher infection risks, as well as the fish species more involved in the transmission of the parasites to man. Finally, these data give very important information regarding life-cycle and epizootiological aspects of these parasites of great economical significance.

Synopsis

Data are reported on some significant results obtained by the use of molecular markers on anisakid systematics, ecology and evolution. The analysis of genetic variation in 27 ascaridoid species has shown a highly significant correlation between mean heterozygosity per locus and the degree of environmental heterogeneity experienced by the parasite. The variability found in ascaridoid nematodes parasitizing only homeothermic vertebrates is similar to that of their hosts (H_e=0.04 in both), while that of ascaridoids parasitizing both poikilothermic and homeothermic species is similar to that found in their poikilothermic hosts (H_e=0.16 and 0.12 in the parasites and hosts, respectively). Available data confirm a major role of natural selection in determining different levels of genetic variability in ascaridoid parasites with different life-history. Moreover, a higher genetic variability was observed in anisakid species from the Antarctic region (average H_e=0.23) than in Boreal congeneric species (H_e =0.13). This appears related to a lower degree of habitat disturbance in the Antarctic region, allowing species to reach higher popula-

tion sizes, with reduced probability of genetic drift phenomena.

A number of morphologically undifferentiated, but reproductively isolated species (sibling species), were detected within morphospecies belonging to different anisakid genera by the use of genetic markers (e.g. the complexes *Anisakis simplex, Pseudoterranova decipiens, Contracaecum osculatum, C. rudolphii*). Estimates of genetic divergence (Nei's D) between anisakid populations and species has shown very low values of D (0.001-0.02) between conspecific populations, in spite of very high geographic distances, with a lack of spatial structuring of genetic variation in geographical races and subspecies. When genetic distance between allopatric anisakid populations reaches values of about 0.2, gene flow is interrupted by intrinsic isolating mechanisms. A number of anisakid genera (e.g. *Contracaecum* and *Anisakis*) have proved to be genetically highly heterogeneous, with intrageneric distances as high as infinity; a splitting of these polyphyletic groups in distinct genera appears needed.

Interspecific hybridization appears to be a very rare event, indicating that efficient barriers to gene flow are operating within the different sibling species complexes studied till now. A single case of introgressive hybridization has been so far detected between *Phocascaris phocae* and *Ph. cystophorae*. Recombinant specimens were found at relatively low frequencies in the harp seal (where "pure" *Ph.phocae* prevails), in the hooded seal and the grey seal (where "pure" *Ph.cystophorae* prevails), while they are largely prevailing over both parental species in the ringed seal. A scenario for the origin and evolution of this hybrid zone is proposed. Two possible evolutionary outcomes are considered: (1) maintenance of a dynamic equilibrium between *Ph. phocae*, Ph. *cystophorae*, and their re-combinants, gene flow being counterbalanced by differential selective pressures; (2) completion of reproductive isolation, giving origin to a stabilized recombinant taxon, adapted to the ringed seal.

Various anisakid morphospecies, often considered cosmopolitan and euriecious, were found by molecular taxonomy to include a number of distinct biological species, ecologically differentiated with respect to their geographic distribution and host preferences. The latter appear due to differential host parasite coadaptation and coevolution as well as to interspecific competition. This acts either reducing the range of potential hosts, or promoting a differential use of resources from a single individual host in sympatric conditions. Examples of niche subdivision are given, involving the *P. decipiens, C. osculatum,* and *Ph. phocae* complexes.

Some final remarks are drawn from the reported data. At the theoretical level, Mayr's peripatric model of speciation, involving geographic isolation of small populations, with a role of both genetic drift and natural selection, apparently fits well for the anisakid cases studied so far, and is probably of more general significance for endoparasites. At the applied level, the genetic identification of anisakid larvae is allowing to relate the appropriate species to the different pathologies they cause to man, as gastric and intestinal anisakiosis, pseudoterranovosis, contracaecosis and other anisakiosis-like antropozoonoses. Moreover, these data give very important information regarding life-cycle and epizootiological aspects of these parasites of great economic significance.

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THIRTY YEARS OF ANISAKID RESEARCH, AND FUTURE TRENDS

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Anisakinosis

Anisakid nematodes of biological and economical importance in the aquatic environment comprise among other groups the genera *Hysterothylacium* in marine fishes; i.e. in "cold" hosts, and *Anisakis*, *Pseudoterranova*, *Phocascaris* and *Contracaecum* in marine mammals, *Contracaecum* also in fish-eating birds, i.e. in "warm" hosts. Their larvae, however, occur encapsulated in and on the viscera or free in body cavity, liver sinusoids and liver parenchyme, and muscles of many species of marine teleosts.

Nematode larvae in fishes, and their adult stages in fish-eating predators, were largely of academic interest until the 1950s; however, the "cod-worm" or "seal-worm", occurring in the musculature of important food fishes was of great concern to the fishing industry in areas with many seals. This changed with the recognition that what is now known as *Anisakis* caused a new human affliction - anisakinosis/anisakiasis - in Holland in the mid-1950s; when published the Japanese scientists and medical profession knew the answer to an old problem. Acquiring medical importance, the new human affliction spawned considerable research efforts in several countries to establish the prevalence and pathology of the condition in man, identify the organism involved and study its occurrence in fish intermediate and pinniped final hosts. The larva involved was initially wrongly identified as *Acanthocheilus* (= *Eustoma* = *Pseudanisakis*) *rotundatus*, which matures in sharks.

As the larva of *Anisakis* - "herring worm" or "whale worm" - was only one of several genera causing anisakinosis, the study of nematode parasites in fishes and whales, seals and aquatic fish-eating birds became important for the medical and veterinary professions, in food inspection and hygiene, legislation and in the fishing industry. Although many epidemiological studies have been made, new knowledge on the general biology, morphology, taxonomy, life cycles and larval stages are the results of basic biological research carried out in several countries. As human infection occurred when raw/undercooked fish, with live larvae, was eaten, it became imperative to study the viability of this larva at various temperatures, pH, salt and curing processes.

Anisakid taxonomy

Let us briefly review the taxonomic history. Originally all the species were members of the genus *Ascaris* Linnaeus, 1758. *Anisakis* was erected by Dujardin (1845), but the important species in marine mammals were described as *Ascaris*-species by Krabbe (1878). Revising the genus *Anisakis*, Davey (1971) accepted three valid marine species: *Anisakis simplex* in "cold seas", *A. typica* in "warm" seas, and *A. physeteris* in the sperm whale. The accumulated knowledge on *Anisakis* - its general biology, taxonomy and anisakiosis/anisakiasis - was reviewed by Smith & Wootten (1978). *Contracaecum* sensu lato was in 1912 erected by Railliet & Henry. The present "cod worm" has been regarded variously as *Terranova* (parasites of elasmobranchs) or *Porrocaecum* (in birds). Hartwich (1957) split from *Contracaceum* the species with "opposed caeca" from fishes, and with the excretory pore at the level of the nerve ring, placing them in *Thynnascaris* Dollfus, 1933. Myers (1959) erected the genus *Phocanema* for the "cod worm" - *P. decipiens*. *Phocascaris* Høst, 1932 is very close to *Contracaceum*, but lacks interlabia and has labial denticulation (Fagerholm & Gibson 1993). Berland (1964) emended the genus *Phocascaris* to accommodate *P. cystophorae* Berland, 1964 which has interlabial knobs.

A truth is said to last about thirty years - give or take a few years. Myers' *Phocanema* became *Pseudoterranova* Mozgovoi, 1951 (see Gibson 1983) and Deardorff & Overstreet (1981) relegated *Thynnascaris* to synonymy with *Hysterothylacium* Ward & Magath, 1917. With these amendments, the present genera *Hysterothylacium*, *Anisakis*, *Pseudoterranova*, *Contracaecum* and *Phocascaris* were, by about 1980, believed to have "come to rest" taxonomically.

New methods

Modern genetic studies on parasites were, in the 1980s, taken up in Rome, Italy. Being "classic morphologists", L. Paggi and P. Orecchia were prompted by their geneticist colleague L. Bullini to complement their morphological studies with genetic ones, using enzyme electrophoresis as their tool. Their results have largely modified the taxonomy of the nematodes in marine mammals.

Paggi, Orecchia, Bullini and co-workers showed that *Anisakis simplex* (Rudolphi, 1809) is composed of two sibling species, provisionally named A and B; their larvae, although lacking the adult morphological characters, can also be identified to species by electrophoresis. Of these two *A. simplex* A is a Mediterranean species, while *A. simplex* B is the common type in the north Atlantic (Orecchia *et al.* 1986). Further, *A. simplex* A was by Nascetti *et al.* (1986) shown to be identical with *A. pegreffii* Campana-Rouget & Biocca, 1955, and the distinctness of *A. physeteris* and its larva (type II) was also established by this method (Orecchia *et al.* 1986; Mattiucci *et al.* 1986).

Pseudoterranova decipiens (Krabbe, 1878) in seals in the northern Atlantic was shown to be comprised of three sibling species, provisionally named A, B and C

(Paggi *et al.* 1991). Type C is restricted as adult to the bearded seal (*Erignathus barbatus*), which is an arctic species; larvae are recorded in the flatfishes *Hyppoglossoides platessoides* and *Reinhardtius hippolossoides*. Type A is mainly parasitic in the grey seal (*Halichoerus grypus*), while type B occurs also in the harbour seal (*Phoca vitulina*). Types A and B occur in Europe, while only type B was recorded from the Canadian Atlantic.

Nascetti *et al.* (1993) found *Contracaceum osculatum* (Rudolphi, 1802) in the North Atlantic to be composed of three sibling species, provisionally named A, B and C. Type C was only found in *H. grypus* in the north-east Atlantic i.e. the Baltic Sea, types A and B occurred in several seal species on both sides of the Atlantic. Chianci *et al.* (1992) found *C. rudolphii* Hartwich, 1964, parasitic in the cormorant *Phalacrocorax carbo* in central and southern Europe, to be composed of two siblings, A and B; in the northern Atlantic *C. septentrionalis* Kreis, 1952 occurs in the two cormorant species (*P. carbo* and *P. aristotelis*).

Markowski (1937) and Punt (1941) described the early development in *H. aduncum;* the first larva moults in the egg. Consequently the II-stage larva, surrounded by the cast first cuticle, hatches from the egg. This has been almost an axiom, and was believed to apply to all the anisakid nematodes we are dealing with. The larvae "with boring tooth" found encapsulated in fishes, and also in some invertebrates, are known to be III-stage. That leaves unexplained where the 2nd moult, producing the III-stage larva, takes place.

Køie & Fagerholm (1993) showed that in *C. osculatum* a second, previously unreported moult, takes place in the egg; consequently the larva hatching from the egg is in its III -stage, not the II-stage. They suggest that development to the III-stage may apply to other members of the Ascaridoidea. Køie *et al.* (in press) found that also in *Anisakis simplex* and *Pseudoterranova decipiens* development to the third stage takes place in the egg.

Many authors have speculated on the life cycle of *H. aduncum,* in any teleost there may be present encapsulated larvae in the viscera, and larvae and adults in the digestive tract. Køie (1993a) found the two first moults to occur in the egg, the small III-stage larva within, about 0.3 mm long, develop in crustaceans into typical III-stage larva. It seems that the fate of the larvae depends upon the size they attain in the invertebrate host. Larvae less than about 2 mm did not survive in fish, larvae about 2-3 mm remained as III-stage larvae in the fish, moving into its body cavity, grew and became encapsulated. Larvae longer than some 3 mm moulted into the IV-stage in the intestinal lumen of the fish. Thus, a two-host cycle occurs when fishes ingest crustaceans harbouring III-stage larvae longer than 3 mm, and a cycle of three hosts when fishes ingest III-stage larvae less than 3 mm.

Causing great economical losses, the "cod-worm" or "seal worm" - *Pseudoterranova decipiens* (Krabbe, 1878) - has received much attention, particularly in Canada. Basic studies on infection and life cycle were carried out by Scott in the 1950s, more extensive and recent ones by, among others, McClelland, Marcogliese and Burt. The knowledge on the population biology of the sealworm and its intermediate and seal hosts was presented in several papers (see Bowen (ed.) 1990). The life cycle of *P. decipiens* in Canadian waters involves small benthic crustaceans as first intermediate hosts, then larger crustaceans become the next hosts. These are preyed upon by fishes, which eventually transfer the larvae to seal definitive hosts; the schematic life cycle graces the front cover of the volume edited by Bowen. In Norway and the Faroe, Aspholm (1991) and Jensen & Andersen (1992), and Køie (1993c) found sculpins (*Myoxocephalus scorpius*) to be heavily infected with *P. decipiens* larvae, while harbour seals do not feed on these small fish; cod, preying on these become infected and transfer the worms to harbour seals.

Future research

What are the future trends in anisakid research? The first priority is, as far as we can judge, a re-study of species/specimen in the genera *Anisakis*, *Pseudoterranova*, *Phocascaris* and *Contracacecum* from all seas by the new biochemical methods, which measure genetic identity/distance, to establish their true taxonomic status. When the specific identity of the various sibling species becomes known, it is possible, on the very specimens used in the genetic study, to analyse morphological characters (lips, spicules and papillae) that may permit morphological identification. One only of the siblings in each genus can retain the original name, e.g. one of the three siblings A, B and C in *Pseudoterranova*. The others must be described, and given new specific names.

The cetacean definitive hosts for *Anisakis* range far and wide, dispersing worm eggs with their faeces. We predict that the number of marine *Anisakis* spp. are few and widely distributed.

P. decipiens sensu lato is known from seals in the Atlantic, the Pacific and from the cold circumpolar northern and southern seas. Some seal species are coastal and rather stationary, others undertake yearly migrations, but all haul out on land or sea ice for long periods to breed and moult. The various populations are geographically isolated, and have probably been so for long periods. Knowing that three sibling species of *P. decipiens* occur in the north Atlantic, one may ask if there are a similar number of siblings in the north Pacific, and some more in the southern hemisphere? The same arguments apply to *Contracaceum* and *Phocascaris*.

Contracaceum species occur in seals and fish-eating birds. Bird and seal parasites "with opposed caeca" must have separated long ago. The head structure of *Contracaecum* species in birds and seals are remarkably similar. A conservative cephalic morphology may be advantageous in a "good" stomach parasite. Emending the definition of *Phocascaris*, Berland (1964), proposed that all *Contracaecum*-like species in seals be transferred to that genus, leaving the bird species in *Contracaecum*. However, it was recently shown that in otariid seals a *Contracaecum* species is found with a morphology close to that of some species occurring in birds (Fagerholm & Gibson, 1987), while some species in birds are morphologically close to those occurring in phocid seals (Fagerholm, 1988). Thus, in the future, numerous problems must be solved before the systematic position, relating especially to the species occurring in birds, of the species of *Contracaecum* and *Phocascaris* can be settled.

The common *Hysterothylacium* species in the North Atlantic is generally believed to represent one species, *H. aduncum*. However, Hartwich (1975) recognised three: *gadi, aduncum* and *auctum*. The modern biochemical techniques may settle the species question. If there is more than one species, as suggested by Fagerholm (1989) it is our guess that one may occur in some of the flatfishes.

H. aduncum lives as adult free in the gut of its fish hosts. Based upon the observation that these nematodes often bore into and mechanically loosen ingested prey, Berland (1980) suggested that the relationship between the symbionts is possibly one of mutualism, rather than parasitism; this idea is difficult or impossible to prove. Could this be the case for the other anisakids. There are many striking illustrations in the literature of *Anisakis* specimens in clusters in craters in the stomach wall. But are all nematodes attached, all the time? If not, can the nematodes "pay for board and lodging" by breaking down ingested prey?

How long do the nematodes live in their mammalian hosts and how do they maximise egg output? One of us (BB) observed that in the hooded seal the largest specimens of *Phocascaris cystophorae* were in the duodenum. What is the general strategy: do the nematodes spend a long time in the stomach to moult, grow and prepare to reproduce, but egg laying is deferred until, when grown, the worm move to the duodenum, lay vast numbers of egg, die and make room for new breeding "recruits"?

What does body size mean? *A. simplex* in the harbour porpoise reaches a moderate "match" size, while the same species in the minke whale reaches at least twice that length. In which ways is a small host able to "tell" its parasites to limit their growth, while in a larger hosts they are permitted realise more of their growth potential.

We shall not be idle for lack of challenges.

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THIRTY YEARS OF *DIPHYLLOBOTHRIUM* RESEARCH AND FUTURE TRENDS

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Abstract

Until the fundamental works of Kuhlow (1953 a, b, c), a German parasitologist, were published, recognition of *Diphyllobothrium* species was based almost exclusively on structural features of the adult worms. For the next ten years several taxonomic reports were published, still merely of a descriptive nature, but utilizing specific criteria of larvae as well as of adult worms. Only from the middle of the sixties was it recognized that the problems encountered in *Diphyllobothrium* taxonomy could not be tackled by merely descriptive studies but had to include experimental work and a critical re-evaluation of the taxonomic criteria used.

For the next ten years (1965 - 1975) experimental work mainly of Scandinavian workers ascertained details of the life cycles, host ranges, morphological variation, etc. of European *Diphyllobothrium* species. This work established three well defined species occurring in freshwater fish, *D. latum*, *D. dendriticum* and *D. ditremum*, and a fourth species, *D. vogeli*, the validity of which is still sometimes subject to discussion. More recent works have shown that these species are widespread in most parts of the northern hemisphere (and South America) and that numerous species previously described under separate names are identical with one or other of these species.

The progress in studies on *Diphyllobothrium* species occurring in marine fish has been slow.

Epidemiological research laid the basis for a rapid drop in the prevalence of human diphyllobothriasis in many endemic areas during the past three decades. It is evident that in addition to *D. latum*, several other species are implicated in human infections in circumpolar regions and in the Pacific area.

Progress in *Diphyllobothrium* taxonomy

Thirty years ago, in 1964, Vik wrote:

> "Although the genus *Diphyllobothrium* Cobbold, 1858 has been revised several times since the type species was described by Cobbold in 1858, the systematics of the group is still in a state of confusion and identification is

> made difficult by discrepancies in original and subsequent descriptions".

And one year later Müller (1965), another eminent student of the same parasite group, wrote:

> "I don't have the slightest idea as to the validity of species and genera in the pseudophyllidean cestodes. I know less about them now than I did fifteen years ago. I do not think we will be in a position to give rigid specific or generic designations to these cestodes until their life-histories and physiology have been worked out in much greater detail."

Even today, although much valuable work has been done during the past three decades, the *Diphyllobothrium* taxonomy is characterized by uncertainty and conflicting information and one is frequently disappointed by the tentative nature of the denomination of species. Several features peculiar to the genus *Diphyllobothrium* make recognition and characterization of species difficult (Bylund, 1975c):

a) the adaption of the adult worm to a very specific milieu, the intestine, has brought about a remarkable elimination or reduction of organ systems which in other invertebrate and parasite groups possess features of taxonomic significance.

b) the *Diphyllobothrium* species are characterized by a poorly developed host specificity, a character accompanied by an enormous range of variability concerning morphological and anatomical structures.

c) except for the first larval stages, these worms lack solid structures, hooks, mouth parts, etc., which in other helminth groups are of major taxonomic significance.

Before the middle of the sixties, taxonomists working on this genus greatly underestimated the vast range of morphological variability of these species and tended to create new species recognized on the basis of minor structural differences.

Until the fundamental studies of Kuhlow (1953 a, b, c) were published, recognition of species was based almost exclusively on structural features of the adult worms. For the next ten years several taxonomical reports were published, still merely of a descriptive nature, but worked out on a broader basis utilizing specific criteria of larvae and adults (Rausch, 1954, 1956; Wikgren and Muroma, 1957; Kozicka, 1958; Chizova and Gofman-Kadoshnikof, 1959; Meyer and Robinson, 1963; Wikgren, 1964). Most of the criteria used in these works were those proposed by Kuhlow (1953 a, b).

From the middle of the sixties it was recognized that the problems encountered in *Diphyllobothrium* taxonomy cannot be tackled by merely descriptive studies. This was strongly emphasized in reports by Rausch (1954), Vik (1964), Wikgren (1964), Stunkard (1965 and Meyer (1966). It was obvious that our inability to draw clear distinctions between many of these species was due to incomplete knowledge or misinterpretations concerning their life-cycles and host specificity. Furthermore, morphological data fundamental for evaluation of relationships between species were inadequate.

Once these concepts were recognized the following decade resulted in remarkable progress in *Diphyllobothrium*-taxonomy. Series of experimental works carried through mainly by Andersen (1971, 1972, a, b, 1973, 1975 a, b, 1978), Andersen and Halvorsen (1978), Bylund (1969, 1971, 1973, 1975 a, b, c, 1977), Halvorsen (1970), Halvorsen and Andersen (1973) and Halvorsen and Wissler (1973 ascertained details of the life-cycles, host ranges and morphological variability of European *Diphyllobothrium* species insufficiently studied and described in previous works. Moreover, by studying larvae and adults reared experimentally under varied but controlled conditions these authors re-tested and re-evaluated the stability and taxonomic significance of the characters so far used and also employed new characters and new techniques for delimitation of species. These studies eliminated most of the confusion until then characterizing the taxonomy of European freshwater *Diphyllobothrium* species and have been largely utilized for re-descriptions and re-evaluation of *Diphyllobothrium* species in other geographical regions.

Freshwater species

Today the *Diphyllobothrium* species occurring in European freshwater fishes are referred to four species: *D. latum, D. dendriticum, D. ditremum* and *D. vogeli*. Of these, the three first mentioned species are very well described and their species validity is undisputed. The validity of the last mentioned species, *D. vogeli*, is still under discussion (Halvorsen, 1970; Bylund 1975c; Andersen and Gibson, 1989). The final host of this species is not definitely known under natural conditions and morphological structures of the plerocercoid larva are very similar to those of *D. ditremum*. On the other hand, the morphology of the adult worm as well as morphological structures of the youngest larval stages (i.e. the embryonic hooks) seem to separate these species distinctly. Comparisons of these two species utilizing modern DNA-techniques are in progress and will elucidate the status and interrelationships between these two species more conclusively (Bylund, unpubl. data).

Due to incomplete knowledge of intraspecific morphological variation and the tendency to split out new species on the basis of minor morphological differences, the taxonomy of *Diphyllobothrium* species in other geographical regions was even more confusing than in Europe. The taxonomic criteria worked out for the delimitation of European *Diphyllobothrium* species, has sucessfully been applied for re-evaluation of freshwater *Diphyllobothrium*-species in some other regions (Andersen et al., 1987). It is apparent that *D. dendriticum, D. ditremum and D. latum* are species widely distributed over most regions of the northern hemisphere and also in South America.The numerous species described under different names may be identical with one of these species. The descriptions of most of these other named species, however, are often so incomplete that they must be regarded as *species inqueriendae* until redescribed in detail from the regions where they were originally recorded.

Marine species

Turning to the marine environment, the situation is still very confusing. Many species are described; but how many are valid? This question cannot be answered today. Most adult marine *Diphyllobothrium* species have been described from pinnipeds (Markowski, 1952 a, b).

The type species for the whole genus, *D. stemmacephalum*, poorly described by Cobbold in 1858, is from a porpoise (*Phocanema phocanema*). Other valid species from cetaceans are *D. macroovatum* as redescribed by Kamo et al. (1980), *D. polyrugosum* (Delyamure and Skrjabin, 1966) and *D. fuhrmani* sensu Yasaki *et al.* (1982).

Andersen (1987) gave a redescription of the type species *D. stemmacephalum* with comments on other marine species. Based on her observations the marine *Diphyllobothrium* species should be referred to four groups:

- one group where the main hosts are seals from the northern hemisphere; some of the these species also infect humans.
- a second group where the main hosts are cetaceans; some of these species are also reported from humans in Japan
- a third group are species which morphologically are very similar to the freshwater types; seals (arctic and antarctic) are their main final hosts; and
- a fourth group, which is found only in antarctic seals.

Specific morphological characters are given for all these groups.Very little is known about the plerocercoids of these marine species, only very few descriptions exist (Tantalean, 1975), and usually the corresponding adult is not known (Andersen, 1977).

From lump fish (*Cyclopterus lumpus*) a larva consisting of only a scolex has been described (Andersen, 1982). Rausch (pers. com.) believed this to be the plerocercoid of *D. cordatum*, a species commonly found in seals, dogs and inuits in Canada. From blue whiting (*Micromestius poutasson)* a plerocercoid has been recorded, which in appearance is very similar to the freshwater species *D. ditremum* (Andersen, 1977).

For the freshwater species, different growth patterns in the final host have been described (Müller, 1959; Andersen, 1978; Yamane *et al.*, 1988): those where the adults generate *de novo*, which means that the larval body is shed before the adult developes from the scolex only; those where the whole larval body developes into an adult; and also intermediate forms. Nothing is known about the marine species with regard to the growth patterns in their final hosts.

Progress in research on human diphyllobothriasis

The *Diphyllobothrium*-problem has attracted particular attention in Finland due to the high prevalence of human diphyllobothriasis. A comprehensive book on all aspects of human diphyllobothriasis was published by von Bonsdorff (1977).

Numerous papers on different aspects of the epidemiology of diphyllobothriasis were published during the sixties and seventies. Works by Wikgren and Muroma (1956), Wikgren (1963), Bylund (1968) showed that pike *(Esox lucius)* perch (*Perca fluviatilis*) and burbot (*Lota lota*) are the main transmitters of *D. latum* to man while coregonids are free from infection with this parasite. Agranovsky (1968) and among others Salminen (1970) carried out studies on the effect of cooking, freezing, smoking, salting and other treatment methods needed in order to render larvae innocuous in infected fish.

The role of sanitary habits and sewage disposal in the epidemiology of human diphyllobothriasis was studied in an endemic area in eastern Finland (Wikström 1975; Bylund *et al.*, 1975). In this district a decline in human infection rate from 18 % to 4.5 % during 1960 - 1978, was accompanied by a considerable increase in the rate of larval infection in the fish (von Bonsdorff and Bylund, 1982). A plausible explanation to this anomaly was the rapid social development which took place in the area with the introduction of modern hygiene with water toilets but with undersized and imperfect sewage systems and purification plants. It was shown that modern purification plants with chemical and biological purification, if properly dimensioned and operated, eliminate 95-99 % of the tapeworm eggs but overloading rapidly reduces the purifying effect.

Owing to the epidemiological research, intensified educational campaigns and improved sanitary systems the infection rate with *D.latum* in man in Finland during the past three decades decreased from more than 10 % to less than 1 %. There is a large number of *Diphyllobothrium* species recorded from man in older literature. During the past three decades many of these have been recognized to be synonymes of *D. dendriticum* (v. Bonsdorff, 1977). We can conclude today, that while *D. latum* is the main agent responsible for human diphyllobothriasis throughout the north temperate zone, *D. dendriticum* is of particular importance as a human parasite in circumpolar and arctic regions (Curtis and Bylund, 1991).

In the Pacific region the patterns behind human infections seem to be even more complex than in other regions. *D. ursi* and *D. dalliae* were recorded from man on the Pacific coast of Alaska and northern Canada; *D. pacificum* is a parasite of sea lions in the southern Pacific ocean but frequently recorded from man in Peru and Chile. Several new *Diphyllobothrium* species infecting man have been described recently from Japan and the Far East: *D. yonagoensis* (Yamane *et at.*, 1981), *D. nihonkaiense* (Yamane *et al.*, 1987), *D. klebanovski* (Muratov and Psokhov, 1988. It appears evident that

Pacific salmon (*Onchorhynchus spp.*) carry several species infective for man. Unless modern taxonomical methods present significant shortcuts for the analysis of species interrelationships, we have to await much more information on the life-cycles, larval stages and biology of these species before their taxonomic validity is confirmed.

Diphyllobothrium larvae as fish pathogens

There are several reports of mass mortalities among salmonid and coregonid fishes caused by *Diphyllobothrium* larvae (Bylund, 1972). Almost without exception the reports dealing with pathogenic effects of *Diphyllobothrium* larvae on their host fish deal with larvae of *D. dendriticum*. Vik (1957) stated that trout severely infected with these larvae were in poor condition; gonad development was retarded and the reproductive potential of the fish thus affected. Similarly the infection caused severe pathogenic effects and mortality of the host fish under experimental conditions (Bylund, 1972). Sometimes fish farmers had to destroy populations of salmon and rainbow trout smolts due to heavy infections with *D. dendriticum* larvae.

A peculiar pattern in the pathogenesis of the larval infection in fish is that the larvae frequently migrate to the heart of the fish; under these conditions even single larvae are able to kill the host fish. This was observed in connection with mass mortalities of fish in natural waters (Bylund, 1972) as well as under fish farming conditions (Rahkonen *et al.* 1993).

It is evident that, due to intensified fish farming, more attention will be focused on *Diphyllobothrium* larvae as fish pathogens in the near future.

Diphyllobothrium-tapeworms in basic biological research

The diphyllobothriid tapeworms have characteristics which make them extremely useful models for studies on basic biological phenomena. They are easily cultured in the laboratory from eggs to adults and especially their capacity of "never ending growth" is unique in the animal kingdom (Gustafsson, 1992). Once established in the final host the worms continue their growth generating new tissue in the neck zone while old tissue is rejected and expelled from the posterior end, like a conveyor belt. From the neck to the posterior end all developmental stages can be studied in the same organism, a fairly unique situation.

These characteristics have been utilized in numerous works on cell division, cell differentiation and recently especially in neurobiology and studies on the evolution of neurotransmitters and the nervous system (reviewed by Gustafsson, 1988 and 1992). Certainly their position in the first group of bilateral organisms (Bilateria) will give these organisms unique potential for studies on evolutionary events in the future too.

Trends in *Diphyllobothrium* taxonomy

Although much valuable work has been done during the last three decades in order to clarify the problems in *Diphyllobothrium* taxonomy, much confusion remains to be solved. There is no doubt that most of the troubles encountered when trying to differentiate between these species is due to misinterpretations of the stability and diagnostic value of morphological features, especially of the adult worms. Many of the criteria still in use, especially for the separation of adult worms, should be classified among those about which Voge (1969) states: "it can be useful in systematics if the systematist is sufficiently well trained to apply it with discrimination and caution"

However, when morphological methods are insufficient for solving taxonomical problems, there are shortcuts and aids available today for elucidating the relationships between species. Chemotaxonomic methods were introduced into *Diphyllobothrium*-taxonomy two decades ago (Bylund, 1977) and are much more sophisticated today - but, are rarely used within this group. Methods introduced by molecular biologists, i.e. DNA-techniques, have been successfully tried (Matsuura *et al.*, 1992) but are poorly developed or applied to these tapeworms. We do not think we will be in a position to establish biochemical taxonomic keys rendering morphological studies unnecessary but in groups where the morphological characteristics of the species overlap owing to external influences, host influences and other factors, modern chemotaxonomic and molecular techniques could greatly facilitate species determination.

Müller (1965) faced with the difficulties encountered in the identification of *Diphyllobothrium* species states:"The problem is one that might lead a respectable taxonomist to give up and go into molecular biology".

In groups like the genus *Diphyllobothrium* even the most respectable taxonomist should not hesitate to avail himself of the help provided by molecular biologists.

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THIRTY YEARS OF FISH TREMATODE (DIGENEA) RESEARCH IN THE NORDIC ENVIRONMENT, AND FUTURE TRENDS

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Introduction

Thanks to the early Scandinavian researchers Müller, Fabricius, Rudolphi, Olsson and Levinsen, who were active in the second half of the eighteenth and in the nineteenth century, and to later ones such as Odhner and Nybelin, the digenean fauna in the Nordic freshwater and marine areas was already well-known by the beginning of the present century. Non-Scandinavian workers, such as Creplin, Looss, Lebour and Nicoll, also contributed by describing species. Therefore, later researchers could focus on ecological and life-cycle studies, even though new species still are found and some taxonomic problems have yet to be solved.

Within the last thirty years our knowledge on the digeneans occurring in the Nordic aquatic environment has expanded significantly and the life-cycles of most shallow water fish digeneans have been elucidated experimentally. Several studies deal with individual parasites, their morphology, ecology and taxonomy.

The Nordic environment comprises the following geographical areas: the Scandinavian peninsula (Norway and Sweden), Finland, Denmark, Iceland and The Faroes plus the surrounding seas. For practical reasons Greenland is also mentioned in this review. In the present review several studies have been omitted which deal with the sporocyst and/or redial stages in molluscs or which do not include fish in their life-cycle. However, although not referred here, these are of importance when the biology of digeneans in general is considered. In the present review only original papers not written in regional national languages, are included. As a rule, abstracts and reports are not included. Papers by non-Scandinavian researchers are included if the parasites have been collected in the above mentioned areas.

The Freshwater environment

Finland and the Bothnian Bay

There has been a gap in the digenean research in Finland between the review by Wikgren (1956) and the studies by Valtonen and co-workers. In the Bothnian Bay the presence of certain parasites can be used as indicators of seasonal changes in feeding habits and migration of the sea-spawning whitefish, *Coregonus nasus*. Metacercariae of the avian digeneans, *Diplostomun spathaceum* (Rudolphi, 1819) occurred in the lens of the eye, *Ichthyocotylurus erraticus* (Rudolphi, 1809) (as *Cotylurus erraticus*) was found in the pericardium of this fish (Valtonen, 1977; Valtonen and Valtonen, 1980). Valtonen *et al.* (1984) examined about 7400 fish from the oligohaline north-eastern part of the Gulf of Bothnia and about 700 from a neighbouring eutrophic lake. Only four species of adult digeneans, *Bunodera luciopercae* (Müller, 1776), *Allocreadium isoporum* (Looss, 1894), *Sphaerostoma globiporum* (Rudolphi, 1802) and *Azygia lucii* (Müller, 1776), all of freshwater origin, were frequently found in both areas. In addition, *Coregonus* sp. and *Salmo salar* were occasionally heavily infected with *Phyllodistomum umblae* (Fabricius, 1780) (syn.: *P. conostomum* (Olsson, 1876)) and the marine *Brachyphallus crenatus* (Rudolphi, 1802), respectively. The digeneans specific to coregonids, *P. umblae*, *Crepidostomum* spp. and *I. erraticus*, and the non-specific digeneans, *A. isoporum* and the metacercariae of *Diplostomum* sp., *D. spathaceum* and *Tylodelphys clavata* (von Nordmann, 1832), were found in coregonids from the Bothnian Bay and two Finnish lakes (Valtonen *et al.*, 1988). The ureter parasite *P. umblae* did not show a clear seasonality in occurrence in *Coregonus* spp. from a lake in north-eastern Finland (Rahkonen and Valtonen, 1987). The gall-bladder and intestinal digeneans, *Crepidostomum* spp., in two species of *Coregonus* and in *Salmo trutta* did not show a clear seasonal pattern and reinvasion occurred throughout the year (Rahkonen and Valtonen 1989). It is surprising that the only digeneans found in migratory *Coregonus lavaretus* from two areas in the Gulf of Bothnia separated by a salinity gradient were metacercariae of the bird digeneans *D. spathaceum* and *I. erraticus* (Fagerholm and Valtonen 1980). The study of *B. luciopercae* from the Bothnian Bay and two lakes in northern Finland showed that in *Perca fluviatilis* the parasites grew and matured during the winter months, became gravid in spring and were usually lost during the summer months (Rahkonen *et al.*, 1984). These results tend to confirm that the parasite, at least in the northern part of its range, has a biannual life-cycle.

In a review on the influence of pollution on parasites of aquatic animals Khan and Thulin (1991) refer to the works by Valtonen and co-workers who observed no adult digeneans in the intestine of roach taken from a lake polluted by pulp and paper mill effluents, whereas the parasites were present in fish obtained from three unpolluted lakes. Similarly, the prevalence of the metacercarial stage of *Tylodelphys clavata* and *Diplostomum* spp. in roach and perch was lower in the polluted than in the other lakes. The difference in the prevalence was attributed to the sensitivity of the intermediate hosts to pollution since those hosts were absent, or present in low numbers only, in the polluted lakes.

Bucephalid digeneans use fishes as both intermediate and definitive hosts.

Two different bucephalid cercariae were found in the bivalve *Anodonta anatina* from Finnish lakes. They encysted in different sites in the fish intermediate hosts. *Rhipidicotyle campanula* (Dujardin, 1845) (syn.: *R. illense* (Ziegler, 1883)) and *R. fennica* Gibson *et al.*, 1992 have *Perca fluviatilis* and *Esox lucius*, respectively, as definitive hosts (Taskinen *et al.*, 1991; Gibson *et al.*, 1992).

Norway

Until the works by Halvorsen there had been no tradition in Norway to examine the digenean fauna of freshwater fish. The occurrence of *Azygia lucii* in *Esox lucius* was studied in a lake in southern Norway. Since no seasonality was found in the infection and since immature parasites were found at all seasons, it was concluded that there is a second intermediate host in the life-cycle of *A. lucii* (Halvorsen, 1968). The helminth fauna of fish in the River Glomma, south-eastern Norway, was studied by Halvorsen (1971, 1972). In the five fish species examined only four digeneans, *Rhipidocotyle campanula* (as *R. illense*), *A. lucii*, *Allocreadium isoporum* and *Bunodera luciopercae*, were found. The author stated that the relationship between the hosts and parasites studied appeared to be fairly constant despite geographical and limnological differences in the localities compared. The reproductive cycles of the endoparasites, including the digeneans, were very uniform with a peak in June showing that water temperature governs the maturation and egg production of the parasites. One of the digeneans, *B. luciopercae* in *Perca fluviatilis*, from two oligotrophic lakes in southern Norway was studied by Andersen (1978) and Skorping (1981). An annual cycle appears to be a characteristic feature of this parasite in different localities.

Bakke (1984, 1985) and Bakke and Lien (1978) used scanning electron microscopy (SEM) to identify specimens of the genus *Phyllodistomum* from the ureters of freshwater salmonids in Sweden and Norway. The arrangement, number and types of papillae and other tegumental structures were used. It was concluded that *P. conostomum* (Olsson, 1876) is a synonym of *P. umblae* (Fabricius, 1780). The morphology of adult *P. umblae* was compared after different treatment procedures including killing and fixation. It was concluded that only methods which induce stretching (and swelling) will result in acceptable specimens for measurements and descriptions (Bakke, 1988).

The charr *Salvelinus alpinus*, resident or sea-migratory, is the dominant or only fish species present in many lakes in northern Norway. *Crepidostomum farionis* (Müller, 1784) was found in resident charr from all five lakes examined, whereas *Phyllodistomum umblae* was found in two of the lakes only. None of the charr examined from lakes in Jan Mayen Island and Spitsbergen harboured digeneans (Kennedy, 1977). *P. umblae*, *Crepidostomum farionis* and *C. metoecus* (Braun, 1900) were recorded in *Salmo trutta* from lakes in northern Norway (Hartvigsen and Halvorsen 1993).

Sweden

Thulin has reexamined and compiled Nybelin's collection of digeneans in rivers and lakes of Sweden (unpubl. obs.). This includes both larval and adult stages as well as a number of new host records.

The eye-flukes *Diplostomum* spp. presents a serious hazard to free-living and cultured fish in freshwater areas and in brackish water (the Baltic Sea, see below). Metacercariae found in the eye-lens of teleosts are usually called *D. spathaceum* even though it may include more than one species (see below, Höglund and Thulin, 1992). Other species occur in the vitreous humour of the eye or in the retina. Pulmonate snails of the genus *Lymnaea* are the first intermediate host. Using SEM, TEM and radiometric assay Höglund (1991) showed that the cercariae of *D. spathaceum* enter the rainbowtrout *Oncorhynchus mykiss* at several sites, but mostly in the gill region. The majority of the penetrating parasites reached the eyes of the host, where they became established as metacercariae within 24 h, thus indicating migration towards the eye region. A specific antibody response in experimentally infected *O. mykiss* was not recorded, even though the decreased recovery of the parasite indicated that repeated exposure to *D. spathaceum* induced some degree of protective immunity in the fish (Höglund and Thuvander, 1990). Since *D. spathaceum* may cause serious economic losses in salmon farming chemical treatment against diplostomiasis has been tried (Bylund and Sumari, 1981).

Denmark

Buchmann and Uldal (1994) studied the effect of the lens dwelling form of *Diplostomum* sp. on the growth of rainbow trout in a mariculture system. The trout were infected in fresh water and later transferred to a mariculture system. Decreased growth was associated with high parasite infection level.

Apart from recent studies on eel and trout parasites, nobody has studied the parasite fauna of Danish freshwater fish since the investigations by O. F. Müller in the second half of the eighteenth century. Among the European countries, the Danish lakes are the least studied with regard to their fish parasite fauna. *Anguilla anguilla* from three lakes in northern Zealand (and brackish - marine localities, see below) have been examined for all parasites. Four species, *Nicolla gallica* (Dollfus, 1941), *Bunodera lucioperca*, *Sphaerostoma* sp. and metacercariae of *Diplostomum* sp. were found in one of the lakes, whereas *Diplostomum* sp. was the only digenean found in the two remaining lakes (Køie 1988a, b). The digenean *Crepidostomum metoecus* was found in *Salmo trutta* from one stream in the island of Bornholm (Buchmann, 1989).

Marine and brackish environment

Norwegian waters

Brinkmann redescribed the gasterostomate digenean *Prosorhynchoides gracilescens* (Rudolphi, 1819) (as *Bucephalopsis gracilescens*), gave some anatomical remarks on *Lecithophyllum botryophorum* (Olsson, 1868), and described encapsulated metacercariae of

Otodistomum sp. in the liver of *Enchelyopus cimbrius*, all from western Norway (Brinkmann 1957, 1977, 1988). Karlsbakk (in press) studied the infections with metacercariae of *P. gracilescens* in *E. cimbrius* from a fjord, the Bergen area. The seasonal fluctuation of the stomach hemiuroids *Hemiurus communis* Odhner, 1905 and *Derogenes varicus* (Müller, 1784) in cod from the Bergen area was studied by Meskal (1967). The thick, non-spinous tegument of the both species, and the sucker muscle cells of *H. communis* were studied at TEM level by Kryvi (1972, 1973). Olsen (1976) recorded *Podocotyle atomon* (Rudolphi, 1802) in *Gobius flavescens* from western Norway.

Other investigations deal with the digenean fauna (or parasite fauna) of a specific fish species. The parasite-fauna of the Atlantic salmon *Salmo salar* from the west coast of Norway was until recently virtually unknown. Adult salmon from the Bergen area and postmolt salmon from the Norwegian Sea, were examined by Bristow and Berland (1991) and Holst *et al.* (1993), respectively. Whereas only the three hemiuroid digeneans, *Hemiurus luehei* Odhner, 1905, *H. levinseni* Odhner, 1905 and *Derogenes varicus*, were recorded in the large salmon (Bristow and Berland, 1991), the small salmon harboured seven digenean species, of which one, the metacercaria of *Apatemon* sp., must have been acquired in fresh water. In addition to metacercaria of *Cryptocotyle lingua* (Creplin, 1825), the five adult hemiuroids, *Hemiurus communis, H. luehei, Brachyphallus crenatus, D. varicus* and *Lecithaster gibbosus* (Rudolphi, 1802), were recorded from the alimentary tract (Holst *et al.*, 1993). Hemmingsen *et al.* (1991) used parasites, including the digeneans *D. varicus, B. crenatus, H. levinseni* and *C. lingua*, as biological tags for *Gadus morhua* in northern Norway. The parasite fauna in cod from three sampling localities indicated that the cod in Balsfjord may comprise a separate population. Because digeneans have a short life span they should be useful for tracing seasonal migration. Lysne *et al.* (1994) found that metacercariae of *C. lingua* (as *Cryptocotyle* spp.) in caged cod were concentrated on the dorsal surface. This was explained by mechanical forces and behaviour of the cod.

Swedish waters

A long didymozoid *Nematobibothrioides histoidii* Noble, 1974 with a world-wide distribution was redescribed by Thulin (1980c) in *Mola mola*, from the west coast of Sweden. Other digeneans found in *M. mola* including specimens from western Sweden were described by Bray and Gibson (1977).

In a series of papers Thulin (1980a, b, 1982) described the external and internal morphology and development of the blood-fluke *Aporocotyle simplex* Odhner, 1900. This digenean inhabits the blood vessels of several species of flatfish and its unique life-cycle was elucidated by Køie (1982, see below). Between 1970 and 1980 Thulin collected digeneans from a great number of marine fish species mainly from the Tjärnö, Gullmar Fjord and Göteborg areas on the Swedish west coast. Much of this material was described and dealt with by Bray and Gibson in their series of papers on the helminth fauna from the north-east Atlantic region. These include *Otodistomum cestoides* (van Beneden, 1871) and the metacercaria of *Otodistomum* sp. (Gibson and Bray; 1977); *Steringophorus furciger* (Olsson, 1868). *S. thulini* Bray & Gibson, 1980, *Steringotrema pagelli* (van Beneden, 1871), *S. ovacutum* (Lebour, 1908), *Monascus filiformis* (Rudolphi, 1819) and *Tergestia laticollis* (Rudolphi, 1819) (Bray and Gibson, 1980); *Hemiurus communis, H. luehei* and *Lecithochirium rufoviride* (Rudolphi, 1819) (Gibson and Bray, 1986); *Zoogonus rubellus* (Olsson, 1868), *Zoogonoides viviparus* (Olsson, 1968) and *Proctophantastes abyssorum* Odhner, 1911 (Bray and Gibson, 1986).

The epidemiology of two species of *Diplostomum* (*D. baeri* Dubois, 1937 in the retina and *D. spathaceum* in the lens) in perch *Perca fluviatilis* from a warm water effluent of a nuclear power station in the northern Baltic and an unheated reference site was studied for a two year period (Höglund and Thulin, 1990). These studies were followed by experimental studies where metacercariae of *Diplostomum* spp. from the retina of the perch and from the lens of the roach, *Rutilus rutilus*, were identified on the basis of adults obtained by feeding various piscivorous birds of the family Laridae (Höglund and Thulin, 1992). The perch form was recognised as *D. baeri* which seemed to have the goosanders, *Mergus merganser*, as principal definitive host, although the metacercariae mature both in herring gull, *Larus argentatus*, and common tern, *Sterna hirundo*. The form living in the lens in roach was identified as *D. spathaceum* and it seemed to be restricted to birds of the family Laridae.

Danish waters

The studies by Køie (1983, 1984) deal with the digenean fauna of dab *Limanda limanda* and cod *Gadus morhua* from Danish and adjacent seas, including the Faroes (see below). In 638 specimens of dab from 11 stations 13 digenean species were recorded. In addition to the metacercariae of *Stephanostomum baccatum* (Nicoll, 1907), *Cryptocotyle lingua, Acanthostomum imbutiforme* (Molin, 1859) and *Otodistomum* sp. encapsulated in the stomach wall, the digestive tract digeneans *Steringophorus furciger, Steringotrema pagelli, Monascus filiformis, Podocotyle atomon, Zoogonoides viviparus, Neophasis anarrhichae* (Nicoll, 1909) (as *N. lageniformis* (Lebour, 1910)), *Derogenes varicus, Lecithaster gibbosus, Brachyphallus crenatus, Hemiurus communis* and the blood-fluke *Aporocotyle simplex* were found (Køie, 1983). In the 850 specimens of cod from 13 stations in Danish and adjacent seas the following metacercariae were found: *Prosorhynchoides gracilescens* (as *Bucephalopsis gracilescens*) in the brain (in the North Sea and Skagerrak), *Lecithochirium rufoviride* (as *Lecithochirium* sp.) encapsulated on the viscera (western Kattegat), *Otodistomum* sp. (The Norwegian Sea), *Diplostomum* sp. in the lens of the eye (the Baltic Sea) and *C. lingua* in the skin (coastal waters). The following adult digeneans were recorded: *Prosorhynchus squamatus* Odhner, 1905, *P.*

atomon, *P. reflexa* (Creplin, 1825), *Opechona bacillaris* (Molin, 1859), *Lepidapedon elongatum* (Lebour, 1908), *L. rachion* (Cobbold, 1858), *Stephanostomum pristis* (Deslongchamps, 1824), *D. varicus*, *L. gibbosus*, *B. crenatus*, *H. communis*, *H. luehei* and *H. levinseni* (see Køie, 1984). It was shown that the presence of a digenean with a known life-cycle (and known distribution of the molluscan host(s)) in an individual fish specimen may give information on where the fish concerned has acquired the infection; the fish has, therefore, been "tagged" by the parasite. Similarly Buchmann (1986) has examined the Baltic cod *G. morhua* caught in the Bornholm Basin for infections with metacercariae of *C. lingua* and *D. spathaceum*. Since the snail host of *C. lingua*, *Littorina littorea*, does not occur east of a line connecting southern Sweden and the island of Rügen, the infected cod must have spent some time in the western part of the Baltic. The low prevalence (1.6%) found in the cod could be explained by the mixing in this area of some of the western cod with a high prevalence of *C. lingua* and the uninfected eastern cod. Since the freshwater snail *Lymnaea peregra*, the first intermediate host of *D. spathaceum*, occurs from the eastern Baltic to the southern coast of Zealand and is common on the shores of Bornholm, the high prevalence (22.5%) of *D. spathaceum* in the eye-lens of the cod was not unexpected (Buchmann, 1986). The intensity of infection (< 53/host) in these cod was too low to affect the sight. However, Guildal (1982) reported that *Zoarces viviparus* from the Baltic south of Zealand were made blind by heavy infections with *D. spathaceum* metacercariae causing cataracts in the lens.

Køie (1988) recorded all parasites, including the digeneans, in the European eel *Anguilla anguilla* from three lakes (see above) and from four brackish-marine areas. In the marine environment eight digenean species were found. Only two of these species, *Deropristis inflata* (Molin, 1859) and *Lecithochirium rufoviride*, are host-specific with regard to the definitive host, the eel, whereas *H. communis*, *B. crenatus*, *D. varicus* and *L. gibbosus* occur in various marine teleosts, as does the metacercaria of *C. lingua*.

Salmo trutta caught in the Baltic near Bornholm were infected with *Podocotyle atomon*, *Brachyphallus crenatus* and metacercariae of *D. spathaceum* (Buchmann, 1989). In addition, flounders *Platichthys flesus* from this area were found to harbour a low *Podocotyle atomon* infection (Buchmann, 1991).

The life-cycles of a large number of marine fish digeneans have been elucidated within the last thirty years. Now the life-cycles of most species which use shallow water teleosts as intermediate or definitive hosts are known. The fish digeneans may in their life-cycle have two, three or four obligatory hosts.

The most simple life-cycles are represented by members of the Fellodistomidae, *Monascus filiformis*, *Steringophorus furciger* and *Steringotrema pagelli*. The large cercariae of these species are ingested by the definitive host, or they enter the definitive host, mainly flatfish, with respiratory currents (Køie, 1979a, 1980). These three two-host cycles are regarded by this author as the most primitive of all known recent life-cycles. The first intermediate hosts, protobranch bivalves of the genera *Nucula* and *Nuculana* may be regarded as living fossils which can be traced back to the Ordovician. No other known molluscan host gener reach even half this age. It is likely that the fellodistomids have retained more primitive features than any other recent digenean. Other fellodistomids have a three-host cycle. Large cercariae from the bivalve *Nucula tenuis* enter, according to a Russian researcher (see Køie, 1980), brittle stars, and the definitive hosts, mainly *Anarhichas lupus*, are infected by ingesting the brittle stars. Since similar cercariae and metacercariae from Danish waters matured to *Steringotrema ovacutum* in flatfish, it was believed that *S. ovacutum* should be considered a synonym of *Fellodistomum fellis* (Olsson, 1868). Experimental infections of *A. lupus* with metacercariae from the stomach of the whelk *Buccinum undatum* from the Øresund, Denmark, have shown that these are metacercariae of *F. fellis* (Køie, unpubl. obs.). Thus the generally accepted life-cycle of *F. fellis* is identical to that of *S. ovacutum*, the latter then being a good species. Bray and Rollingson (1985) demonstrated using enzyme electrophoresis that *S. ovacutum* and *F. fellis* are not synonymous. The first intermediate host, probably a mytilid bivalve, of *F. fellis* is unknown. Similar to *F. fellis* the related wolffish digenean, *Steringophorus agnotum* (Nicoll, 1909), uses *B. undatum* as the second intermediate host (Køie, unpubl. obs.).

Another two-host life-cycle is represented by another wolffish digenean, *Neophasis anarrhichae*. This species (as *Neophasis lageniformis*) (Acanthocolpidae) has *B. undatum* as the only intermediate host, and *A. lupus* acquires the infection by ingesting infected whelks. Adult specimens, as well as the cercariae, are provided with flattened, serrated spines (Køie, 1973).

The two-host life-cycle of blood-flukes such as *Aporocotyle simplex* (Sanguinicolidae) has probably evolved by development into adults in the second intermediate host, thus eliminating the former final host. The furcocercous cercariae, which develop in the terebellid polychaete *Artacama proboscidea*, penetrate the skin of the flatfish definitive hosts, of which *Hippoglossoides platessoides* is the most important. The immature specimens reach the branchial vessels and the heart via the lymphatic system. *A. simplex* is the only known digenean species which does not have a molluscan intermediate host (Køie, 1982). The adult *A. simplex* was redescribed from the west coast of Sweden by Thulin (1980a).

The common flatfish digenean *Zoogonoides viviparus* (Zoogonidae) has the whelk *Buccinum undatum* as the first host. Its cercaria was studied at the ultrastructural level (TEM and SEM) by Køie (1971). Encysted metacercariae have been found in a polychaete (Orrhage, 1973), various polychaete species, molluscs and brittle stars (Køie, 1976). The cercaria of the gadoid digenean *Stephanostomum* *pristis* (as *S. caducum* (Looss, 1901), see Køie, 1984) (Acanthocolpidae) develops in the prosobranch *Natica alderi* and encysts in fish belonging to the family Gobiidae (Køie, 1978). The cercaria of another "gadoid" digenean, *Lepidapedon elongatum* (Lepocreadiidae) develops in the small prosobranch *Onoba aculeus* and encysts in various polychaetes, molluscs and brittle stars. The cercaria differs from all known lepocreadiid cercariae in being non-ocellate and not being able to swim (Køie, 1985). An ocellate, trichocercous cercaria developing in the prosobranch *Nassarius reticulatus* is believed to be the cercaria of *L. rachion* (Køie, thesis abstract). A similar ocellate, trichocercous cercaria of the related *Opechona bacillaris* (Lepocrea-diidae) develops in the prosobranch *Nassarius pygmaeus*. It penetrates various planktonic, non-crustacean invertebrates where the non-encysted metacercaria is found. Various planktophagous fish, such as *Scomber scombrus*, *Cyclopterus lumpus* and gadoids, act as definitive hosts (Køie, 1975). The digenean *Podocotyle reflexa* (Opecoelidae) has the whelks *B. undatum* and/or *Neptunea antiqua* as the first intermediate hosts (see Gibson and Bray, 1984). The encysted metacercariae occur in amphipods and decapods (Køie, 1981).

The life-cycles of most hemiuroids from Nordic shallow seas have been elucidated during the last few years. Hemiuroids have a complicated cystophorous cercaria. The cercarial body is injected into the haemocoel of a crustacean intermediate host, most often a copepod (Køie, 1979b, 1989, 1990c, 1990e, 1991, 1992a, 1995). Most species are not very specific with regard to the fish definitive host, and, if they occur in the stomach, they may be transferred from one host to another via predation. The free-swimming cercaria of the ubiquitous stomach digenean *Derogenes varicus* (Derogenidae) develops in the prosobranch genus *Natica*. Small fish become infected by ingesting calanoid copepods, larger fish definitive hosts by predation on other fish (Køie, 1979b). *Magnibursatus caudofilamentosa* (Reimer, 1971) Køie and Gibson, 1991 (Derogenidae), on the other hand, has a non-motile cercaria which develops in the prosobranch *Rissoa membranacea*. Harpacticoid copepods are second intermediate hosts. The adult occurs on the operculum or gills of *Gasterosteus aculeatus* (Køie and Gibson, 1991, Gibson and Køie, 1991, see also below). The cercaria of the intestinal species *Lecithaster gibbosus* (Lecithasteridae) occurs in the small opisthobranch genus *Odostomia* (Køie, 1979). The non-motile cercaria of the eel stomach parasite *Lecithochirium rufoviride* (Hemiuridae) develops in the prosobranch *Gibbula cineraria*. Harpacticoid copepods or malacostracans may act as second intermediate hosts. The small metacercariae in the copepods have to grow further in the viscera of a fish host before they are infective to the eel. Metacercariae in malacostracans may develop to infectivity in these crustaceans. *L. rufoviride* has thus, depending on the crustaceans hosts, a conventional three-host life-cycle or a four-host life-cycle (Køie, 1990c). The host-specific parasite of *Scomber* sp., the large stomach digenean *Lecithocladium excisum* (Rudolphi, 1819), and the less host-specific stomach digeneans *Hemiurus luehei*, *H. communis* and *Brachyphallus crenatus* (all Hemiuridae) have small free-swimming very similar cercariae which develop in four species of the bullomorph opisthobranch genera *Philine* and *Retusa* (Køie, 1990e, 1991, 1992a, 1995).

When fish harbour metacercariae they act as second intermediate hosts. Shallow water fishes may function as hosts of species which have avian definitive hosts. The most well-known is *Cryptocotyle lingua* (Heterophyidae) causing "black-spots" in the skin of fish and with piscivorous birds such as gulls as definitive hosts. Cercariae from *Littorina littorea*, and various developmental stages of metacercariae as well as adults of *C. lingua* were studied by SEM (Køie, 1977). Another heterophyid metacercaria (*Pygidiopsis ardeae* Køie, 1990) was found in the viscera of shallow water fish such as *Gasterosteus aculeatus* and *Pomato-schistus microps*. The small ocellate cercariae developing in *Hydrobia* sp. penetrated the gills where some encysted, but most scattered throughout the body via efferent branchial arteries and the dorsal aorta to encyst mostly in the mesenteries, spleen and on the surface of other viscera (Køie, 1990d). The small adult heterophyids found in the experimentally infected chickens and pigeons were identified as *Pygidiopsis* sp. The natural definitive host is the grey heron *Ardea cinerea*, the type-host of *P. ardeae* (Køie, 1990a, see below). The metacercaria of *P. ardeae*, similar to that of *C. lingua*, grows considerably in the fish host, and the simple pointed spines of the cercaria become flattened, scale-like and multiserrated (Køie, 1992b). *Mesorchis denticulatus* (Rudolphi, 1802) (Echinostomatidae), a common parasite in *Larus* spp. in northern Europe, infects the fish intermediate host in a completely different way. The large, red-tailed gigantocercous cercariae, which develop in *Hydrobia ulvae*, are actively ingested by small fish or carried to the respiratory current into the gill chambers. They encyst in the gill filaments of small euryhaline fish such as *G. aculeatus* and *P. microps* (Køie, 1986). The infective metacercariae have conspicuous collar spines and smaller pointed spines occur on most of the body surface (Køie, 1987).

Greenland, Iceland and the Faroes and the surrounding seas

Greenland

There has been a long tradition for Nordic researchers to study the Greenland parasite fauna. In this aspect the work by Brinkmann (1975) is important. In addition to summarizing previous records of trematodes (including the Monogenea) he gave detailed descriptions of the 24 digeneans found in the 16 teleost species examined, most from the Disco area, western Greenland. Køie (1979, 1980) studied the larval stages of some fish digeneans in molluscs from the Disco area.

Iceland

The digenean fauna of Iceland is little

known. Most important is the work by Brinkmann (1956), who reviewed the 14 known digeneans recorded from the Icelandic teleost species examined (15). Bray and Gibson (1986) redescribed some of these as well as reporting some new records. These include *Pseudozoogonoides subaequiporus* (Odhner, 1911) Bray & Gibson, 1986, *Panopula spinosa* (Zubchenko, 1978) Bray & Gibson, 1986 and *Brachyenteron pycnorganum* (Rees, 1953) Overstreet & Pritchard, 1977.

Skírnisson *et al.* (1993) recorded the avian digenean *Cryptocotyle lingua*, which use teleosts as intermediate hosts, in the Icelandic Arctic fox. The finding of this species in Iceland is unexpected, since the snail host, *Littorina littorea*, does not occur in Iceland. However, recently cercariae of *C. lingua* have been recorded in *L. obtusata* and metacercariae found in some littoral fishes (Eydal, pers. obs.). To the list of marine fish digeneans should be added the flatfish blood-fluke *Aporocotyle simplex*, since cercariae apparently identical to those of *A. simplex* have been found in a polychaete, the terebellid *Lanassa nordenskioeldi*, from an east Icelandic fjord (Køie and Pedersen, 1988).

The digenean fauna of Icelandic freshwater fishes have been examined by Blair (1973) and Frandsen *et al.* (1989), who examined *Gasterosteus aculeatus* and *Salvelinus alpinus*, respectively. The sticklebacks harboured metacercariae of the families Strigeidae and Diplostomidae which mature in birds. Mature specimens of *Diplostomum* sp. and *Apatenom gracilis* (Rudolphi, 1819) were recovered in ducklings fed with infected sticklebacks. The metacercariae were found in the humour of the eye, retina, eye lens and the brain (Blair, 1973). In the polymorphic Arctic charr from Thingvallavatn only two digeneans were found. The authors demonstrated the important influence diet and habitat has on the transmission of parasites to the four Arctic charr morphs. The large benthivorous charr were most heavily infected with the intestinal adult allocreadiid *Crepidostomum farionis*, whereas the highest parasite burden with the diplostomid *Diplostomum* sp. was found in the small benthivorous charr. Metacercariae of *Diplostomum* sp. were located in the vitreous humour of the eye, where they may interfere with light passing through the eye lens to the retina (Frandsen *et al.*, 1989). The digenean fauna in some intertidal and sub-littoral molluscs from a North Iceland fjord was examined by Sannia and James (1977). As expected most were larvae of species with avian definitive hosts, only *Prosorhynchus squamatus* and *Podocotyle atomon* have teleosts as definitive hosts.

The Faroe Islands

The digenean fauna of the Faroe Islands is even less well known than that of Iceland. In the 12 specimens of *Limanda limanda* examined (Køie, 1983) the small rectal digenean *Zoogonoides viviparus*, the stomach digenean *Derogenes varicus* and the skin metacercarie of *Stephanostomum baccatum* were found. The 40 specimens of *Gadus morhua* from two different depths harboured, in addition to metacercariae of *Prosorhynchoides gracilescens* (as *Bucephaloides gracilescens*) in the brain and metacercariae of *Lecithochirium rufoviride* (as *Lecithochirium* sp.) encapsulated in the viscera, adult *Prosorhynchus squamatus*, *Podocotyle reflexa*, *Lepidapedon elongatum*, *L. rachion*, *D. varicus* and *Hemiurus levinseni* (Køie, 1984). About 50 teleost species from shallow water to a depth of 1540 m off the Faroes have been examined. At least 35 digenean species were found in these teleosts (Køie, unpubl. obs.). *Glomericirrus macrouri* (Gaevskaja, 1975) and *Lecithocladium excisum* in the stomachs of *Coryphaenoides rupestris* and *Scomber scombrus*, respectively, from Faroese waters, were redescribed by Gibson and Bray (1986).

New species from the Nordic countries and the surrounding seas

The known Finnish freshwater digenean fauna has been enriched by two new species. *Rhipidocotyle fennica* Gibson *et al.*, 1992 (Bucephallidae) is described from the intestine of *Esox lucius* in central Finland. Its cercaria develops in the bivalve *Anodonta anatina* and the metacercaria occurs in the skin and fins of *Rutilus rutilus*. This new species was found in connection with a study on bucephalid digeneans parasitising molluscs and fishes in Finland (Taskinen *et al.*, 1991, Gibson *et al.*, 1992).

Crepidostomum wikgreni Gibson & Valtonen, 1988 (Allocreadiidae) was described from the gall-bladder and intestine of the whitefish *Coregonus acronius* in Lake Yli-Kitka in north-east Finland. It has larger eggs but is otherwise morphologically similar to *C. farionis*, with which it occurs sympatrically and sometimes concurrently. The authors suggested that the new species had arisen from *C. farionis* after deglaciation and since about 8,400 years ago, at which time the waters of the Lake Kitka System were isolated from those of the rest of Finland and flowed eastwards into the White Sea Basin. The isolation of this basin appears to have been maintained by watersheds running north-south close to the Fenno-Russian border and east-west through central Karelia. The authors suggested that the present occurrence of *C. farionis* in Lake Yli-Kitka is due to a recent reintroduction (Gibson and Valtonen, 1988).

Steringophorus thulini Bray & Gibson, 1980 (Fellodistomidae) was found in the intestine of *Enchelyopus cimbrius* from the Skagerrak (Bray & Gibson, 1980). It also occurs in other gadoids from more than 1000 m depth.

Anomalotrema koiae Gibson & Bray, 1984 (Opecoelidae) occurs in the intestine of scorpaenids, mainly *Sebastes* spp., and the gadoids, *E. cimbrius* and *Onogadus argentatus*, from the Skagerrak and Faroese waters (Gibson & Bray, 1984; Køie, unpubl. obs.). Its cercaria probably occurs in the prosobranch snails *Buccinum undatum* and/or *Neptunea antiqua*, and the metacercaria is believed to develop in shrimps (See Gibson & Bray, 1984).

Magnibursatus caudofilamentosa (Reimer, 1971) Gibson & Køie, 1991 (Derogenidae) occurs in the branchial chamber of *Gasterosteus aculeatus* from Danish shallow waters (Gibson & Køie, 1991). The finding of a halipegine derogenid in Danish waters was unexpected, since the closest fish halipegines, including a congener, occur in the Mediterranean/Black Sea region, and halipegines were thought to be absent from northern European fishes. It was suggested that the genus *Magnibursatus* in the past had a much wider distribution in the Mediterranean zoogeographical region, and that its distribution extended north along the Atlantic coast into the boreal region (Gibson & Køie, 1991).

Pygidiopsis ardeae Køie, 1990 (Heterophyidae) matures in the grey heron *Ardea cinerea*, but the metacercariae occur in shallow water fishes (Køie, 1990a, d, see above). The type-locality is the Isefjord area, Zealand, Denmark.

Van der Land (1967) found four specimens of a blood-fluke in one of 40 rabbitfish *Chimaera monstrosa* from the Trondheim Fjord. *Chimaerohemecus trondheimensis* Land, 1967 (Sanguinicolidae) differs from the other marine blood-flukes found in the Nordic environment, *Aporocotyle simplex* and *A. spinosicanalis* Williams, 1958, by having among other traits, a bifurcate intestine which was considered a primitive feature (van der Land, 1967).

Future trends

Recent studies have shown that in some cases a species may have more than one type of cercariae. This means that some species should be split into two or more entities. Since in these instances descriptions of adults based on their morphology is not sufficient, biochemical analysis such as enzyme electrophoresis, DNA-profiles, southern blotting may in the future be used to separate dubious species, as has been done successfully in the case of some digeneans (Bray and Rollinson, 1985) and in studies on numerous anisakid nematode species (by Paggi and co-workers, see review-article by Paggi and Bullini 1994).

Further life-cycle studies

A number of common shallow water digeneans still have unknown life-cycles. The problem with the anarhichadid gallbladder digeneans *Fellodistomum fellis* and *Steringophorus agnotum* is mentioned above. Both species and other fellodistomids from the north-east Atlantic have recently been redescribed by Bray and Gibson (1980) based on material partly from Nordic waters. Also the anarhichadid digenean *Lepidophyllum steenstrupi* Odhner, 1902, which occurs in the urinary bladder and has recently been redescribed based on material partly from Iceland and the Faroes (Bray and Gibson, 1986), has an unknown life-cycle. No life-cycle within the genus *Otodistomum* has been elucidated. These large digeneans whose mature specimens occur in the stomach of elasmobranchs, and of which two species have been recorded from Scandinavian waters (Gibson & Bray, 1977; Køie, 1983), use teleosts as the second intermediate host. The smallest specimen recorded measured 780 µm in length, occurring in the lumen of the stomach of a small cod, and probably representing the body of a recently ingested cercaria (Køie, 1984).

Within the family Hemiuridae the life-cycle of *Hemiurus levinseni* still has to be elucidated. This species is very common in the Arctic-boreal Atlantic and has recently been redescribed based on material from among other sites, Greenland and northern Norway (Gibson and Bray, 1986). With the recently acquired knowledge that two other species of *Hemiurus*, in addition to closely related hemiurids, use bullomorph opisthobranch snails of the genera *Philine* and *Retusa* as the first intermediate hosts (Køie, 1990e, 1991a, 1992, 1995) it would be relevant to examine related snails for the cercaria of *H. levinseni*. A large cystophorous cercaria found in the prosobranch snail *Natica pallida* in Danish waters (Køie, 1990b) was erroneously believed to be the cercaria of *H. levinseni*.

Even though the didymozoids (Didymozoidae) are extremely numerous no didymozoid life-cycle has been elucidated. Even though they mainly have a tropical and subtropical distribution, a few species occur in boreal pelagic fishes, such as *Scomber scombrus*. *Halvorsenius exilis* Gibson *et al.*, 1981 was described from this fish from the North Sea (Gibson *et al.*, 1981). The finding of didymozoid larvae in ctenophores and small gobies from the Øresund and the Gullmar Fjord (Køie & Thulin, unpubl. obs.) indicates that snail hosts are infected with larval didymozoids in Scandinavian waters.

Further specific research on pathogenic effects of trematodes on different host categories within the Nordic environment should be encouraged.

Based on the accumulated knowledge on digenean life-cycles further studies on fish stocks and biological tagging using digenean markers should be implemented. Likewise, the accumulating results regarding interaction between pollution and parasites, including digeneans, indicate the possibility of using fish parasites as indicators of pollution.

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THIRTY YEARS OF GYRODACTYLUS RESEARCH AND FUTURE TRENDS

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Abstract

The 162 years since the description of *Gyrodactylus elegans* Nordmann, 1832 can be divided into five periods. The first three periods are shortly presented as a historical background for the next two periods, embracing the last 30 years. Frequently, during the first period new *Gyrodactylus* species were incorrectly named *G. elegans*. The second period (approx. 1860 to 1905) rendered a number of important publications on the anatomy and embryology of *Gyrodactylus*. During the third period (approx. 1905 to 1962), many new *Gyrodactylus* species were described and important faunistic investigations were performed. However, the species characters were still a problem. The fourth period (from 1962 to 1975) rendered important publications on relevant species characters and a number of insufficiently known *Gyrodactylus* species were redescribed. Research on *Gyrodactylus* during the last (present) period has been much experimentally based, e.g. in England on *G. turnbulli*, in Canada on *G. salmonis* and *G. colemanensis*, in Norway on *G. salaris*. The effect of *G. salaris* gyrodactylosis on wild Norwegian Atlantic salmon, and fear for further spread within Norway, and between the Baltic, the East-Atlantic and the West-Atlantic stocks of *Salmo salar* caused the initiation of important experimental research on *G. salaris*: taxonomy, host specificity, resistance and susceptibility, investigations on macroenvironmental influences and reproductive capacity. Future research fields on the genus *Gyrodactylus* are discussed.

Introduction

In 1832, the genus and the first species of *Gyrodactylus* were described by Alexander von Nordmann, professor of zoology in Helsinki and Odessa and a member of several scientific societies. The species, *Gyrodactylus elegans* Nordmann, 1832 was "kaum 1/9 einer Linie lang", i.e. less than bout 0.3 mm long. It was found on gills of *Abramis brama* (L.), a cyprinid fish species with a wide Eurasian distribution. No locality, however, was given. *Gyrodactylus* belongs to the class Monogenea. It is of interest to note that von Nordmann placed the gyrodactylids among the "Cestoideen". About a century later Bychowsky (1937, 1957) stated that the monogeneans are more closely related to cestodes than to digeneans.

In broad outlines, the 162 years since the first *Gyrodactylus* description is divided here into five periods. The first three periods will be shortly presented as a historical background of the following two periods, embracing approximately the last 30 years.

Gyrodactylus research prior to 1962

The period 1832 to approx. 1860

The external appearance of most *Gyrodactylus* species is rather similar. Generally, the species are differentiated by the morphology of the hard parts of the opisthaptor and have to be studied at a high magnification. Before 1860, the light microscope and microscopical technique were insufficiently developed for taxonomy. This may explain, why during the first period, most records of *Gyrodactylus* were incorrectly published as studies on *Gyrodactylus elegans*.

During this period, Siebold, v. (1849) revealed the viviparity of *Gyrodactylus* and Wagener (1860) established the remarkable positions of different developmental stages within each other, in the uterus of *Gyrodactylus*.

The period from approx. 1860 to 1905

During this period, microscopes and the preparation and section technique for microscopy were further developed, and skillful scientists published a number of important anatomical works on *Gyrodactylus*. Especially impressing is Kathariner's (1904) work on the development from egg to adult of a *Gyrodactylus* species. Furthermore, due to comparative anatomical studies, Wagener (1860) and Kathariner (1895) established differences between species, e.g. regarding anchors and ventral bars.

The increased importance of fish culture during the last part of the 19th century, resulted in a corresponding increase in the interest in fish parasites, and in both Europe and North America the knowledge about *Gyrodactylus* species as pathogens was increased. Frequently, however, the infecting species were wrongly named *G. elegans* or called *G.* sp.

The period from approx. 1905 to 1962

Faunistics and taxonomy: During this period, microscopes and microscopic techniques were further developed. Few penetrating anatomical *Gyrodactylus* papers, however, were published. The previous period, based on anatomy seems replaced by a period with an interest in faunistic work. The anchors and ventral bars were used as species characters (e.g. Bychowsky, 1933 a, b, 1936, 1948; Mueller, 1936; Alarotu, 1944). Drawings of the shape of these body parts, however, were rarely correctly presented, which makes comparisons and species identification difficult. The figures of the family Gyrodactylidae in "Synopsis of the Monogenetic Trematodes" (Sproston, 1946) demonstrates these problems.

Eurasian and North American Investigations: Several new species were described. The Leningrad school increased the knowledge about the Eurasian *Gyrodactylus* fauna (e.g. Bychowsky and

Poljansky, 1955; Gusev, 1955; Markewitsch, 1951; Shulman and Shulman-Albova, 1953; Zhukov, 1960). Thanks to Czech investigations (e.g. Ergens, 1959, 1960; Zitnan, 1960), new species of the Central European fauna were described. Hargis Jr. (1953, 1955) increased the numbers of North American species. In China, Ling Mo-en (1962) and in India, Tripathi (1957) contributed with descriptions of new species.

Works on the reproduction of *Gyrodactylus* were published. Thus Bychowsky (1957) presented results from investigations of a species on stickleback and Thurnbull (1956) described the life cycle of a species on guppy, *Poecilia reticulata* (Peters).

Early Gyrodactylus records from Scandinavia and adjacent areas: The first Scandinavian reports on *Gyrodactylus* were from four fish species in Denmark (Krøyer, 1834-1840, 1852-1853). The first report from Sweden was made by Olsson (1893) on *Gyrodactylus* specimens in gill mucus from *Phoxinus phoxinus* (L.). Nybelin (1925) mentioned (personal communication by H. Nordqvist) the finding of *Gyrodactylus* on *Cyprinus carpio* L. Later on, Nybelin found members of the genus on four other fish species in Sweden (Nybelin´s collection; see Malmberg, 1957). Neither in the Danish nor in the Swedish reports figures were included. Drawings, however, were included in the first *Gyrodactylus* species descriptions from Finland by Alarotu (1944). Her material from seven fish species was mainly captured in the Gulf of Finland at the Zoological Station, Tvärminne. At this station, *Gyrodactylus* had for decades been used for demonstrations in courses (Alarotu, 1944). An earlier report from fish in the Baltic was given by Wagener (1910) including descriptions (with drawings) of *Gyrodactylus* species from six fish species.

Although the drawings by Alarotu and Wagener are incomplete, such drawings may nevertheless be useful. Thus, on the basis of drawings and data about hosts and locations, some of their described species were again discovered and redescribed (Malmberg, 1964). The same applies to to the drawings in a much earlier description, i.e. the description by Levinsen (1881) with rather complete drawings of the anchors and the ventral bar of *G. grönlandicus* from *Myxocephalus scorpius* (L.), captured at Greenland.

Swedish Gyrodactylus research: In 1951, the investigations on *Gyrodactylus* on Swedish fishes were initiated with field investigations at Aneboda fiskeriförsöksstation (Malmberg, 1957). There the late Professor Orvar Nybelin, the first Swedish specialist on monogeneans, performed a large part of his *Dactylogyrus* investigations. The base for the Swedish *Gyrodactylus* investigations has since constantly been the Department of Zoology, Stockholm University.

In 1950 and the spring 1951, the above *Gyrodactylus* investigations were preceded by studies of an African gyrodactylid, *Macrogyrodactylus polypteri* Malmberg, 1956. Mainly phase contrast microscopy on live worms was performed. The studies aroused great interest in the shape of protonephridial system of the Monogenea. In an attempt to study the nervous system of *M. polypteri*, staining *in vivo* with methylene blue and fixation by means of ammonium picrate-glycerin was tried. The experiences from these studies of *M. polypteri* were important to the following monogenean studies. Furthermore, it was favourable to start with a worm about five times longer than most *Gyrodactylus* species (Malmberg, 1956).

The *Gyrodactylus* studies in Aneboda were followed by comparative field studies in Sweden at several localities, covering fresh, brackish or salt water (Malmberg, 1957). In 1958, fish from brackish water in Finland (Tvärminne), brackish and freshwater in North-Western Germany (Kiel) and salt water in Northern Norway (Tromsö) were investigated (Malmberg, 1970). Phase contrast microscopy was used for studies *in vivo* and for anatomical studies on fixed specimens. The fixation used, was ammonium picrate-glycerin (see below).

The comparative phase contrast studies of *Gyrodactylus* revealed not only a large number of new species with different protonephridial systems but also the presence of several incomplete species descriptions in the literature. For approaching the species discrimination problem, a comparative study was performed, using specimens collected and fixed in formalin and then stained in eosin. In this way, the ventral bar, especially the shape of the ventral bar membrane was found to be an important species character. However, specimens used for *in vivo* studies of the protonephridial systems were found to be superior to the eosine stained specimens. At the end of the analysis, such a specimen was fixed between a slide and cover glass by adding a drop of saturated ammonium picrate-glycerin to the edge of the cover glass. After about a week, the specimen could be used for comparative opisthaptoral studies under a phase contrast microscope.

On a basis of this "ammonium picrate-glycerine method" (Malmberg, 1957; 1970) 15 species and five subspecies of *Gyrodactylus* were described (Malmberg, 1957). The presentation of comparative drawings of e.g. the protonephridial systems, anchors, ventral and dorsal bars and marginal hooks extended the numbers of taxonomical characters. Many of the drawings appeared later in two works on the classification of helminths (Gusev *et al.*, 1962; Yamaguti, 1963). However, the very species specific shape of the marginal hook sickle was not yet fully estimated (Malmberg, 1957).

The last thirth years of *Gyrodactylus* research

The period between 1962 and 1975

Symposium in Prague: In 1962, eleven years after the initiation of the above Swedish *Gyrodactylus* investigations, an important symposium was held in Prague: "Parasitic worms and aquatic conditions". Several scientist working on the Monogenea attended the symposium, including Professor B.E. Bychowsky, and many members of his school in Leningrad (Saint Petersburg), and Professor Jack Llewelly, Birmingham, England. Bychowsky was the most important scientist on the Monogenea of his time. He introduced larvae and larval development into the discussion on evolutionary relationships within the Monogenea. He also found the Monogenea to be more closely related to the Cestoda than to Ddigenea/Trematoda (see Bychowsky, 1937, 1957). Headed by Llewellyn, there was an English school working on form and function, larval development and evolution, mainly using saltwater monogeneans. One of the organizers of the symposium, Dr. Radim Ergens, today one of the most important taxonomists on the genus *Gyrodactylus*, was working experimentally with monogeneans, at the Parasitological Institute in Prague.

At the symposium, the basic results of the above comparative studies on *Gyrodactylus* were presented. Thus different types of protonephridial systems, pharynx and cirrus, ventral and dorsal bars and marginal hooks were presented, as well as results regarding seasonal variations, host and organ specificity, and macro- and microenvironmental relations. Attempts to reidentify incompletely described species were also presented. For a further development of the taxonomy and systematics of the Monogenea, Bychowsky recommended this type of studies (Malmberg, 1964; Discussion).

The Prague Symposium significantly increased the exchange of ideas and material between parasitologists, including "The Monogeneans" (i.e. scientist working on monogeneans). Although the Symposium was held in 1962, the proceedings of the meeting were not published until 1964 - thirty years ago.

A monograph on Gyrodactylus: The Swedish investigations presented in Prague were increased and later on published as: "The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea)" (Malmberg, 1970). In this work, 29 species were described on material from freshwater (mainly Sweden), brackish water (the Baltic: Sweden, Finland, North-Western Germany), and from salt water (Sweden, Northern Norway). The most important species characters, i.e. the shape of marginal hooks, anchors and ventral bars were described and intraspecific and seasonal variations of these characters were presented. On a basis of the type of protonephridial systems and the opisthaptoral hard parts, the genus was divided into six subgenera and a number of species-groups. Results from field experiments were also included. Finally, all earlier described *Gyrodactylus* species were tentatively organized into subgenera and species groups (Malmberg, 1970; Table 40). The list of references in this monograph, called "the *Gyrodactylus* bible", covered the majority of publications on *Gyrodactylus* from 1832 to May 1969.

Other Gyrodactylus research: During the sixties many new *Gyrodactylus* species were described. The knowledge about the Central-European fauna was enlarged: Ergens and Zitnan (Ergens, 1985) described several new species, and in East-Germany, Gläser (1969) initiated his *Gyrodactylus* studies. In USA, Kritsky, Mizelle, Rogers and Wellborn (see Mizelle and Kritsky, 1967) contributed with new North-American species, Paperna (1964, 1968) increased our knowledge on *Gyrodactylus* in Israel and Africa, and Hargis Jr. and Dillon (1968) described species from the Antarctic. Hargis *et al.* (1969) presented a bibliography of the monogenean literature of the world.

Ergens (1965a, b) also presented important analyses of the development and the variation of hard parts in the haptor of two *Gyrodactylus* species. He likewise made significant attempts to redescribe a number of incompletely described species, in many cases using type specimens from the collection of monogeneans at the Zoological Institute in Leningrad/Saint Petersburg (e.g. Ergens, 1969, 1975).

Important results from work on the reproduction biology (Braun, 1966) and reproduction and mortality (Lester and Adams, 1974a) of different *Gyrodactylus* species, and effects of a *Gyrodactylus* species on the epidermis of *Gasterosteus aculeatus* L. (see Lester, 1972; Lester and Adams, 1974b) were published.

At the end of the sixties, scanning (SEM) and transmission (TEM) electron microscopical investigations of different body parts of *Gyrodactylus* opened a new field of anatomical investigations (e.g. Lyons, 1969a, b, 1970; Kritsky, 1971, 1976).

The period since 1975

Gyrodactylus salaris and the Norwegian Atlantic salmon: In 1975, it was found that the parr of salmon, *Salmo salar* L., in a northern Norwegian river was mass infected by a *Gyrodactylus* species. During the period 1975-1979, *Gyrodactylus* infection was established in an increasing number of Norwegian rivers and hatcheries, causing heavy mortality (Johnsen and Jensen, 1991). Thus a fish species of great economic value for the natural fishery, aquaculture, as well as a valuable sportfish industry - in natural waters - had become affected by gyrodactylosis. The salmon reproduction in the infected rivers was severely influenced, and within a few years following introduction of the parasite, the parr population of a river was drastically diminished. The event resulted in unique scientific and economic measures directed at increasing the knowledge about a *Gyrodactylus* species.

The parasite was first observed in a central salmonid farm on the west coast and then in a river (Johnsen, 1978). Until know, it is reported from a total of 38 Norwegian rivers and 37 fish farms, comprising 11 salmon hatcheries along the western and north-western coast and 26 inland rainbow trout farms in the south-eastern part of the country (Mo, 1994).

Generally, within about two years after introduction of the parasite, the native salmon parr population in an infected river was drastically diminished (e.g. Mo, 1994). In two infected rivers, however, the percentage salmon parr surviving, seemed to be higher than in other affected rivers (Mo, 1992; Jansen and Bakke, 1993a, b).

In 1980, a *Gyrodactylus* committee was established in Norway with the main objective to initiate a survey on the distribution of the parasite in Norway, and research on the taxonomy and biology of the species. The base for these investigations has been the Zoologisk Museum, University of Oslo .

Taxonomic research by Tanum (1983) revealed the parasite to be *G. salaris* Malmberg, 1957. Further taxonomic work, e.g. regarding the shape and seasonal variation of the species characters in the opisthaptor were performed by Mo (1991a, b) and by Mo and Appleby (1990).

Experimental work on the biology of the parasite has been an important part of the Norwegian research program. Thus the host specificity of *G. salaris* towards different salmonids (e.g. Bakke, 1991; Bakke *et al.* 1990, 1991b; 1992a, b; Bakke and MacKenzie, 1993), and towards certain non-salmonids (e.g. Bakke and Sharp, 1990; Bakke *et al.*, 1991a) were examined. Further, the site specificity of *G. salaris* on salmon parr (Jensen and Johnsen, 1992; Mo, 1992), as well as its effect on the fish epidermis (Sterud, 1992; Malmberg, 1993) were studied. The temperature was found to be and important factor, controlling the reproduction rate of the parasite (Jansen and Bakke, 1993a, b). Furthermore, the dispersal strategy (Bakke *et al.*, 1992b), and the population age structure of the species (Harris *et. al.*, 1994) were analysed, and its epidemiology described (e.g. Johnsen and Jensen, 1992).

Most likely, *G. salaris* was introduced several times to Norway from fish farms within the Baltic Area, via imports of salmon (parr and smolt) and rainbow trout *Oncorhynchus mykiss* (Walbaum), at first in the early 1970´s (Malmberg, 1989) and later on, during the first half of the 1980´s (Mo, 1994). Apparently, salmon culture and restocking of rivers played an important part for the dispersal of the parasite within Norway: restocking of several rivers with parr from an infected, central farm correlated to a following outbreak of gyrodactylosis in the same rivers (Johnsen and Jensen, 1986, 1991; Malmberg, 1989; Mo, 1994). Halvorsen and Hartvigsen (1989) presented critical views on such introductions and spreading of the *G. salaris* in Norway.

On the basis of an observation in a Swedish salmonid farm, Malmberg (1989) supposed the Baltic salmon to be more resistant than the Norwegian Atlantic salmon against *G. salaris* gyrodactylosis. Experimental studies on their resistance by Bakke *et al.* (1990) proved that salmon parr specimens of Baltic stock had a better ability than parr specimens of Atlantic stock, to resist a *G. salaris* infection. Other comparative studies, however, gave no direct support to the presence of resistance differences between the two stocks (Bakke *et al.*, 1992b).

For preventing further spreading of *G. salaris*, efforts were taken to eradicate the parasite from infected Norwegian fish farms and rivers (Mo, 1994). So far, *G.*

salaris has been eliminated from 34 of the 37 infected farms, mainly by destocking, draining and disinfecting the fish containers. 21 of the 38 infected rivers have been treated against the parasite, mainly by killing all fish with rotenone and restocking with uninfected salmon. At present, 10 of the treated rivers are declared free from *G. salaris* and another 10 are hopefully re-establishing a salmon population. In only one of the 21 rivers have the eradication measures failed (Mo, 1994).

Gyrodactylids on salmonids: The possible import of *G. salaris* from the Baltic area to Norway caused a special interest in an increased knowledge about the *G. salaris* situation in Sweden and Finland. Thus in 1988, a project was initiated, dealing with gyrodactylids on salmonids in Swedish natural waters and fish farms (Malmberg and Malmberg, 1993). In Finland, fish farms with salmon and/or rainbow trout were monitored (Aalto and Rahkonen, 1994). Hitherto, these investigations indicate, that Swedish and Finnish stocks of the Baltic group of *Salmo salar* are relatively resistant and tolerant to the *G. salaris* gyrodactylosis (cf. Bakke *et al*., 1990)

The fear for further spreading of *G. salaris* caused an increased interest in this and other species of *Gyrodactylus* also in other countries, e.g. Russia, Germany, Czechoslovakia, UK, Canada and USA. At the "Second International Symposium on Monogenea, Montpellier, France, 1993, two reviews on gyrodactylids were presented, one on *Gyrodactylus* species and gyrodactylosis on salmonids (Malmberg, 1993) and another (Harris, 1993) on the reproduction biology in the Gyrodactylidae

Additional research on gyrodactylids since 1975: The knowledge about the occurrence of *Gyrodactylus* in different areas of the world was considerably increased. A new edition of the "Key to parasites of freshwater fish of the USSR" was published. Part 2 includes good descriptions of several *Gyrodactylus* species (Ergens, 1985). The Canadian *Gyrodactylus* fauna was presented by Beverley-Burton (1984). Investigations of the South-American monogenean fauna revealed many new species, e.g. the presence of oviparous gyrodactylids (Harris, 1983; Kritsky and Boeger, 1991; Boeger *et al*.,1994). The gyrodactylid genus *Isancistrum* de Beauchamp, 1912 was rediscovered and a new species described from squids (Llewellyn, 1984). Harris and Tinsley (1987) increased the knowledge about *Gyrdicotylus* Vercammen-Grandjean, 1960, parasitizing the African clawed toad.

In England, M.E. Scott and her coworkers made several penetrating studies on the epidemiology of a *Gyrodactylus* species on guppy, covering different aspects, e.g. changes in the aggregation of the parasite species (Scott, 1987), the reproduction potential (Scott, 1982), the temperature dependent reproduction (Scott and Nokes, 1984), the population dynamics and experimental epidemiology (Scott and Anderson, 1984; Scott, 1985), the duration of refractoriness (Scott and Robinson, 1984), and the importance of fish genetics (Madhavi and Anderson, 1985). Other important publications on reproduction, population dynamics and biology in *Gyrodactylus* also appeared (e.g. Harris, 1989; Harris *et al.*, 1994; Gelnar, 1987, 1991). A comprehensive review was given by Harris (1993).

The growth in fish culture increased the need for better disinfectant methods against monogeneans, including *Gyrodactylus*. Different chemicals (Thoney and Hargis Jr, 1991), e.g. formalin were employed as an effective treatment in fish farms. Prolonged use of formalin, however, is not recommended. During the last 20 years, several new, more suitable, effective treatments have been introduced (Schmahl, 1993). Treatment of *Gyrodactylus* infected fish in natural waters require, however, other methods. In Norway, treatment of salmonid rivers with rotenone have been practised (Johnsen and Jensen, 1991; Mo, 1994).

Results from several new SEM and TEM studies appeared. Thus new methods for SEM studies of the opisthaptoral hard parts (Mo and Appleby, 1990; Shinn *et al.*, 1993) and effects of the opisthaptor (Cone and Odense, 1984; Cone and Cusack, 1988) and the pharynx (Malmberg, 1993) on the host skin were published. TEM studies of the ultrastructure of sperms, tegument, cephalic glands, protonephridia and intestine in certain gyrodactylids were performed (e.g. Kritsky, 1976, 1978; Kritsky and Krudenier, 1976; Kritsky *et al.*, 1994; Justine *et al.*, 1985; Rohde, 1989; Malmberg, 1993; Malmberg and Lilliemarck, 1993). Justine (1993) published a list of ultrastructural investigations on monogenean species, including gyrodactylids.

The increased knowledge about *Gyrodactylus* and other gyrodactylid genera resulted in new discussions on the interrelationship within the family Gyrodactylidae and its position within the Monogenea. On the basis of chaetotaxi, Lambert (1980) gave the Gyrodactylidae a separate position within the Polyopisthocotylea, while on the basis of spermatology, Justine *et al.* (1985) included the family in the Monopisthocotylea. On the basis of the development of the haptor/ opist-haptor and its hard parts, the development of the protonephridial systems, the type of spermatozoa, and the type of marginal hooks (hinged or unhinged) within the Monogenea, Malmberg (1990) discussed the evolution of the Monogenea and included e.g. the Gyrodactylidae, the Enoplocotylidae, the Anoplodiscidae, the Acanthocotylidae, the Tetraonchoididae and the Bothitrematidae in a new subclass, the Articulonchoinea (hinged marginal hooks). Boeger and Kritsky (1993) using phylogenetic systematics enclosed the Gyrodactylidae, the Anoplodiscidae, the Tetraonchoididea and the Bothitrematidae in the Gyrodactylidea.

Future trends

Our knowledge about the world distribution of *Gyrodactylus* is still rather limited and has to be increased. At present, most studies deal with species of the European fauna. Hopefully, the very interesting records from Africa and South-America will inspire further investigations within these two areas and other little investigated areas, such as Australia, New Zealand and East-Asia.

The rapid development of computer techniques might result in an increased use of image analysis in taxonomic studies. This will considerably increase the capacity to measure and reproduce the precise shape of the taxonomically important opisthaptoral hard parts. Furthermore, the statistical treatment of different data and the printing out of images and different kinds of diagrams will be more efficient.

The use of scanning and transmission electron microscopy for comparative anatomical, cytotaxonomical and spermatological investigations will certainly result in new discoveries, which in turn will improve evolutionary discussions and the understanding of speciation within the Gyrodactylidae and other monogeneans.

Aquaculture of salt water fish species, an increasing industry, will continue to cause problems with ectoparasite as *Gyrodactylus*. This will certainly inspire to further investigations of the marine species of *Gyrodactylus*, and treatments against pathogenetic species. The influence of *G. salaris* on wild stocks of the Atlantic group of salmon might continue to provoke further field and laboratory investigations regarding fish resistance to ectoparasite and the process of epidemiology. The spread of *G. salaris* to Norway and its consequences shows the risk of spreading pathogens to new areas and might inspire better disinfection routines in connection with fish transportation.

There is an urgent need for an increased knowledge about the ecology of *Gyrodactylus* species, e.g. host specificity, temporary hosts, transmission strategies, seasonal variations, reproductive capacity and strategies, and population dynamics. Studies of processes behind the unique gyrodactylid viviparity may improve our knowledge about speciation and evolutionary processes and reveal opportunities to influence the reproduction of pathogenetic species. Let us hope that *G. salaris* sets the lesson, so responsible authorities will contribute to the realisation of the necessary basic research and scientific programs.

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PARASITES OF FISH OF ECONOMIC INTEREST IN NORHTERN EUROPE, A LITERATURE ANALYSIS

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Introduction

The early investigations of fish parasites in European fresh-, brackish- and marine- waters date back several centuries. In other words, fish parasitological research has a history analogous to that of the parasitological science as a whole reaching back to the first naturalists. Thus, when analysing literature relating to parasites of fish of economic value in Northern Europe, one has to review the situation for as long as the last three centuries. A bibliography which has recently been compiled by the present authors (Pugachev & Fagerholm, in press), provides the basis of the analysis presented in this review.

Bibliography

A bibliography on fish parasites and parasitic disease of fish of N. Europe, was compiled (Pugachev & Fagerholm, in press) as it was considered valuable to produce a bibliography by which the Russian literature can be made more easily accessible, as titles are translated into English. Also titles of publications published in Nordic languages have been translated. It was feasible to produce such a work now because of the efficient means available to deal with bibliographic information which is provided by present day computers and software.

Although the bibliography, upon which the results are based is not claimed to be complete (some 2000 references included), the information given describe the trends in the scientific activities in this field. Published papers up to the early 1990s were included.

Database

One aim of this bibliography was to use the literature which was analysed as a basis for a database on parasites of fish in N Europe. This work is well advanced. In this virtual data-base, a full in-text indexing will be possible. In the work special emphasis was put on the early literature which today, sofar, has not been systematically introduced to any mainframe computers, while current studies efficiently can be retrieved from on line databases.

The criteria used for introducing references into the bibliography, and the database, were that the papers should include parasite- and parasitic disease data of fish from N Europe (Region from

Ural in the east to Greenland in the west, and from the Baltic Sea in the south to the Arctic Ocean in the north). Although, in the Atlantic, information from areas south of the Faroes is generally not included, in order to retain important data on taxonomy, distribution and biology of the parasites, numerous exceptions from this rule was made. In addition numerous old general studies on fish parasites were included.

Results, including some general comments

The number of publications increased constantly from 1700 up to 1940 with a distinct increase in 1850-1900. The effects of the Second World War resulted in a decrease in the production comparable to output of the period 1800-1850.

The number published papers increased rapidly in 1950-1980 but even more so in 1970-1980. During last decade (1980-

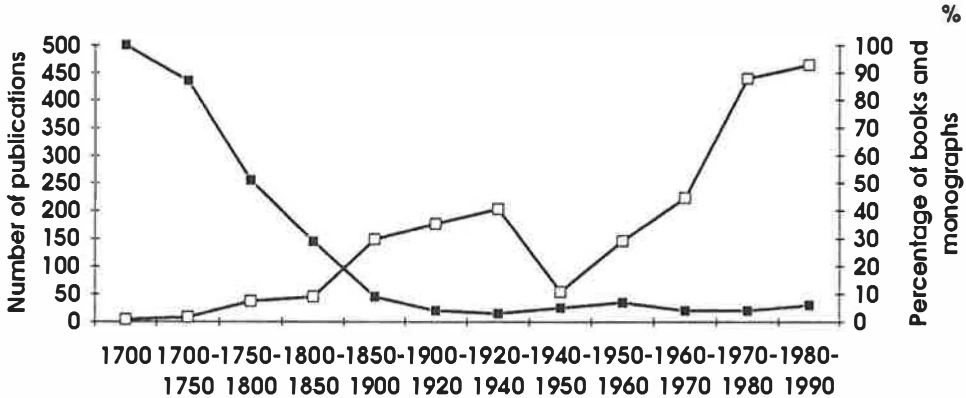

Fig 1. Number of published paper dealing with parasites of fish in N. Europe (open squares), and the proportion (%) of monographs or books in relation to published papers. (closed squares) during the periods shown.

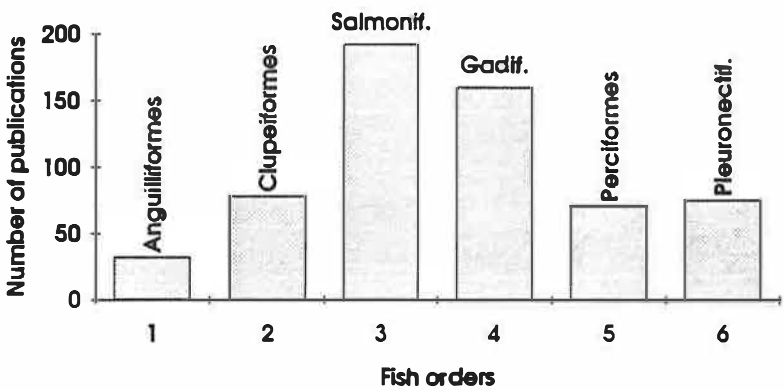

Fig. 2. The number of publications on parasites of certain orders of fish in literature from N. Europe.

1990) the number of papers published was similar to that of the previous one (Fig. 1).

Based upon the literature which was analysed it can be observed that fishes of the orders Salmoniformes and Gadiformes were the most extensively studied while the order Clupeiformes and the large order Perciformes did not attract the same attention (Fig. 2). There are some fish genera even among Gadiformes and Pleuronectiformes which still have not been studied or have attained only little any attention by researchers (e.g. *Gadiculus, Brosme, Echiodon, Lycodes, Microstomus*).

The Cestoda, Trematoda, Monogenea and the parasitic Crustacea were extensively studied, while Nematoda was less so (Fig. 3). Myxosporidia was the leading

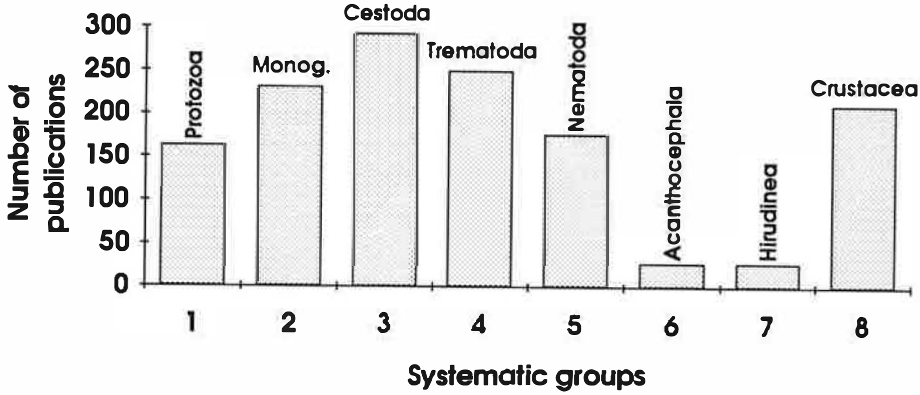

Fig. 3. Number of papers dealing with certain parasite groups in studies on fish parasites from N. Europe.

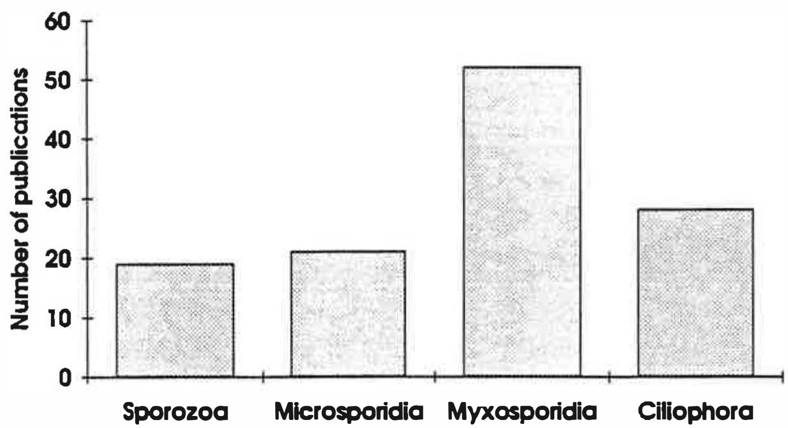

Fig. 4. Number of papers published on certain sporozoan parasites of fish in N Europe.

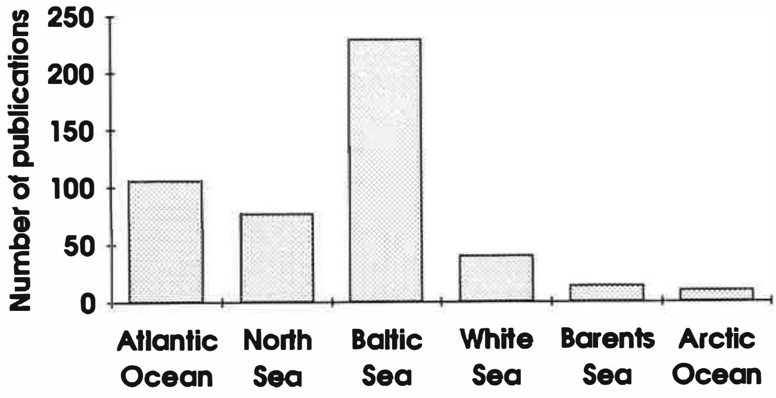

Fig. 5. Number of papers on parasites of fish from certain N European water regions.

group among the Protozoa (Fig. 4). It can be noted that in the case of parasitic protozoans, including Myxosporidia, relatively few studies have been done in the Scandinavian region.

A prominent portion of the publications has dealt with the parasite fauna of Baltic Sea fishes while the number of studies from the White Sea, the Barents Sea and the Arctic Ocean is low (Fig. 5). Freshwater fish parasites were extensively studied in Russia, Norway and Finland, while in Denmark (hardly any studies, not included in figure) and Sweden more attention was paid to the study of marine fish parasites (Fig. 6).

In spite of the fact that especially during the last three decades, a rapidly increasing number of papers have been published there still remains work to be done with regard to the geographical distribution of parasites, the parasite fauna of certain specific hosts and the structure of parasite communities. The

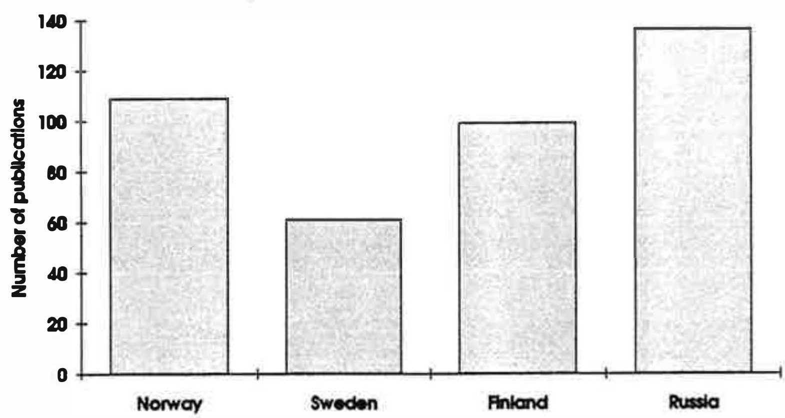

Fig. 6. Papers on freshwater parasites of fish from certain areas in N Europe.

lack of regular monitoring programs of fish parasites even in the case of commercially valuable fishes is also striking.

Studies relating to taxonomy, morphology and life cycles have been dominating (Fig. 7). However, recently the number of studies dealing with other subjects, such as parasite ecology, problems related to fish disease, pollution studies and genetics have been increasing.

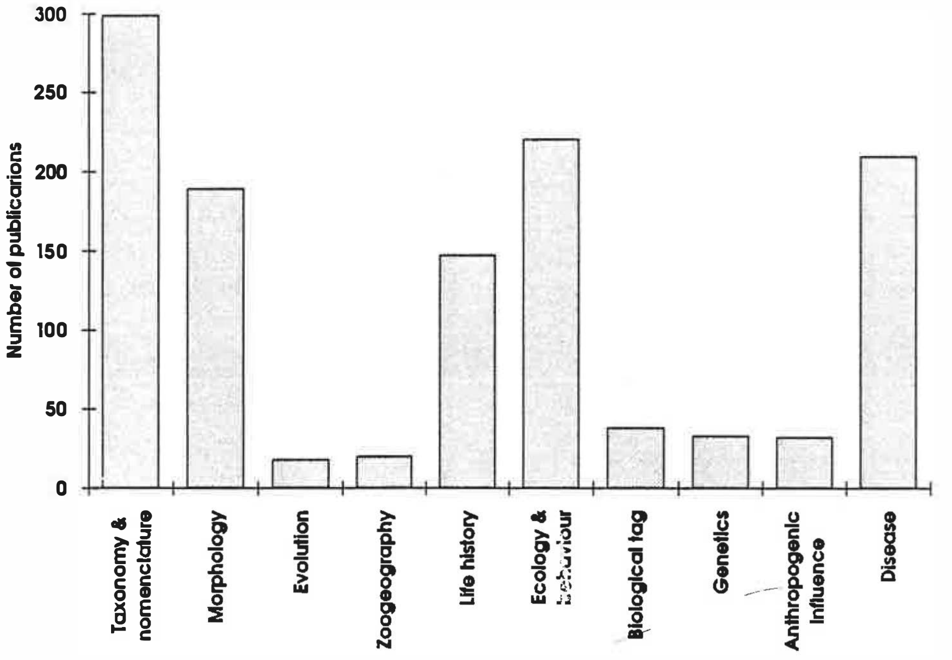

Fig. 7. Different subject areas dealth with in studies on parasites of fish in N Europe

As was shown (Fig. 1), the relative number of books or monographs published during the periods reviewed has decreased and has remained at the same low level as it was already at the start of the century. It appears that during the last few decades we have produced primary data but we still have to sum up the results obtained and, when possible, draw some general conclusions.

According to the present analysis it is possible to predict that there will be a decrease in the number of publications during next decade. Furthermore, it seems reasonable to anticipate a further substantial increase in the use of computer technology in the dissemination of scientific information.

References

Pugachev O, Fagerholm H-P. A bibliography and an index list on parasites and parasitic disease of fish in Northern Europe. Åbo: Åbo Akademi Press (In press)

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LARVAL ANISAKINE NEMATODES AS BIOLOGICAL INDICATORS OF COD (*GADUS MORHUA*) POPULATIONS IN THE SOUTHERN GULF OF ST. LAWRENCE AND ON THE BRETON SHELF, CANADA

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Abstract

Atlantic cod (*Gadus morhua*) exploited in the southern Gulf of St. Lawrence, Canada, during spring, summer and fall, belong to distinct southwestern and southeastern Gulf populations. In late fall, both populations migrate to the southwestern slope of the Laurentian Channel and then through the Cabot Strait and into the North Atlantic. They overwinter along the edge of the Breton Shelf where they mix with resident cod. From a management standpoint, techniques for discriminating between migrant Gulf and resident cod, and thereby determining their relative rates of exploitation in the Breton Shelf winter fishery, have become increasingly important with recent declines in cod stocks. Present results show that larval anisakine nematodes, *Pseudoterranova decipiens*, *Anisakis simplex* and *Contracaecum osculatum*, surveyed in 4,360 cod from the southern Gulf of St. Lawrence and the Breton Shelf, were suitable as biological indicators for host stock discrimination. Multivariate analyses of infection levels revealed that southwestern Gulf, southeastern Gulf and resident Breton Shelf cod differed significantly in regard to levels of one or more anisakine species. Analyses of anisakine levels also indicated that cod sampled from the Cabot Strait in late fall were migrants from the southwestern Gulf, while those collected from the edge of the Breton Shelf in mid-winter were resident cod rather than overwintering migrants from the southern Gulf.

Introduction

Biological indicators, such as vertebral counts (McKenzie and Smith 1955) and length-at-age profiles (Lambert 1993), and results of artificial tagging experiments (Martin and Jean 1964; Kohler 1975; Lambert 1993) suggest that there are two populations of Atlantic cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. During late spring, summer and fall, one population occupies the southwestern Gulf off the Gaspé Peninsula, northern New Brunswick and Prince Edward Island while the second population is confined to the southeastern Gulf, off the eastern end of Prince Edward Island, the northern Nova Scotian mainland, and the west coast of Cape Breton Island (Fig. 1).

In late fall, cod from the southwestern and southeastern Gulf migrate to the southern slope of the Laurentian Channel and thence, eastward, through the Cabot Strait (Lambert 1993). Gulf cod ultimately overwinter in Laurentian Channel slope-waters to the east of Cape Breton Island, along the edge of the Breton Shelf. Here, they apparently mix with the resident Breton Shelf cod. Given the depleted state of southern Gulf and, especially, local Breton Shelf cod stocks in recent years (Sinclair 1993), their ability to discriminate between migrant Gulf and resident cod, and thereby determine the relative rates of exploitation in the Breton Shelf winter fishery, has become increasingly important from a management standpoint.

Parasites have been widely used as natural indicators providing information on movements and discreteness of fish host stocks for fisheries management (Williams *et al.* 1992). Suitable candidates for biological tags include larval anisakine nematodes such as "sealworm", *Pseudoterranova decipiens*, and "herring-" or "whaleworm", *Anisakis simplex*, which, as a consequence of their economic and medical importance, have frequently been surveyed in North Atlantic fisheries (Scott and Martin 1957; Templeman *et al.* 1957; Young 1972; Platt 1975; McClelland *et al.* 1983a; 1983b; 1985; 1987; 1990; Brattey *et al.* 1990; Brattey and Bishop 1992; Jensen *et al.* 1994). As is apparent from survey data, levels of larval anisakine infection vary dramatically over the geographic range and in different populations of a given host species, thereby satisfying the most important criterion for selection of a biological tag (Williams *et al.* 1992). Platt (1976), for example, advocates use of larval sealworm levels in distinguishing lightly infected migrant cod from Greenland from the more heavily infected resident cod in Icelandic fisheries. An inventory of Norwegian cod (Hemmingsen *et al.* 1991) reveals that prevalences (percentages of host samples or populations infected with a given parasite) of larval sealworm and larvae of a second anisakine, *Phocascaris* sp., could be used to separate Balsfjord and Ullsfjord cod, respectively, from Barents Sea cod. Chenoweth *et al.* (1986) similarly demonstrated that *A. simplex* larvae showed the greatest potential as biological indicators in discriminating southern Nova Scotian from New England herring stocks. The most successful application of larval anisakine levels in fish stock discrimination, however, is Arthur and Albert's (1993) analyses of prevalences and abundances (mean counts of a parasite species in a host sample or population) of *P. decipiens* and *A. simplex* larvae, and larval nematodes belonging to the anisakine tribe, Contracaecinea, in Greenland halibut (*Reinhardtius hippoglossoides*). In the latter study, geographic origins of individual fish from the St. Lawrence estuary, Gulf of St. Lawrence, and Labrador are identified through discriminant function analysis of infection levels of the three larval anisakines, a larval digenean, *Otodistomum* sp., and a juvenile acanthocephalan, *Corynosoma strumosum*.

Between February 1980 and November 1981, cod from the southern Gulf of St. Lawrence and the Breton Shelf were surveyed for *P. decipiens*, *Anisakis* sp. and *Contracaecum* sp. larvae (McClelland *et al.* 1983a). The latter two species of larval anisakines were subsequently identified as *A. simplex* and *C. osculatum* through structural comparisons with preserved nematodes of known identity, and by cultivation to an identifiable stage *in vitro* (McClelland *et al.* 1985). Independent ANOVAs of abundances of each nematode species (McClelland *et al.* 1983a), indicated that *A. simplex* and *C. osculatum* were significantly more numerous in cod from the southern Gulf than they were in Breton Shelf cod and, hence, that these two nematode species might be useful as natural tags for migrant southern Gulf of St. Lawrence cod overwintering on the Breton Shelf.

The main objective of the 1980-81 survey, however, was merely to document infection levels of larval sealworm and related nematode species in southern Gulf of St. Lawrence and Breton Shelf cod, not to seek out biological tags for host populations. In the present study, we investigate the suitability of larval anisakines as natural tags for southern Gulf and Breton Shelf cod populations, according to the various criteria outlined by Williams *et al.* (1992) and, especially, with regard to disparities in levels of infection in different host populations. To this end, multivariate procedures were employed in reanalysing variations in, not only abundances, but also prevalences of larval anisakines recorded in southern Gulf of St. Lawrence and Breton Shelf cod in the 1980-81 survey.

Materials and Methods

Samples of round cod (*Gadus morhua*) were collected from commercial draggers and longliners in the southern Gulf of St. Lawrence and Breton Shelf fisheries (Fig. 1, Table 1). Cod were selected according to an orthogonal sampling design in which they were stratified by total length (TL) into 10 categories (≤30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65, 66-70, ≥71 cm TL) containing uniform numbers of fish. It was difficult, however, to fill all length categories of some samples. Cod ≤45 cm TL, for example, were seldom caught by longliners while large market and steak cod (≥66 cm TL) were scarce in the catches of small inshore draggers.

Samples were transferred to the DFO, Halifax Fisheries Research Laboratory, where they were either stored on ice for fresh examination, or frozen at -17 °C and examined at a later date. Total lengths of each fish were measured to the nearest cm and weights, to the nearest 0.1 kg. The fish were then sexed, eviscerated and filleted. The viscera were scanned with unaided eye and nematodes detected therein were tentatively identified and counted. Nematodes from the body cavity were placed in labeled vials of 0.9% saline and their identities were subsequently verified by microscopic examination. Fillets and napes (hypaxial

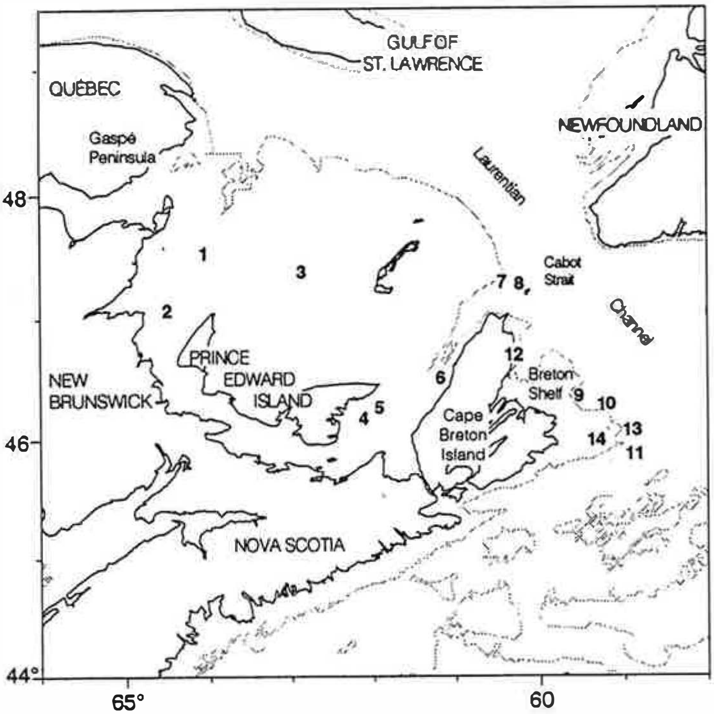

Fig. 1. Sampling locations for the 1980-81 survey for larval anisakines in cod (*Gadus morhua*) from the southern Gulf of St. Lawrence and Breton Shelf.

musculature surrounding the body cavity), as well as flesh which remained on the frames, were inspected for larval nematodes by slicing the flesh (Power 1961). Nematodes in the flesh clearly identifiable as larval sealworm (*Pseudoterranova decipiens*) were counted *in situ* while those of uncertain identity were examined microscopically before being enumerated.

An additional sample of 164 cod in the 50-60 cm TL range was collected from Scatari Bank on the Breton Shelf on October 23, 1981. This sample was used to investigate the possibility of post mortem migration of nematodes from the viscera to the flesh of round cod stored on ice for prolonged periods prior to examination, and to assess the efficiency of our routine procedure for detecting nematodes in the flesh. A random subsample of 56 of the cod were eviscerated at sea and the viscera and body of each fish were stored on ice in separate polybags labeled with the specimen number. The remaining cod were stored in the round, on ice, until examined four days later. Each of the 164 cod sampled were measured, weighed, sexed and examined for nematodes following procedures above but, in this instance, all nematodes detected were removed from host tissues. Flesh from the 56 cod which had been gutted at sea and from 57 of those stored round for four days on ice were then digested in a solution consisting of 5 g of 1:10,000 pepsin per litre of 1% hydrochloric acid. Fillets of individual cod were placed in four beakers with two L of solution and napes in one L beakers with 0.5 L of solution. After 2-3 h of incubation at 35 to 40° C and frequent stirring, the contents of each beaker were strained through a series of sieves with mesh size ranging from 5 to 0.3 mm. Nematodes recovered from the sieves were identified by microscopical examination.

Quantitative terms such as prevalence (P), number of infected fish expressed as a percentage of the number of fish sampled, and abundance (A), mean nematode count for all fish sampled (including uninfected specimens), are defined according to Margolis *et al.* (1982). Prevalence and abundance of the larval nematodes *P. decipiens, Anisakis simplex* and *Contracaecum osculatum* were analysed as dependent variables in two-way (cod length x cod sample) MANOVAs (SPSS-X). For analysis of prevalence, individual cod were encoded in binary form, infected fish being assigned the value "1" and uninfected fish, "0" (Neter *et al.* 1985). To permit analysis of nematode abundance, frequency distributions of worm counts, positively skewed to varying degrees, were brought closer to normality by a $\log_{10}(n+1)$ transformation (Platt 1975). Empty cells were eliminated by reducing the number of cod length strata from 10 (above) to seven, all fish ≤45 cm in length being included in a single length stratum. The sample from Cheticamp, Nova Scotia (no. 6, Table 1) lacked fish ≥61 cm in length and, hence, was omitted from analyses.

Individual and pooled cod samples were compared by fixed factor design, *a priori* contrasts based on MANOVAs for nematode prevalence and abundance. Samples were pooled only when there was a possibility that they had been drawn from the same population and when preliminary contrasts revealed that they had similar levels of nematode infection.

Results

Larval nematodes belonging to three anisakine species, *Pseudoterranova decipiens, Anisakis simplex* and *Contracaecum osculatum,* were identified and counted from 14 samples of cod (a total of 4,360 fish) from the southern Gulf of St. Lawrence, Cabot Strait and Breton Shelf. As larval anisakine levels invariably increased with host length (Figs 2-5), cod samples were partitioned into 10 cm length groups for summary presentation of nematode prevalences and abundances in Table 1.

Pseudoterranova decipiens

Larval sealworm levels were greatest in cod collected from the southeastern Gulf of St. Lawrence near Souris, Prince Edward Island in late April and early May 1981 and from Frenchman's Shoal on the Breton Shelf in late July '81 (Table 1, Fig. 2). The lowest *P. decipiens* levels occurred in cod taken along the southern edge of the Laurentian channel, in the Cabot Strait in late November and early December 1980, and in cod sampled from southern Breton Shelf (Louisbourg) off

Table 1. Prevalences and abundances of larval anisakines in cod (*Gadus morhua*) from the southern Gulf of St. Lawrence and Breton Shelf.

Host				Larval anisakines detected					
				P. decipiens		*A. simplex*		*C. osculatum*	
Sample no.[1]	Location and date	Length range (cm)	n	prev.	abun.	prev.	abun.	prev.	abun.
1	Shediac Valley,	≤40	22	27	0.41	41	0.50	5	0.05
	September 20, 1980	41-50	52	44	0.62	77	1.63	15	0.25
		51-60	37	51	1.41	62	1.16	33	0.95
		61-70	14	79	2.71	64	1.71	57	3.14
		≥71	10	100	10.50	90	8.00	50	4.20
2	Pt. Escuminac,	≤30	46	26	0.26	11	0.15	0	0
	New Brunswick,	31-40	72	38	0.49	28	0.38	1	0.01
	June 17, 1981	41-50	92	54	1.12	49	0.98	1	0.02
		51-60	74	68	1.77	76	2.09	28	0.91
		≥61	23	100	5.09	96	6.09	70	5.26
3	Bradelle Bank,	≤50	64	39	0.73	44	0.70	0	0
	November 6,1981	51-60	63	65	1.11	76	2.63	17	0.37
		61-70	53	72	2.15	81	3.91	36	1.51
		≥71	29	97	17.31	86	5.52	62	3.69
4	Souris,	≤40	20	30	0.45	60	1.20	0	0
	Prince Edward Island,	41-50	65	60	1.62	38	0.68	5	0.06
	April 28, 1981	51-60	62	79	4.45	74	1.56	11	0.48
		61-70	44	84	9.16	68	2.07	14	0.39
		≥71	13	100	25.31	77	6.38	54	4.38
5	Souris, P.E.I.,	≤40	11	36	0.91	18	0.27	0	0
	May 6, 1981	41-50	67	70	2.36	33	0.63	4	0.07
		51-60	69	75	4.23	55	1.49	14	0.54
		61-70	43	88	11.53	49	1.28	19	0.37
		≥71	20	95	15.60	70	4.15	25	0.70
6	Chéticamp,	≤40	83	34	0.52	19	0.24	0	0
	November 24,1980	41-50	168	40	0.74	39	0.54	1	0.01
		≥51	38	58	1.24	50	0.82	0	0
7	Cabot Strait	≤50	13	8	0.08	62	1.46	8	0.23
	November 24, 1980	51-60	110	39	0.63	67	2.45	24	0.97
		61-70	69	36	0.90	81	3.01	59	3.96
		≥71	29	72	3.10	97	8.93	62	13.17
8	Cabot Strait	≤50	84	36	0.45	45	0.83	4	0.06
	December 11, 1980	51-60	154	45	1.01	63	1.48	10	0.29
		61-70	33	61	2.68	76	2.27	48	3.09
		≥71	24	96	8.46	83	6.75	50	10.79
9	Edge of Breton Shelf	≤40	98	39	0.71	27	0.44	3	0.09
	February 10, 1981	41-50	134	45	1.16	47	0.80	2	0.10
		51-60	123	46	1.61	52	1.34	4	0.08
		61-70	108	73	3.96	66	1.68	21	0.76
		≥71	43	95	15.58	74	3.88	19	1.21
10	Edge of Breton Shelf	≤40	132	14	0.17	31	0.71	0	0
	March 20, 1981	41-50	148	36	0.75	56	1.30	3	0.09
		51-60	150	53	1.94	63	1.77	11	0.34
		61-70	124	77	3.43	68	2.59	26	2.84
		≥71	52	96	7.79	81	2.79	29	2.08
11	Louisbourg Hole,	≤30	29	14	0.14	10	0.24	0	0
	February 22, 1980	31-40	108	33	0.73	7	0.08	3	0.05
		41-50	123	22	0.58	24	0.67	1	0.01
		51-60	205	51	1.97	29	0.79	2	0.09
		61-70	85	52	2.25	27	1.26	11	0.91
		≥71	27	63	5.22	22	1.26	11	0.59
12	Cape Smokey,	≤40	11	45	1.00	55	1.09	9	0.18
	Nova Scotia,	41-50	59	68	1.53	54	1.64	7	0.12
	May 27, 1981	51-60	71	62	2.76	75	2.32	13	0.41
		61-70	63	71	3.22	75	2.21	3	0.05
		≥71	26	96	13.92	77	5.23	12	2.65
13	Louisbourg offshore,	≤50	59	4	0.81	25	0.37	0	0
	June 30, 1981	51-60	92	50	1.03	43	0.87	2	0.16
		61-70	90	68	2.56	50	1.12	2	0.02
		≥71	46	87	6.46	61	2.72	0	0
14	Frenchman's	31-40	42	43	0.93	14	0.24	0	0
	Shoal,	41-50	70	59	2.00	16	0.29	0	0
	July 23, 1981	51-60	67	87	3.76	46	0.63	0	0
		61-70	71	85	9.28	49	1.08	1	0.14
		≥71	33	97	11.24	76	4.36	18	1.21

[1] see Figure 1.

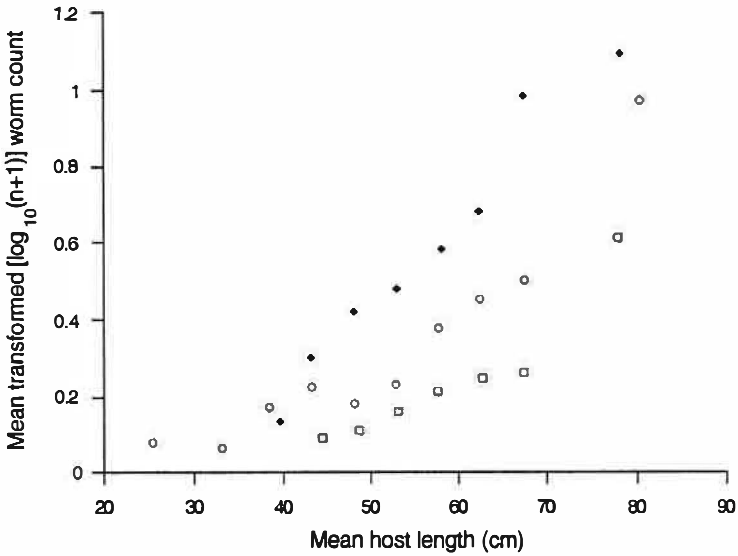

Fig. 2. Mean transformed [$\log_{10}$(n+1)] counts of *Pseudoterranova decipiens* larvae in 5 cm length groups of cod from pooled southeastern Gulf of St. Lawrence (♦), southwestern Gulf (O) and Cabot Strait (□) samples.

shore in February 1980 and June '81. Routine inspections of cod yielded a total of 10,366 sealworm larvae ranging from 8 to 60 mm in length; 90% were found in fillets, 7% in napes and 3% in body cavities. Frequencies of sealworm in body cavities and napes, however, increased with host length. Of 3,529 *P. decipiens* in small (≤60 cm TL) market cod and scrod, 96, 3 and 1% occupied the fillets, napes and body cavities respectively while respective sealworm frequencies in the fillets, napes and body cavities of the largest cod (≥71 cm TL) were 82, 12 and 6%. Although sealworm larvae were found throughout the body cavities, encapsulated or free on various organs (gastrointestinal tract, liver, spleen, gonads, etc.) and associated mesenteries, the majority, 223 (65%) of 342, were encapsulated on the liver or on the pyloric caecae and supporting mesenteries.

Distribution of larval sealworm in cod tissues did not seem to have been influenced by post mortem migrations of nematodes from the viscera to the flesh in iced round fish. Using routine examinations, a total of 398 *P. decipiens* were recovered from a sample of 164 cod (50-60 cm in length) from Scatari Bank (Breton Shelf, Oct. 23, 1981). Only two sealworm larvae were found in the viscera, however, and both of these nematodes occurred in cod which had been stored in the round for four days. The parasite was absent in viscera from 56 cod gutted at sea.

Subsequent digestion of the flesh from 113 Scatari Bank cod yielded an additional 46 *P. decipiens* larvae for a total of 339 found by routine (slicing) examination and digestion procedures. Hence, 14% of the larval sealworm in the flesh escaped detection during routine inspections.

Anisakis simplex

Larvae of *A. simplex* were generally more prevalent and abundant in cod from the southern Gulf of St. Lawrence and Cabot Strait than they were in Breton Shelf Cod (Fig. 3). The lowest infection levels occurred in winter and summer samples from the Louisbourg Hole area (sample nos. 11 & 13, Table 1) at the southeastern extremity of the Breton Shelf. Larvae of *A. simplex* were 12 to 35 mm in length and, of 6, 214 recovered from cod samples, 61% were found encapsulated on the liver, and 31% encapsulated on the mesenteries of the pyloric caecae. They were seldom found in the fillets (1%) or napes (1%). Frequency of the parasite on caecal mesenteries increased with host length. In fish ≤50 cm in length, 76% (926 of 1,214) of the *A. simplex* larvae occupied the liver, and 17%, the caecal mesenteries, but in fish ≥71 cm in length, 49% (746 of 1549) of the nematodes were found on caecal mesenteries, and only 42% on the liver.

The experiment in which Scatari Bank cod, 50-60 cm in length, were either gutted at sea (n=56) or stored in the round for four days (n=108), failed to provide conclusive evidence of port mortem migration of *A. simplex* larvae in iced round cod. After routine inspections, 3 (8%) of 37 larvae recovered from "gutted" cod, and 9 (7%) of 123 from "round" cod were found in the flesh. While 2 of 3 *A. simplex* larvae in the flesh of "gutted" cod were detected in the napes, however, 7 of 9 larvae found in the musculature of round cod were from the fillets.

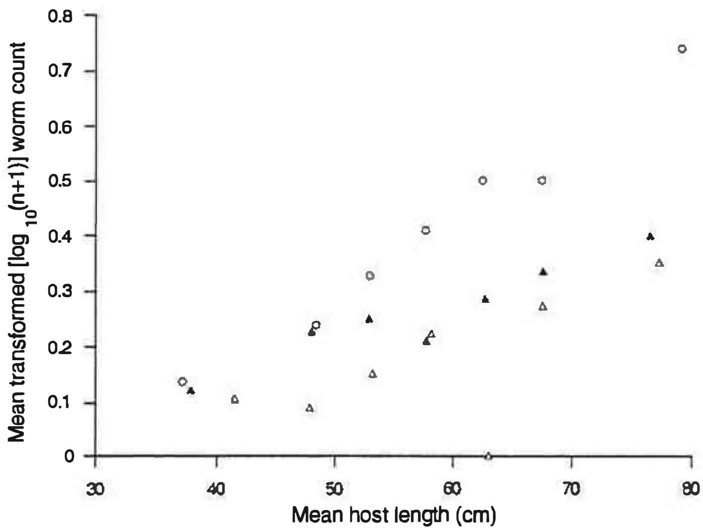

Fig. 3. Mean transformed [$\log_{10}(n+1)$] counts of *Anisakis simplex* larvae in 5 cm length groups of cod from pooled southwestern Gulf of St. Lawrence (O), Breton Shelf winter (▲) and Breton Shelf summer (Δ) samples.

Subsequent digestion of the fillets and napes of 56 "gutted" and 57 of the "round" cod revealed that 15 (68%) of 22 *A. simplex* larvae in the flesh had escaped detection during our routine inspections. With nematodes recovered by digestion included, 9 (21%) of 43 larvae from "gutted" cod and 13 (24%) of 55 from "round" cod were detected in the flesh. Of the nematodes found in the flesh, the majority (6 of 9) occupied the napes of gutted cod, and the fillets (8 of 13) of round cod.

Contracaecum osculatum

While large market and steak cod (≥71 cm TL) were often heavily infected with *C. osculatum* larvae, this parasite was seldom found in scrod (≤50 cm TL) (Table 1). Infection levels were greatest in cod from the southern Gulf of St. Lawrence (especially the southwestern Gulf) and Cabot Strait summer and fall fisher ies but relatively low in samples from the Breton Shelf winter and summer fisheries (Figs. 4 & 5).

A total of 2,749 *C. osculatum* larvae, 7-23 mm in length, were detected in 4,360 cod, the great majority (96%) being encapsulated on the pyloric caecae and associated mesenteries. A total of three (<<1%) were found in the musculature, two in napes, and one in fillets. The parasite was lacking in the Scatari Bank sample used to investigate post mortem migration of nematodes and efficiencies of routine examinations.

Multivariate analysis of larval anisakine levels in cod.

Bartlett's test of sphericity indicated that correlations among independent variables i.e. levels of *P. decipiens*, *A. simplex* and *C. osculatum* infection were highly significant ($P \leq 0.00001$) and that

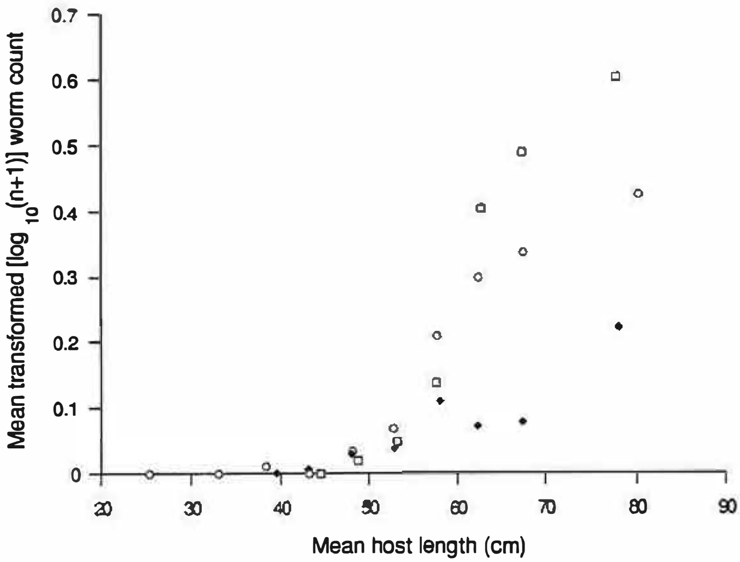

Fig. 4. Mean transformed [$\log_{10}(n+1)$] counts of *Contracoecum osculatum* larvae in 5 cm length groups of cod from pooled southwestern Gulf of St. Lawrence (♦), southwestern Gulf (O) and Cabot Strait (□) samples.

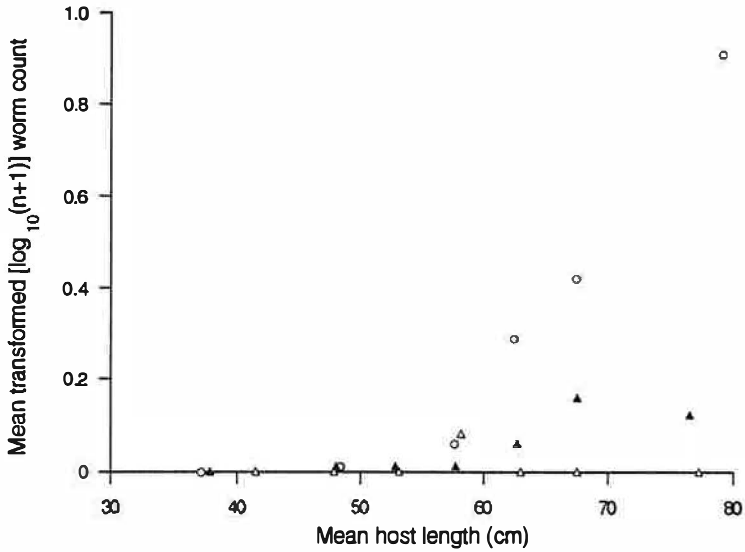

Fig. 5. Mean transformed [$\log_{10}(n+1)$] counts of *Contracoecum osculatum* larvae in 5 cm length groups of cod from pooled southwestern Gulf of St. Lawrence (O), Breton Shelf winter (▲) and Breton Shelf summer (Δ) samples.

multivariate analyses were therefore appropriate for comparing nematode levels in our cod samples. Host length, sample, and length—sample interaction effects in respective MANOVAs for nematode prevalence and abundance were highly significant ($P \leq 0.00001$) in each case, with *C. osculatum* levels making the greatest contribution to disparities among samples and to length—sample interactions for both prevalence and abundance. While *C. osculatum* larvae also made the greatest contribution to differences in nematode prevalence and among length groups, variation in nematode abundance with host length was primarily attributable to larval sealworm.

Contrasts of individual and pooled cod samples based on two-way MANOVAs of larval anisakine levels are summarised in Tables 2 to 4 by indicating the significance of the contribution of each anisakine species to overall disparities in nematode prevalence and abundance in the samples compared. Significance of interactions between dependent variables were also computed for each contrast and, in some instances, appear in the text below.

Contrasts of southern Gulf of St. Lawrence and Cabot Strait cod samples revealed that *P. decipiens, A. simplex* and *C. osculatum* larvae were uniformly prevalent and abundant in samples taken from the southwestern Gulf (Pt. Escuminac, Shediac Valley & Bradelle Bank) summer and fall fishery, but that southwestern and southeastern Gulf samples (Souris, P.E.I.) differed in regard to larva sealworm and *C. osculatum* levels (Table 2). A comparison of pooled southwestern and pooled Souris samples confirms that there are significant disparities in larval sealworm abundance (P≤0.01), in prevalence (P≤0.001) and abundance (P≤0.001) of *C. osculatum* larvae and in the *P. decipiens—C. osculatum* interactions for prevalence (P≤0.001) and abundance (P≤0.001).

Larval anisakine levels in cod taken from the Cabot Strait in late fall were consistent with those found in southwestern Gulf cod, but differed significantly from Souris cod in respect to prevalence and abundance of *P. decipiens* and *C. osculatum* (Table 2). Cabot Strait and

Table 2. Contrasts[1] of larval nematode levels in samples of cod from the southern Gulf of St. Lawrence and Cabot Strait.

Sample(s)	Nematode sp.	Contrasts with samples from: Cabot Strait[2]		Bradelle Bank		Shediac Valley		Pt. Escuminac	
		prev.	abun.	prev.	abun.	prev.	abun.	prev.	abun.
Souris[3]	*P. decipiens*	***	***	—	***	—	**	—	—
	A. simplex	*	—	—	—	—	—	—	—
	C. osculatum	***	***	—	—	***	***	***	***
Pt. Escuminac	*P. decipiens*	—	—	—	—	—	—		
	A. simplex	—	—	—	—	—	—		
	C. osculatum	—	—	—	—	—	—		
Shediac Valley	*P. decipiens*	—	—	—	—				
	A. simplex	—	—	—	—				
	C. osculatum	—	—	*	—				
Bradelle Bank	*P. decipiens*	—	—						
	A. simplex	—	—						
	C. osculatum	—	—						

[1] based on two-way (host length x host sample) MANOVA; Scheffé significance at 1 (*), 0.1 (**) and 0.01 (***) %.
[2] sample nos. 7 and 8 pooled.
[3] sample nos. 4 and 5 pooled.

Souris cod also differed (P≤0.001) in regard to *P. decipiens—C. osculatum* interactions for prevalence and abundance.

As is evident in contrasts of samples taken from the southern edge of the Laurentian Channel and February and March '81 and from the Cape Smokey inshore in late May '81, larval anisakines were uniformly prevalent and abundant in cod from the northern (Sydney Bight) portion of the Breton Shelf (Table 3). Similarly, larval anisakine levels in cod sampled from the rim of the Louisbourg Hole in February 1980, were consistent with those found in cod collected along the nearby edge of the Breton Shelf in late June '81. Offshore cod from the Louisbourg Hole, however, differed significantly from inshore cod taken near Louisbourg (Frenchman's Shoal, July '81) in regard to larval sealworm levels. Significant disparities in the infection levels of *A. simplex* and *C. osculatum* are also revealed when cod from the northern Breton Shelf (Sydney Bight) are contrasted with those from the southern (Louisbourg) portion of the Shelf.

Larval anisakine levels in Breton Shelf

Table 3. Contrasts[1] of larval nematode levels in cod samples from the Breton Shelf.

Sample(s)	Nematode sp.	Contrasts with samples from							
		Louisbourg offshore (summer)		Frenchman's Shoal (summer)		Cape Smokey (spring)		Louisbourg Hole (winter)	
		prev.	abun.	prev.	abun.	prev.	abun.	prev.	abun.
Edge of	*P. decipiens*	—	—	**	***	—	—	—	*
Breton Shelf[2]	*A. simplex*	—	*	—	*	—	—	***	***
(winter)	*C. osculatum*	***	***	*	—	—	—	***	***
Louisbourg	*P. decipiens*	—	—	***	***	**	***		
Hole (winter)	*A. simplex*	—	—	—	—	***	***		
	C. osculatum	—	—	—	—	—	—		
Cape Smokey	*P. decipiens*	—	—	—	—				
(spring)	*A. simplex*	**	***	**	***				
	C. osculatum	—	—	—	—				
Frenchman's	*P. decipiens*	*	***						
Shoal	*A. simplex*	—	—						
(summer)	*C. osculatum*	—	—						

[1] based on two-way (host length x host sample) MANOVA; Scheffé significance at 1 (*), 0.1 (**) and 0.01 (***) %.
[2] February and March samples (nos. 9 and 10) pooled.

cod were not consistent with those found in pooled southwestern Gulf of St. Lawrence and Cabot Strait samples (Table 4). Cod taken from the Breton Shelf offshore near the southern slope of the Laurentian Channel in February 1980, and in February, March and June '81, differed significantly from migrant southwestern cod in regard to levels of *A. simplex* and *C. osculatum* infection and interactions of infection levels of both of the latter species with larval sealworm levels ($P \leq 0.001$). Contrasts of southwestern Gulf cod with inshore cod from Cape Smokey indicated significant disparities in prevalence and abundance of *C. osculatum* and in *C. osculatum* interactions with *P. decipiens* and *A. simplex*. There were significant differences in levels of each of the three species of larval anisakine and in *P. decipiens* interactions with *A. simplex* and *C. osculatum* ($P \leq 0.001$) when southwestern Gulf cod were contrasted with cod from the Louisbourg inshore (Frenchman's Shoal).

Similar disparities in larval anisakine levels are also revealed when Breton Shelf and southeastern Gulf (Souris) samples are compared (Table 4). However, Souris cod differed from cod from the Louisbourg offshore in regard to each of the three species of nematode while differing from cod from the northern (Sydney Bight) Breton Shelf only in respect to larval sealworm levels. Contrasts of samples from the southeastern Gulf and the Louisbourg inshore indicated disparities in *A. simplex* and *C. osculatum* infection levels but the differences were not highly significant.

Table 4. Contrasts[1] of nematode levels in cod samples from the southern Gulf of St. Lawrence and Breton Shelf fisheries.

Breton Shelf cod sample(s)	Nematode sp.	Contrasts with cod from the southern Gulf of St. Lawrence: Western contingent[2] prevalence	Western contingent[2] abundance	Eastern contingent[3] prevalence	Eastern contingent[3] abundance
Edge of Breton Shelf[4] (winter)	*P. decipiens*	—	—	*	***
	A. simplex	—	***	—	—
	C. osculatum	***	***	—	—
Louisbourg Hole (winter)	*P. decipiens*	—	—	***	***
	A. simplex	***	***	***	***
	C. osculatum	***	***	***	***
Cape Smokey (spring)	*P. decipiens*	—	—	—	**
	A. simplex	—	—	—	—
	C. osculatum	***	***	—	—
Frenchman's Shoal (summer)	*P. decipiens*	*	***	—	—
	A. simplex	***	***	*	*
	C. osculatum	***	***	*	*
Louisbourg offshore (summer)	*P. decipiens*	—	—	**	***
	A. simplex	***	***	—	*
	C. osculatum	***	***	***	***

1 based on two-way (host length x host sample) MANOVA; Scheffé significance at 1 (*), 0.1 (**) and 0.01 (***) %.
2 Pt. Escuminac, Shediac Valley, Bradelle Bank and Cabot Strait samples (nos. 1, 2, 3, 7 and 8) pooled.
3 Souris samples (nos. 4 and 5) pooled.
4 February and March 1981 samples (nos. 9 and 10) pooled.

Discussion

In a recent review article, Williams *et al.* (1992) list various guidelines for the selection of parasites as "biological tags" for fish stocks. Preferably, levels of infection would vary significantly in different host populations but have long term temporal stability in a given population. The parasite would have a direct life cycle and be long lived in the host. While it is not necessary that a fish parasite be nonpathogenic, it would not influence host behavior. Finally, the parasite would be site specific and easy to detect and identify.

Larvae of anisakine nematodes of marine mammals seem to satisfy some of the criteria above. Most importantly, levels of infection often vary significantly within the geographical range, and in different populations of a fish host species (Scott and Martin 1957; 1959; Templeman *et al.* 1957; Young 1972; Platt 1975; 1976; McClelland *et al.* 1983a; 1983b; 1985; 1987; Brattey *et al.* 1990; Brattey and Bishop 1992; Hemmingsen *et al.* 1991; Jensen and Idås 1992; Jensen *et al.* 1994). As larval anisakines may also survive for years in the fish host (Smith 1984a; Hemmingsen *et al.* 1993), it follows that there would be little likelihood of dramatic seasonal fluctuations in infection levels. Levels in a given host population may change significantly over the course of many years (McClelland *et al.* 1987) but probably remain stable over the time frame of a given survey.

Although parasites with single host life cycles are preferred, those with complex indirect cycles may also prove useful as biological indicators (Williams *et al.* 1992). Indeed, geographical disparities in the infection levels of larval anisakine nematodes in fish probably result largely from differences in the distributions and migratory patterns of intermediate and definitive hosts. Larval sealworm levels in fish, for example, have frequently been shown to increase with proximity to colonies of definitive harbour (*Phoca vitulina*) and grey seal (*Halichoerus grypus*) hosts (Scott and Martin 1959; McClelland *et al.* 1983b; Marcogliese and McClelland 1992; Jensen *et al.* 1994). The relatively high levels of larval sealworm infection recorded in cod from the southeastern Gulf of St. Lawrence and southern Breton Shelf inshore in the present study are probably attributable to large concentrations of grey seals which breed in the southern Gulf and haul out on Scatari Island and other smaller islands and ledges along the southeastern coast of Cape Breton (Stobo *et al.* 1990). Disparities in levels of larval *C. osculatum* in cod from the southwestern and southeastern Gulf of St. Lawrence and Breton Shelf on the other hand, are clearly related to the seasonal distribution of harp seals (*Phoca groenlandicus*) in the more southerly waters of Atlantic Canada. Harp seals visit the southern Gulf of St. Lawrence in winter and early spring and whelp on ice lying primarily between the north shore of Prince Edward Island and the Laurentian Channel (Sergeant 1991). They greatly outnumber other seal species found in Atlantic Canadian waters and, individually and collectively, host the greatest numbers of mature *C. osculatum* (McClelland *et al.* 1983a; 1985; Brattey and Ni 1992). MANOVAs of nematode prevalences and abundances reveal that, of the three species of larval anisakines surveyed in the present study, *C. osculatum* contributed most to disparities in nematode levels among samples, i.e. geographic variation, and to the sample-host length interaction.

Although there is no direct evidence that larval anisakines influence the behavior of their fish hosts, metabolic byproducts excreted by sealworm larvae are known to include volatile ketones which may act as local anaesthetics in surrounding host musculature (Ackman and Gjelstad 1975). Further, experiments with captive European smelt (*Osmerus eperlanus*) infected with *P. decipiens* (Sprengel and Lüchtenberg 1991) show that maximum swimming speed of the host declines with increasing intensity of nematode infection. Thus, in sufficient densities, the parasite may cause the host to become sluggish, thereby impairing its ability to avoid capture by natural predators or fishing gear. As a consequence of selective capture of infected hosts by mobile fishing gear e.g. trawls and seines, infection levels in hosts sampled during parasitological surveys might be greater than those found in the host population at large.

Of the three species of larval nematode used as natural indicators here, *C. osculatum*, which occurred almost exclusively (96%) on pyloric caeca and caecal mesenteries of cod, best satisfies the criterion of site specificity in the host. Larvae of *A. simplex* were found predominantly (91%) on the liver and pyloric caeca of cod during examinations in which the flesh was inspected by slicing but subsequent digestion of fillets and napes revealed that frequency of infection in the flesh was much higher (>20%) than indicated in our routine inspections. As other studies (Smith 1984b; McClelland *et al.* 1990) have shown, distribution of *A. simplex* in the body cavity and flesh varies with species and size (age) of host, and a large proportion (40-60%) of these nematodes may occupy the flesh (especially the napes) of marine fish. Similarly, larval sealworm distribution in fillets, napes and body cavity also varies with host species and size or age of host and may even vary in different populations of the same host species (Young 1972; Platt 1975; McClelland *et al.* 1985; 1990; Brattey *et al.* 1990). In smaller (younger) fish, sealworm occurs primarily in the fillets or, in some cod populations may be evenly distributed between fillets and napes. As indicated in present results, frequencies of *P. decipiens* in the napes and body cavity increases with size (age) of host. The majority of larval sealworm may occur in the napes or body cavities of mature marine fishes such as European cod (Young 1972; Platt 1975) and monkfish (*Lophius americanus*), cusk (*Brosme brosme*), and white hake (*Urophysis tenuis*) from Atlantic Canadian waters (McClelland *et al.* 1990).

The manner in which fish is stored prior to examination may also influence the distribution of larval anisakines in host tissues. Experiments performed by Smith and Wootten (1975), for example, indicated significant migration of *A. simplex* larvae from the viscera to the skeletal musculature of iced herring (*Clupea harengus*) examined 37h after capture. There was no evidence in the present study that either *A. simplex* or *P. decipiens* larvae moved from the viscera to the flesh of round cod after four days storage on ice, but comparisons of nematode distributions in the flesh of gutted and round cod seems to indicate that larvae of both species of nematodes migrated from the napes to the fillets when cod was stored in the round.

As larval anisakines are usually large enough to be seen with the naked eye, one might assume that they would readily qualify as biological tags from the standpoint of ease of detection. While this may be true for nematodes lying on visceral organs and mesenteries or shallowly embedded in tissues, those deeply embedded in skeletal musculature are often difficult to find. Powers (1961) observed that three quarters of the nematodes (mainly sealworm larvae) in cod fillets escaped detection by candling procedures routinely employed in fish processing plants. The more laborious procedure of slicing the flesh, used in examinations of fillets and napes in the present study, proved fairly efficient for finding larval sealworm as only 14% of the nematodes of this species escaped detection, but was far less effective for the detection of *A. simplex* larvae, most of which (68%) were found only by subsequent digestion of the flesh. Pepsin—HCl digestion is clearly the most efficient procedure for detecting parasites in the musculature, but in terms of time, labour and material cost, this approach may be impractical for use in large scale surveys, or surveys of larger fish such as mature cod.

Larval anisakines may often prove unsatisfactory as biological markers in terms of ease of identification. This is especially true when two or more anatomically similar but reproductively isolated sibling species share the same geographical range and hosts (Paggi *et al* 1991; Nascetti *et al.* 1993). Sibling species can be identified by electrophoretic procedures, but as only small numbers of nematodes (subsamples) can be sorted in this manner, such an approach may not be feasible in large scale surveys. Fortunately, it seems unlikely that we encountered more than one sibling of each of the three nematode species surveyed in the present study. Only sibling B of *P. decipiens* has been reported in fish and seals from Northwest Atlantic waters south of Labrador (Paggi *et al.* 1991; Brattey and Stensen 1993), and, according to Brattey and Bishop (1992), *A. simplex* larvae from eastern Canadian fisheries also belong to a single species, sibling B. While information on the sibling species status of larval *C. osculatum* from northwest Atlantic fish is lacking, all *C. osculatum* identified from Northwest Atlantic harp, harbour, ringed (*Phoca hispida*) and bearded seal (*Cystophora cristata*) were sibling B (Brattey and Stensen 1993; Nascetti *et al.* 1993). Sibling A is the predominant sibling of *C. osculatum* in bearded seal (*Erignathus barbatus*) but bearded seal frequent waters off Labrador and northern Newfoundland and are rare visitors to the Gulf of St. Lawrence. A small minority of the *C. osculatum* in eastern Canadian grey seal are also sibling A but, Sibling B being far more numerous in the latter host. As shown in Arthur and Albert's (1993) study of Greenland halibut (*Reinhardtius hippoglossoides*), failure to determine the specific identity of larval anisakines may not preclude their use as indicators. Arthur and Albert successfully employed infection levels of Contracaecinea, an anisakine tribe including structurally similar *Contracaecum* and *Phocascaris* spp. larvae, in distinguishing the geographic origin of individual hosts.

Results of our analyses of larval anisakine levels are consistent with other biological indicators such as vertebral counts (McKenzie and Smith 1955) and length-at-age profiles (Lambert 1993), and evidence from artificial tagging experiments (Martin and Jean 1962; Kohler and Fitzgerald 1975; Lambert 1993) which reveal the existence of distinct cod populations in the southwestern and southeastern Gulf of St. Lawrence, and on the Breton Shelf. Nematode levels in cod collected in the southwestern Gulf were remarkably uniform despite the fact that the fish were sampled at widely separated locations and at different times during late spring, summer and fall. Pooled southwestern Gulf cod samples, however, differed significantly from southeastern Gulf cod in regard to *P. decipiens* and *C. osculatum* levels, and from local Breton Shelf cod sampled in late spring and summer, in respect to levels of *A. simplex* and *C. osculatum* infection.

The fact that disparities in nematode levels in contrasts of southeastern Gulf (Souris, P.E.I.) cod and cod from the Breton Shelf inshore (Cape Smokey and Frenchman's Shoal) were not highly significant, may be attributable to the presence of southeastern Gulf fish in our samples from Breton Shelf summer fisheries. Whereas both southern Gulf cod populations perform annual migrations along the southern slope of the Laurentian Channel and overwinter along the Breton Shelf, the western contingent seems to move en masse while the movements of the eastern population are not as well defined (Lambert 1993). Cod tagged in the southeastern Gulf are found on the Breton Shelf throughout the year.

The activity of the mobile gear fishing fleet is indicative of the seasonal migration of southwestern Gulf cod through the Cabot Strait to the Breton Shelf area (Lambert 1993). Fleet activity reveals that cod from the southwestern Gulf reach the Cabot Strait area in early December. The presence of southwestern Gulf cod in the Strait area at this time of year was also evident in the consistency of larval anisakine levels in our late November—early December Cabot Strait samples with levels found in cod sampled in the southwestern Gulf from June to November.

In light of the results of the present study, concern that there may be significant exploitation of severely depleted, resident cod stocks in the Breton Shelf winter fishery (Sinclair 1993) seems well founded. Analyses of larval anisakine levels in our winter cod samples from the edge of the Breton Shelf indicate that these samples differed significantly from migrant cod sampled in the southern Gulf of St. Lawrence and Cabot Strait, but were indistinguishable from samples of resident cod collected from the Breton Shelf in summer. Hence, our winter samples from the Breton Shelf probably consisted primarily of resident cod. It is unlikely that the results of our analyses, however, are indicative of the extent in which local cod is exploited in the winter fishery on the Breton Shelf. Other biological factors, tagging data and observations on fishing activity show that cod taken in the Cabot Strait—Breton Shelf area in late fall and winter are predominantly migrant Gulf cod, which are intensively exploited by the mobile gear fleet as they move through the Cabot Strait along the southern slope of the Laurentian Channel (Lambert 1993). Our samples were collected from chartered vessels in February and March of 1980 and '81, weeks after commercial cod quotas were reached, and the fishery closed. Contact with concentrations of migrant Gulf cod, maintained through continuous exploitation by the mobile gear fleet, was lost with the closure of the fishery and, at the time of our surveys, the whereabouts of migrant Gulf cod on the Breton Shelf was not known.

In summary, the present analyse show that larval anisakine nematodes, which are frequently monitored in marine fish populations as a consequence of their economic and medical importance, are useful as natural tags for southern Gulf of St. Lawrence and Breton Shelf cod stocks, and complement other biological approaches (morphometrics, meristics, biochemical genetics, etc.) and artificial tagging experiments in revealing movements and discreteness of cod populations. The sensitivity of the parasitological approach might be improved by discriminant function analyses of nematode levels, although this would probably entail the identification and monitoring of additional parasite species which are suitable as natural tags for cod.

Acknowledements

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NOTES FROM THE EDITOR

There will be a supplementary issue of the Bulletin with the proceedings from the joint Baltic-Scandinavian Symposium on Parasitic Zoonoses and Ecology of Parasites, which was held in Vilnius, Lithuania 7-8 September 1994. This will probably be printed before the end of this year.

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Bull Scand Soc Parasitol 1994; 4: 117-122

TRANSMISSION OF *HYOSTRONGYLUS RUBIDUS* IN HOUSED PIGS

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Abstract

Four specific pathogen free (SPF) helminth naive pigs were inoculated each with 14,000 third stage larvae of *Hyostrongylus rubidus*. After 3 days they were moved to a disinfected indoor pen with concrete floor and deep straw litter, together with four helminth naive SPF pigs. The pigs were kept together in the same pen for 122 days. During this period, faecal samples were collected at weekly intervals from all pigs in order to monitor the excretion of helminth eggs. Eggs of *H. rubidus* were excreted by the inoculated pigs 22 days post inoculation (p.i.), and were observed in the non-inoculated group from around day 77 p.i. Excretion remained at a steady level in both groups until the time of slaughter, i.e. day 128 p.i., where *H. rubidus* was found in both groups. It is concluded that *H. rubidus* can be transmitted among traditionally indoor-reared pigs in Denmark.

Introduction

Although infections with *H. rubidus* have earlier been described in Denmark (Jacobs, 1967), cases of hyostrongylosis have not been reported over the last decades. In a recent joint Nordic survey, *H. rubidus* was not recorded in any of the countries (Roepstorff *et al.*, 1991). Pig production in Denmark has changed over the past decades from traditional pen-keeping farming to large-scale, indoor intensive production, e.g. in the form of specific pathogen free (SPF) units having a high level of hygiene. This production may be sectionalised, and pigs often do not remain in the same pen for longer periods of time. These and other factors may explain why hyostrongylosis has not been recorded for many years.

However, along with the ongoing development of industrialized large-scale herds, the so-called ecological (organic) pig farming has recently attracted some interest, and a number of such farms has been established. The management of these farms includes outdoor as well as indoor rearing of pigs under relatively poor hygienic standards. Recently it was demonstrated that *H. rubidus* may be transmitted under outdoor conditions in Denmark (Dangolla *et al.*, 1994), but it is unknown to what extent indoor transmission may take place. In England, some field observations indicate that indoor transmission may occur in pigs under unhygienic conditions (Connan, 1967), but similar observations have not been made in Denmark. The aim of this study was to investigate the ability of *H. rubidus* to be transmitted among housed pigs in Denmark under sub-optimal hygienic conditions.

Materials and methods

Eight 10-week old specific pathogen free (SPF) female pigs, approximate weight 30 kg, were purchased from a large pig farm. The pigs were divided into two groups of four (A and B), and moved to two separate pens. The pigs of group A were inoculated each with 14,000 *H. rubidus* third stage larvae (L3), while the pigs of group B remained non-inoculated. Three days after inoculation, all pigs were moved to a clean pen in a traditionally built stable with no previous history of hyostrongylosis. The floor of the pen was solid concrete, without slats. Straw was used as bedding, and the pen was cleaned only twice a week. The pigs were fed full-constituent pelleted fodder, according to a standard feeding regimen, and water was provided ad libitum via drinking nipples. The room temperature of the stable was in the range of 16°-19°C.

Faecal samples were collected at approximately weekly intervals until the time of slaughter. The samples were examined for the number of eggs per gram of faeces (EPG) by a modified McMaster technique using saturated NaCl-glucose as flotation medium. The lower detection level of this technique was 20 EPG. Faecal cultures were set up to obtain third stage larvae for identification (Henriksen & Korsholm, 1983).

The pigs were slaughtered on day 128 post infection at a live weight of approximately 120 kg, and the gastrointestinal tract was obtained. Aliquots of 20% stomach contents were sieved (mesh size 212 µm) for recovery of adult stages of *H. rubidus*. Macroscopic changes in the stomach mucosae were recorded. The stomachs were suspended in physiological saline solution (0.9% NaCl) at 37°C to allow migration of worms from the mucosa into the solution. After an incubation period of approximately two hours, the saline solution was poured into a sieve (mesh size 212 µm), trapping all worms, which were subsequently counted. Finally the stomach mucosae were scraped off and digested in a stomacher, in a pepsin/hydrochloric acid solution (Grønvold *et al.*, 1989). The digested mucosae were sieved (mesh size 38 µm) to recover and determine the numbers of larvae. Hence the total number of worms was calculated.

Statistical analysis:

The geometric mean number of eggs excreted by the two groups was calculated for each sampling day. In order to normalize data and stabilize the variances for statistical analysis, the EPG values and worm counts were transformed to logarithmic values, using the formula **ln(X+1)**, where X was EPG or worm count and **ln** the natural logarithm function. The transformed data from the two groups were compared by means of the Student's t-test. The null-hypothesis implied that the mean number of eggs excreted by the two groups and the number of adults in the two groups did not differ significantly.

Results

Fig. 1 illustrates the geometric mean faecal egg excretion of *H. rubidus* in group A and group B.

Eggs in low numbers were detected in the faeces of the animals in group A from day 22. The EPG continued to rise, reaching a peak of approximately 500 EPG by day 69, whereupon it decreased. Between day 90 and day 118, the mean EPG counts were between 35 and 50, but increased again to a mean value of almost 400 towards the end of the study period.

Figure 1. Geometric mean of EPG of H. rubidus in inoculated (Group A) and non-inoculated (Group B) pigs housed in the same pen.

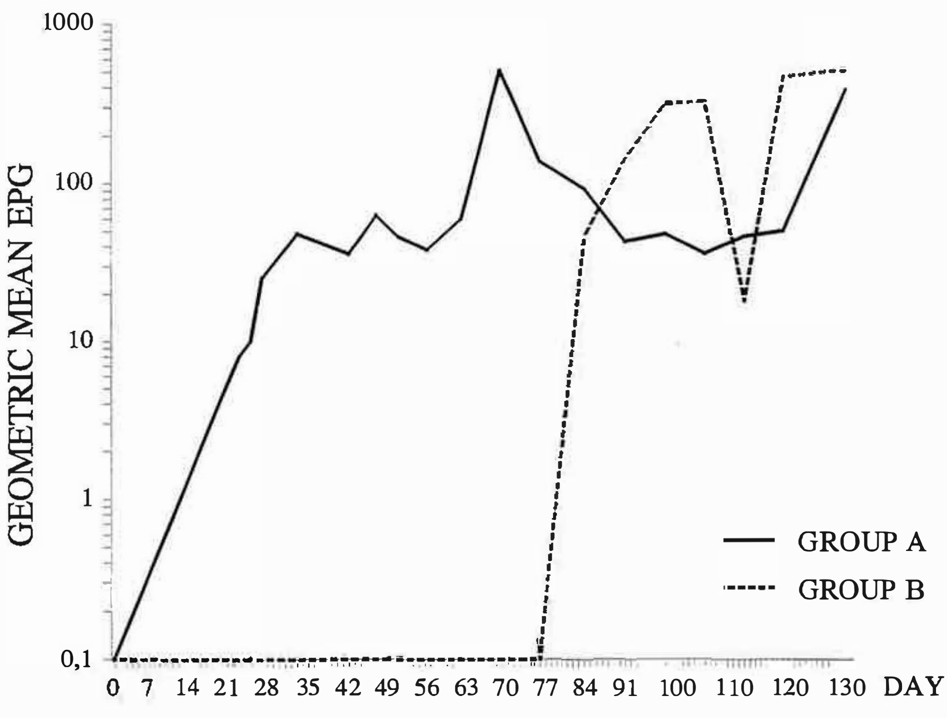

Table 1. Total number and the geometric mean of adult H. rubidus recovered at slaughter in pigs artificially inoculated (Group A) and non-inoculated (Group B) with H.rubidus and housed in the same pen.

Group A, inoculated				Group B, non-inoculated			
Pig no.	*H. rubidus* males	*H. rubidus* females	*H. rubidus* total	Pig no.	*H. rubidus* males	*H. rubidus* females	*H. rubidus* total
43	55	58	113	47	3	3	6
44	108	117	225	48	1	1	2
45	374	425	799	49	27	41	68
46	299	343	624	50	20	40	60
Geometric mean	160±2.5	177±2.5	336±2.5	Geometric mean	6.3±4.8	8.4±6.5	16.2±5.7

In group B, eggs were recorded from around day 77, after which time the EPG levels were in the range from 100 to 500. At the end of the study, *H. rubidus* was recovered from the stomachs of artificially infected (group A) as well as non-artificially infected pigs (group B), see table 1. Less than 5% of the worms were immatures. The geometric mean of adult worms recovered from the infected group (336±2.5) significantly ($p<0.05$) exceeded the geometric mean of worms recovered from the originally non-infected group (16.2±5.7).

Hyperaemia, hypertrophy, and hyperplasia were observed in the stomach mucosa of both groups, but none of the experimental animals showed signs of clinical hyostrongylosis.

Discussion

This study, carried out under indoor conditions, showed that transmission from infected pigs to previously non-infected pigs in the same pen, took place within a couple of weeks. Towards the end of the study period, the egg excretion of the two groups was roughly similar, which fact contrasted the size of the worm burdens of the two groups, where the number of worms found in group A was significantly higher than that of group B. An explanation for this may be that the fecundity of the worms in the previously infected group (A) had been impaired by host immunity due to the longer period of exposure, which is in line with similar findings of a related trichostrongyle, *Ostertagia ostertagi*

(Michel, 1963).

Environmental conditions such as temperature and relative humidity exert a direct influence on the rate of hatching of eggs and development of larvae. Conditions for optimal development are 15°-20°C and a relative humidity of 80-95% (Alicata, 1935, Fossing *et al.*, 1994, Rose & Small, 1982). The temperature in the stable under investigation was 16°-19°C, and the pigs were kept on deep straw litter, ensuring a high level of humidity on the floor of the pen. This may explain the successful development and transmission of *H. rubidus* in the present study. Since the pigs were housed in a pen with solid floor, and mechanical cleaning was done only twice a week, a high concentration of eggs and larvae was expected to be maintained on the floor of the pen.

Pens with slatted floors, where contact between the pigs and their faeces may be at a minimum, allow a less ready transmission of infection (Connan, 1986). Furthermore, frequent removal of litter and dung from the pens in modern confinements, and maintenance of high levels of hygiene in general, will essentially have the same effect.

In this study, the animals were kept together in the same pen throughout a study period of 128 days. However, under modern management systems, production is often divided into several separate entities, e.g. farrowing, weaning, fattening, etc., and therefore the animals rarely remain in the same pen for very long. At each stage the animal is moved on to a thoroughly disinfected pen with no, or few, parasites. This routine effectively helps keeping infection at a minimum throughout. Previous studies indicate that the highest infection levels are generally observed in sows (Busse, 1992, Pattison *et al.*, 1980), most likely due to the fact that sows are kept in the same pens for longer periods of time, permitting an accumulation of helminth eggs and favouring transmission.

Sensitivity to management practice, combined with a low bio-potential (Jacobs & Andreassen, 1967), may leave *H. rubidus* at a disadvantage in the modern pig production systems, and the occurrence of *H. rubidus* may thus be seen predominantly in pigs kept on deep litter in traditional herds with poorer hygiene. The system of ecological farming with deep straw litter, pens with solid floors, and probably also access to an outside yard, provides a micro-environment suitable for the development and transmission of infective stages of *H. rubidus*.

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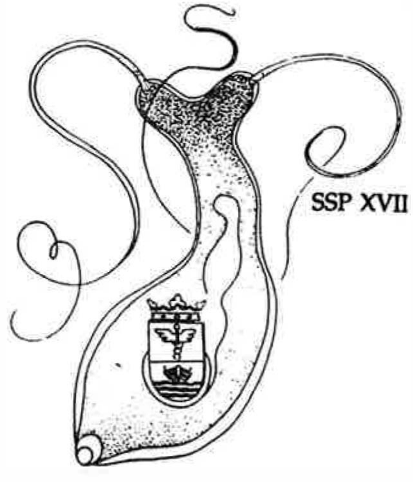
ANNOUNCEMENT

17th SCANDINAVIAN SYMPOSIUM OF PARASITOLOGY



UNIVERSITY OF JYVÄSKYLÄ, FINLAND 15-17 JUNE 1995

Welcome to Jyväskylä

The Scandinavian Society for Parasitology is pleased to invite you to the SSP XVII, the 17th Scandinavian Symposium of Parasitology, which will take place at the University Main Campus, 15 - 17 June 1995. The campus is located in the centre of the city, within easy walking distance from a range of comfortable hotels. Jyväskylä is a lively university town 300 km north of Helsinki. It is located in the heart of the beautiful Finnish lake district.

Scientific programme

The symposium will accept contributions on all aspects of parasitology. The following themes have been chosen for plenary lectures:

* Evolutionary ecology in parasitology
* Pathogenesis in human parasitic diseases
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All contributions should be submitted as word-processed manuscripts on floppy disk, accompanied by two exactly matching print-outs of good reading-quality. The preferred storage medium is a 3½ or 5¼ inch disk in MS-DOS or MS-DOS compatible format. The text should be written in WordPerfect or other word processing programs convertible to WordPerfect. **With a Macintosh computer, save the file in the MS-DOS compatible option.** Please indicate the word processor (and version) used to generate the file, the type of computer, the operating system, and the formatted capacity of the diskette.

Short articles/communications should not exceed 4 printed pages, including tables, figures, and references, and may contain a maximum of 2000 words if there are no figures or tables. The first page should show the title of the article, and the name(s) of the author(s). The authors' addresses should be given, and the complete correspondence address with telephone and telefax number (if available). The text should follow, without subheadings, but a short summary, maximum 100 words, may be included.

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Figure legends must be included on the diskette, but the **figures will be handled conventionally.** They should be marked on the back with the title of the article and name of the (first) author.

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Lund-Larsen TR, Sundby A, Kruse V, Velle W. Relation between growth rate, serum somatomedin and plasma testosterone in young bulls. J Anim Sci 1977; 44: 189-94

Books and other monographs:

Austin B, Austin DA. Bacterial fish pathogens: disease in farmed and wild fish. Chichester: Ellis Horwood, 1987

McFerran JB, McNulty MS, eds. Acute virus infections of poultry: a seminar in the CEC programme, Brussels 1985. Dordrecht: Martinus Nijhoff, 1986. (Current topics in veterinary medicine and animal science 37)

Sosialdepartementet. Tsjernobyl-ulykken: Rapport fra Helsedirektoratets rådgivende faggruppe. Oslo: Universitetsforlaget, 1987 (Norges offentlige utredninger NOU 1987: 1)

Thornhill JA. Renal endocrinology. In: Drazner FH, ed. Small animal endocrinology. New York: Churchill Livingstone, 1987: 315-39

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BULLETIN OF THE SCANDINAVIAN SOCIETY FOR PARASITOLOGY

VOL. 4 No. 2 **CONTENTS** **OCTOBER 1994**