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BULLETIN OF THE SCANDINAVIAN SOCIETY FOR PARASITOLGY

The Bulletin is a membership journal of the Scandinavian Society for Parasitology. This is, however, the last regular issue of the issue, for more information, see the note from the president, page 69, and the society's home page: www.hi.is/pub/ssp/

Scandinavian Society for Parasitology (Nordisk Förening för Parasitologi)

Society Board:

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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WELCOME BY THE PRESIDENT OF THE SCANDINAVIAN SOCIETY FOR PARASITOLOGY

By KARL SKIRNISSON

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Dear colleagues,

On behalf of the Scandinavian Society for Parasitology, I welcome you to our 21st symposium in Bergen, this almost thousand years old, charming city which is not only famous for rich culture and history, but also for several renowned parasitologists. The Society is not meeting in Bergen for the first time. In 1976 Scandinavian parasitologists also gathered in the city during the 8th symposium of the Society, at which time the parasites brought together 47 parasitologists.

We all know that members of the Scandinavian Society for Parasitology form a heterogeneous group of scientists with different background and interests. Some of us studied veterinary or human medicine, but in recent decades an increasing number of our members have finished their degree in biology. Most SSP members are active in research, either working at universities or other governmental institutions, and some work for private companies. The diverse background, and different activities, is often considered as a weakness of our Society. Of course, this is true to some extent. We all know e.g. how important it is to attend, or to organize meetings on an international level where our special, often quite narrow, field of work is the main subject. However, it is an advantage when not too many parasitologists from a certain region, dealing with more or less the same fauna and problems, are brought together and everyone has got the opportunity to attend lectures on research in various fields and view all posters exhibited. Through the decades this has probably been the main characteristic and strength of the SSP meetings. I am convinced that these arrangements have systematically inspired and improved the parasitological skills of the SSP members and also increased their panorama. And in this context we should not forget the importance of creating circumstances where colleagues and friends have the opportunity to meet, discuss and enjoy life.

During the General Assembly arranged at the end of our symposium, the already prepared joining of the Scandinavian and the Baltic Societies for Parasitology is planned. This joining of our societies is expected to have valuable advantages in future years with e.g. increased membership numbers, better scientific communication and improved cooperation between colleagues working in the Scandinavian and the Baltic countries. However, it is clear that the success and future of the enlarged Society will mainly depend on the activity and interest of our members in the next few years.

It is an honour and privilege to thank the local organizing committee (Arne Levsen, Per Jakobsen, Are Nylund and Arne Skorping) for arranging the 21st SSP

Symposium. They have set up a very interesting scientific programme, which covers broad issues highly relevant to medical, veterinary as well as general parasitology, and they have also arranged a lovely social programme, which enables us to meet, discuss and enjoy ourselves. A special welcome is directed to the invited speakers. The SSP Board is also indebted to various agencies and sponsors who contribute to the success of our meeting. Last, but not least, we thank the participants attending the symposium. We hope that you bring back home new knowledge and some fresh ideas, and also the intention to attend the next SSP meeting, which probably will be arranged in Vilnius in 2005.

INVITED LECTURES

THE SPECIES CONCEPT IN *GYRODACTYLUS*

By P.D. HARRIS

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The genus *Gyrodactylus* is composed of a large number of morphologically conserved species differing primarily in biological properties such as site infected, host identity and pathogenicity. Many species are rare and appear to cause little harm; others, particularly *G. salaris*, are highly pathogenic. The origin of the Norwegian *G. salaris* epidemic has led to concerns for the potential of these organisms for speciation and host switching. This paper will critically review our understanding of the species concept in *Gyrodactylus*, in order to clarify their potential for genetic change.

Only 400 species of *Gyrodactylus* have been described, but specificity patterns suggest many more exist infecting bony fishes. Gyrodactylid taxonomy utilises three classes of characters. Morphology, especially the morphometry of the attachment hooks and bars, has been most extensively used. Molecular loci have been available since the mid-1990's, particularly the ITS1 and ITS2 sequences which homogenise by concerted evolution, making them sensitive indicators of gene pool boundaries. By and large, the two taxonomies have coincided, although several ambiguities have emerged. Some taxa (*Gyrodactylus salaris* and *G. thymalli*) have identical ITS, but are morphologically quite distinct. There has also been a trend to describe new taxa which appear morphologically identical to existing species, but have distinct ITS sequences, suggesting that numerous "cryptic" *Gyrodactylus* species may exist. In these cases it is not clear whether the species pairs are truly morphologically indistinguishable, or whether high resolution morphometric analysis could separate them. The third set of characters used in gyrodactylid taxonomy is biological. These are rarely explicit, but host identity and site of infection are often implicit in species identification. Molecular data indicate that species pairs are not found on the same host, suggesting that host shifts are important isolation mechanisms. Species infecting different sites on the same host are usually more distantly related, suggesting that they are the result of several radiations of gyrodactylid species.

We have little understanding of gyrodactylid reproductive biology, or of its implications for the species concept. ITS sequences are stable across considerable geographical distances, even when barriers to dispersal (different watersheds, open sea) exist. This strongly suggests sexually reproducing species with assortative mating. In *G. salaris* and *G. thymalli* morphological divergence proceeded more rapidly that ITS sequence change, suggesting that these taxa are sexually and genetically variable. In *G. turnbulli* and *G. gasterostei* on the other hand, there is little underlying genetic

variance at loci contributing to hooks and bars, suggesting that extensive bottlenecking has occurred. More generally, it is assumed that host-induced phenotypic variance is slight, but few studies have demonstrated this experimentally. Host identity may influence morphometrics, and morphometric genes may be closely associated with genes for host choice, leading to hitch-hiking at these loci. Biological characteristics involved in isolation, such as host preference, pathogenicity and mate choice, may in part be phenotypic and maternally determined.

There are several unanswered questions in relation to the gyrodactylid species concept. In particular, it is paradoxical that *G. salaris* could expand its range so rapidly with such significant pathogenic effects when most gyrodactylid-host interactions appear stable and lack the genetic variance for selection to act upon. Our understanding of the species concept based on *G. gasterostei* and *G. turnbulli* as morphologically delimited and genetically uniform, with distinct ITS sequences and a particular specific host, is overturned when considering the highly pathogenic, morphologically labile, genetically variable non-host specific *G. salaris*. Reconciling these two views of the gyrodactylid species concept requires more detailed study of sibling species pairs. *G. salaris/G. thymalli* may be the best example of such a pair, but others (e.g. *the G. arcuatus*-like complex infecting gobies) may be more tractable to experimental investigation. Within these systems, consideration of the ability of gyrodactyids to hybridise, and the role and extent of maternal effects in determining assortative mating or host choice is needed. Finally, further taxonomic work on the genus must integrate both molecular and morphological approaches, and where possible, include the biological characterisation of new taxa.

THE *ANISAKIS* PROBLEM IN GERMANY/NORTHERN EUROPE

By H. KARL

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The presence of *Anisakis* sp. and other nematodes in food fish has been known for a long time, but it was not until the late fifties that this was recognised as a public health problem in Europe. Furthermore, the public awareness of parasites in fish may cause severe economic losses as the "nematode crisis" in Germany in 1987 has shown.

In commercially exploited marine fish species from the North Atlantic, the parasites of primary human health concern are *Anisakis simplex* and *Pseudoterranova decipiens*, commonly known as the "herring or whale worm" and the "cod worm", respectively. If live nematode larvae are ingested by man, they may penetrate into the wall of the gastrointestinal tract and cause acute inflammation (Anisakiosis or Anisakidosis). Additionally, allergic reactions to *Anisakis* specific antigens of both live

and dead worms have been reported, especially from southern Europe and Japan. However, the occurrence of *Anisakis*-related allergy in northern European countries has so far not been thoroughly investigated.

Approximately 97 % of all anisakiosis cases reported world wide are caused by *Anisakis simplex*, mainly due to the consumption of raw or inadequately processed fish, molluscs (squid) or crustaceans (shrimp and prawn). Fish acquire the infection by ingesting nematode larvae in their prey. In fish, the majority of the nematodes are found on the visceral organs, especially the stomach, intestine and liver. However, several studies have shown that at capture *Anisakis* larvae are also embedded in the belly flaps and/or the fillets. In saithe from the North Atlantic, and in mackerel from the Bay of Biscay, we found 10 % and 14 % of the nematodes located in the musculature, respectively. Of more than 3000 herrings investigated, on an average 4 % of the nematodes were embedded in the flesh. Other studies reported *Anisakis* larvae in the musculature of cod, Alaska pollock, haddock, blue whiting, whiting and pike-perch (Arthur *et al,* 1992, Brattey & Bishop, 1992, Smith, 1984, Karl *et al,* 2002).

Therefore, it seems inevitable that food fish infected with live nematodes enter the market. Preventive safety measures during catch handling, processing and household preparation are consequently imperative. Immediate evisceration of the fish directly after catch was recommended as an early measure to prevent migration of larvae from the viscera into the flesh. However, recent studies on several fish species indicate that no such post mortem migration occurs. Also, exploitation of apparently less infected fish stocks cannot guarantee parasite free products, as seen from an investigation on herring from different grounds of the North Sea, the Baltic Sea, the Irish Sea and waters west of British Isles (Karl, unpublished).

Consequently, safety measures have to be implemented. The EU regulation 91/493/EWG has laid down requirements for handling of fishery products on shore and on board of factory vessels, with regard to nematodes in fish prior to market release. Any visible parasites have to be removed, and all fish intended for raw consumption (sushi and sashimi) has to be kept frozen at –20°C for at least 24 hours. Additional processing requirements have been included in the German and other national fish hygiene directives regulating the marinating, smoking and salting procedures. Common household preparation habits have been studied within an EU-project (FAR UP-1-18), and food safety recommendations are available.

Today, no official viability test for fish nematodes exists, but a standardised method is in preparation by the Codex Alimentarius Committee on fish and fishery products. Current regulations can protect the European consumer effectively against infections with live nematodes. However, dead nematode larvae may still be found in marine food products, and, although not a direct health hazard, rendering them much less appealing to the consumer.

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MOLECULAR PHYLOGENY OF MICROSPORIDIA WITH EMPHASIS ON SPECIES INFECTING FISH

By F. NILSEN

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Since the description of the first microsporidium species in 1857, this group of intracellular parasites have been classified together with several unrelated taxa. Some of the characteristics for the microsporidia are intracellular development, lack of mitochondria in all developmental stages and spores containing a polar tube. Microsporidia possess several features atypical for eukaryotes. It was discovered relatively early that the microsporidia possessed ribosomes of an unusual size and studies indicated that their 70S ribosomes were closer to the bacteria than typical eukaryotes. Sequencing of the small subunit (SSU) rRNA confirmed the special anatomy of the microsporidia ribosomes as the *Vairimorpha necatrix* sequence was shorter than similar sequences from bacteria. Phylogenetic analysis revealed a very basal position of the microsporidia in the eukaryotic tree. The basal position of the microsporidia was later confirmed using EF-1α and EF-2 and it was believed that the microsporidia was one of a few protozoans that split off the eukaryotic tree prior to the acquisition of mitochondria. This led to the creation of the kingdom Archezoa for eukaryotes that were assumed to be ancient amitochondrial. However, studies using other protein coding genes questioned the "microsporidia early" hypothesis as these studies showed that microsporidia where related to fungi. Currently, Microsporidia is a phylum within the fungi (i.e. the subkingdom Eumycota). Current classifications of the microsporidia are based upon morphology and ultrastructure of spores and lifecycle stages. Utilisation of molecular techniques in phylogeny has improved the understanding of relationship between many groups of organisms. When similar methods were used for microsporidia, it was soon realised that many of the important characters used in the classification of these parasites were not producing monophyletic taxa. Microsporidia infects a wide variety of hosts, ranging from other unicellular organisms to man. They are particular common in insects and arthropods but fish is also a common group of hosts. Where as the species infecting insects appear to be a heterogeneous group, the majority of microsporidia infecting fishes seems to be closer to each other, judged from rDNA phylogenies. In the presentation, current status of microsporidia phylogeny and classification will be presented with emphasis on species infecting fish.

THE IMPACT OF PARASITISM ON THE BIODIVERSITY OF INTERTIDAL ECOSYSTEMS

By ROBERT POULIN

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Few field investigations have demonstrated that parasitism can influence the composition and structure of natural animal communities. Typically, the impact of parasites is believed to extend only to species within the community that serve as hosts for the parasites, as well as other species that interact directly with these host species. For instance, some parasites mediate competitive interactions between animal species, by depressing the abundance of one competitor more than that of the other; the net effect at the community level is that the relative abundances of a few species are different from what they would be in the absence of the parasite. Thus, the effect of any given parasite is often restricted to only a subset of the community. In many situations, however, parasites can impact key members of the community and thus indirectly affect the entire community. We demonstrated community-wide impacts of a single parasite in a New Zealand intertidal community. Juvenile stages of the trematode *Curtuteria australis* encyst in the foot of cockles, *Austrovenus stutchburyi*, and impair the burrowing ability of cockles. This results in heavily-infected cockles remaining stranded at the surface of the sediments, where they are easy prey for oystercatchers, the trematode's definitive host. Cockles are the dominant bivalves in New Zealand soft-sediment intertidal zones. Normally, they are buried 2-3 cm under the sediment surface. In areas of intense parasitism, however, large numbers are either protruding from the sediments, or merely lying at the surface. Parasitism therefore results in an increase in the heterogeneity of the sediment surface structure, with likely effects on seabed hydrodynamics and sediment bioturbation and deposition. The trematode indirectly causes microhabitat changes that should affect the settlement and survival of all other macrofaunal benthic species. To test this, we manipulated the density of surface cockles in experimental plots in a replicated block design. Benthic communities were then allowed to adjust over a 6-month period covering one austral summer. At the end of the experiment, there were significantly more species of macrofaunal invertebrates (polychaetes, molluscs, crustaceans, etc) in the treatments with added surface cockles than in control plots. In addition, the density (numbers per core sample) of various taxa also varied among plots, with densities observed in plots with added surface cockles being higher than those in control plots. Analysis of sediment samples taken in all plots indicates significant differences in sediment characteristics, such as mean particle size, between the treatments. The combined influence of the added surface structure and the altered sedimentation regime is the likely proximate explanation for the higher invertebrate colonization rates observed in plots with added surface cockles. These results illustrate well how a single parasite species can have community-wide effects on the diversity and abundance of non-host organisms.

VACCINES AND THE EVOLUTION OF PARASITE VIRULENCE

By A.F. READ[1], S. GANDON[2], S. NEE[1], & M.J. MACKINNON[1]

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As exemplified by the spread of drug resistance and vaccine-escape mutants, parasite evolution is of substantial practical significance. Less frequently considered by those responsible for public and animal health is the possibility that the evolution of pathogen virulence could also be affected by medical and veterinary interventions such as chemotherapy, vaccination and enhanced resistance through animal breeding. Our work with animal and mathematical models of malaria shows that at least some candidate vaccines could prompt the spread of more virulent malaria on timescales comparable to that of the spread of chloroquine resistance. This evolution, which would not be seen in a short-term clinical trial, would erode the population-level benefits of a vaccination programme and put unvaccinated individuals at greater risk of death. Data on the past evolution of some other pathogens is consistent with the general idea that clinically relevant virulence evolution can occur. Even though virulence surveillance is not easy, particularly for human pathogens, there is a very real need to understand how virulence evolution is altered by both inadvertent and well-intentioned modifications of pathogen ecology.

SUBMITTED PAPERS - ORAL PRESENTATIONS

EFFECTS OF PARASITISM BY HAEMATOPHAGOUS ECTOPARASITES ON REPRODUCTIVE SUCCESS IN THE PIED FLYCATCHER (*FICEDULA HYPOLEUCA* PALLAS)

By A. BREISTØL & G. HÖGSTEDT

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Effects of ectoparasites on nesting Pied Flycatchers (*Ficedula hypoleuca*) were explored in a population in Southern Norway. The ectoparasites were mainly fleas (Siphonaptera) and mites (Acari). To evaluate the effects the parasites had on their hosts, a partial crossfostering experiment was performed between 15 pairs of nest boxes. In each pair one nest box was fumigated with a solution of pyrethrin (an insecticide harmless to birds) while the other remained a control. The chicks were measured every second day and haematocrit levels were determined when the chicks were 13 days old. After fledging all nests were collected and parasite load determined. There was no significant difference in nesting success, body mass or wing length between fumigated and control nests. However, there were significantly higher haematocrit levels in fumigated broods compared to control broods. Also the nestling period was significantly shorter for the control group. Hence, nestlings in parasite-infected nests fledge with less developed wings, which may increase predation risk compared to parasite-free chicks. The conclusion is therefore that the effects of parasites on this population of pied flycatcher are moderate, but potentially important for reproductive success. However, the lack of differences in growth variables may be due to favorable nesting conditions this year and/or compensation in feeding rates from the parents.

RESPONSES IN FISH HOSTS AGAINST MONOGENEANS – AN UPDATE

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It has been estimated that the taxon Monogenea comprises more than 24,000 species. The majority are parasitic on teleosts. This makes this class one of the most successful fish parasite groups suggesting that evasion mechanisms are well developed among monogeneans. Nonetheless, during the latest 70 years a number of studies have elucidated the capability of fish hosts to mount a response against these parasitic in-

fections. A range of papers have been presented demonstrating acquired or innate resistance of fishes belonging to many teleost orders to these platyhelminths. Best characterized is the response in cyprinids, anguillids, percids and salmonids. Attempts to vaccinate fish against monogenean infections have been conducted at several occasions with some success. Despite this the mechanisms involved in the responses are not clearly defined. Recent work has elucidated various factors of the host immune system that are likely to be at least partly responsible for the anti-monogenan activity in teleosts. Thus, humoral factors such as antibodies, complement components and lectins are known to be active in host-monogenan associations. Cellular factors comprise leukocyte action and mucous cell activity. It is probably an intricate interplay of various factors that is responsible for the host response and a model of the reactions in the teleost epidermis is presented and discussed. The implications for host specificity of these interactions between fish and monogenean are discussed.

SEVERE MALARIA: PROTECTION AGAINST THESEQUESTRATION OF PLASMODIUM FALCIPARUM-INFECTED ERYTHROCYTES BY VACCINATION WITH A PFEMP1-DBL1A VACCINE

By Q. CHEN, F. PATTERSSON, A. VOGT, B. SCHMIDT, P. LILJESTRÖM & M. WAHLGREN

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A family of parasite antigen known as PfEMP1 plays an important role in the binding of infected erythrocytes to human receptors in the micro-vasculature and causes severe/cerebral malaria. They are strongly implicated as virulence factors. PfEMP1 expressed by parasite isolates causing severe malaria is highly immunogenic and commonly recognised by human immune sera. We have been exploring the possibility of using a Duffy-binding like (DBL) domain of PfEMP1 from a multi-adhesive parasite with a phenotype associated with severe malaria as an anti-severe malaria vaccine candidate. DBL1α domain is present in all PfEMP1 molecules and is the most conserved functional domain in the PfEMP1 molecule. A mini-PfEMP1 gene encoding a DBL1α domain and a transmembrane sequence was constructed to mimic PfEMP1expression, transportation, *in vivo* folding and presentation pathways. The mini-gene was cloned into a Semliki forest virus (SFV) vaccine vector and packaged into RNA particles. We have immunized more than 200 small animals (mice, rats and rabbits) with DBL1α RNA particles and generated very encouraging results. Antibodies to the DBL1α domain are able to recognise life-infected erythrocytes, disrupt preformed malaria rosettes and block parasite adhesion in micro-vasculature in vivo. The data support the further investigation of using PfEMP1 as an anti-severe malaria vaccine candidate.

GENETIC VARIATION BETWEEN *GYRODACTYLUS SALARIS* MALMBERG, 1957 POPULATIONS IN NORTHERN EUROPE

By C.O. CUNNINGHAM[1], T.A. MO[2], A.J.A. McBEATH[1] & C.M. COLLINS[1]

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Gyrodactylus salaris has been the most intensively studied member of the genus due to its' deavastating effects on natural populations of Atlantic salmon, Salmo salar L., in Norway. The species has also been reported in several other European countries. Some of these reports have been misidentifications and the use of molecular techniques has improved diagnosis of this pathogenic species. These methods are now being applied to the study of different populations of G. salaris. No intraspecific variation has been found in a variable region of the small subunit (18S) ribosomal RNA (rRNA) gene or the internal transcribed spacer (ITS) region of the rRNA gene array from many specimens of G. salaris from Norway, Sweden, Finland, Russia and Denmark. Random Amplified Polymorphic DNA (RAPD) demonstrated genetic differences between G. salaris from different rivers in Norway, but the method is difficult to reproduce.

Analysis of the intergenic spacer (IGS) that separates individual units of the rRNA gene array revealed the presence of two regions of repetitive DNA that strongly resemble satellite DNA, short repeated sequences that can be highly variable and useful as population markers. Analysis of the sequence of this region from several different populations of G. salaris was carried out to investigate the utility of these sequences for population discrimination. Population differences have been detected and correspond to the patterns expected from limited introductions of the parasite to Norway.

PATTERNS IN MACRO- AND MICROSCALE DISTRIBUTION OF TREMATODE INFECTIONS IN MUDSNAIL *HYDROBIA VENTROSA* POPULATIONS IN ICELAND

By K.V. GALAKTIONOV[1], E.V. KOZMINSKY[1] & K. SKIRNISSON[2]

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In Iceland the mudsnail *Hydrobia ventrosa* occurs in brackish saltmarsh ponds in a few isolated areas. This study analysed the influence of several abiotic and biotic variables on the distribution of trematode infections in *H. ventrosa*, inhabiting ponds on the Melabakkar and Galgahraun saltmarshes in SW Iceland.

Eleven trematode species were found to infect the snails; *Microphallus pirum*, *M. breviatus*, *M. pseudopygmaeus*, *M. claviformis*, *Maritrema subdolum* (Microphallidae), *Cercaria Notocotylidae* sp. 11 Deblock, 1980, *C. Notocotylidae* sp. 12 Deblock, 1980, *C. Notocotylidae* sp. 13 Deblock, 1980 (Notocotylidae), *Cryptocotyle concavum* (Heterophyidae), *Himasthla continua* (Echinostomatidae) and *Psilostomum brevicolle* (Psilostomatidae). Six species were recorded in Galgahraun (with predominance of *M. breviatus*), but nine in Melabakkar (different predominant species in different ponds). Final hosts of most of these species are water birds. Different avifauna in the study areas explains the different parasite fauna composition. In Melabakkar both infection composition and prevalence in different ponds showed an extremely wide range of variation. Examination of correlations between abiotic (distance of each pond from the sea, pond elevation above chart datum, size, average depth, salinity and some characters of the littoral zone and sediments) and biotic (snail density in the ponds, vegetation cover) variables and the trematode prevalence revealed that the trematode prevalence was higher in ponds which were situated higher above datum, had relatively lower banks and had a more sloping littoral. We suggest that birds visited such ponds more often because they were more suitable for them as a source of food (sloping littoral), and/or these ponds provided a better field of vision (afforded safety) and maybe because they also provided an easier take-off (due to more intensive wind). The principal components analysis indicated that the infection parameters were influenced by two different factors. One of them (F2) influenced the Simpson's indices of diversity and evenness, while the other (F3) influenced the trematode prevalence in the ponds. In our opinion, F2 expresses the "diversity of the avifauna in the study area" and F3 the "number of birds" or the "level of the parasite load of the birds".

As a general conclusion, we propose that trematode infections of mudsnails in Icelandic saltmarsh ponds are almost exclusively determined by the species composition, abundance and behaviour of the different final hosts, i.e. the birds that inhabit the area.

EVIDENCE FOR THE MAINTENANCE OF SEXUAL REPRODUCTION THROUGH RED QUEEN DYNAMICS IN THE BARK BEETLE *IPS ACUMINATUS* (CURCULIONIDAE, SCOLYTINAE)

By STEPHANIE HAMM, L.R. KIRKENDALL, A. SKORPING

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The Red Queen model for the maintenance of sex predicts that parthenogenesis should be favoured in populations with a low risk of parasitism, whereas clonal females should on average be more parasitized than sexual ones where the two female types coexist. We found some evidence for this in *Ips acuminatus* (Curculionidae, Scolytinae) populations in Scandinavia. Here sexual and clonal females live sympatrically, but the proportion of parthenogenetic individuals per population is highly vari-

able. Bark beetles were parasitized by two nematode species, one occurring in their body cavity, the other in their digestive tract. At sites where both nematode types were prevalent, *Ips acuminatus* populations with a high fraction of sexually reproducing females (over 40%) occurred. In these populations, the total nematode load, as well as the infection load of each nematode species alone, were higher than in populations with a high fraction of clonal females, and clonal females were more infected by body cavity nematodes than sexual ones. In other populations, we found no difference in infection load between sexual and clonal females. Theoretically, clonal diversity might erode any advantage to sex where parasites are transmitted horizontally (between families). Due to the special biology of *Ips acuminatus,* probably mostly vertical transmission occurs in sexually biased populations, whereas horizontal transmission is assumed to be higher in clonal populations. We therefore suggest that in the latter populations parthenogenetic females might be better able to defend parasites than clonal females in sexually-biased populations.

MOLECULAR SYSTEMATICS AND PHYLOGEOGRAPHY OF *GYRODACTYLUS* (MONOGENEA) PARASITIZING SALMONIDS IN NORWAY AND SWEDEN

By H. HANSEN, L. BACHMANN & T.A. BAKKE

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To shed light on the taxonomic riddle of the closely related species Gyrodactylus salaris Malmberg from Atlantic salmon (Salmo salar) and G. thymalli Zitnan from grayling (Thymallus thymallus) and to investigate the epidemic of G. salaris in Norway, the mitochondrial cytochrome oxidase I (COI) gene was studied. Approximately 800 bp of CO1 from 76 Gyrodactylus specimens from 32 salmonid host populations of S. salar (27), T. thymallus (4), and O. mykiss (1) in Norway, Sweden and Latvia were sequenced.

The obtained COI sequences indicated a substantial intraspecific differentiation of G. salaris and G. thymalli. Twelve haplotypes were identified which group into five well supported clades; three clades with parasites from Atlantic salmon and two clades with parasites from grayling. The basal nodes linking the five clades together are only weakly supported, and there is no support for the monophyly of all G. salaris haplotypes and the monophyly of all G. thymalli haplotypes. The lack of monophyly indicates that instead of retaining G. salaris and G. thymalli as two species, they could instead be treated as one polytypic species or as a complex of more than two sibling species.

The mtDNA data imply that several clades of both G. salaris and G. thymalli have been introduced into Norway. A minimum of three introductions of G. salaris and two introductions of G. thymalli are supported. The present results on G. salaris are con-

gruent with earlier hypotheses of anthropochore introductions of the parasite into Norway.

THE DISTRIBUTION AND ECOLOGY OF *GYRODACTYLUS* SPECIES ON STICKLEBACKS IN THE UK

By P.D. HARRIS[1] & J. CABLE[2]

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Gasterosteus aculeatus occupies a variety of habitats in the UK, ranging from mountain tarns to intertidal rock pools, making it ideal for comparative studies of the factors influencing its gyrodactylid fauna. *Gyrodactylus gasterostei*, *G. arcuatus*, *G. branchicus* and *G. alexanderi* infect *G. aculeatus.* The most widespread is *G. arcuatus*, ranging from fully marine habitats (rock pools, lagoons and drainage ditches) to pure freshwater. *G. branchicus* is rare and may have been confused with *G. rarus* in the past. It is limited to intertidal rock pools in the North, and to salt marsh drainage ditches in the South East. *G. gasterostei* is widespread in southern England, but is stenohaline, and does not infect marine sticklebacks. A shift in site specificity occurs in these gyrodactylids. In both freshwater and marine habitats, monospecific infections of *G. arcuatus* occur on the skin, fins and gills of the host. In freshwater, in the presence of conspecific *G. gasterostei* infection, *G. arcuatus* becomes restricted to the gill chamber, moving onto the skin when *G. gasterostei* is rare. Conversely, in marine habitats where *G. branchicus* is found in the gill chamber, *G. arcuatus* is restricted to the skin. The fourth species infecting *G. aculeatus* is the enigmatic *G. alexanderi.* Originally recorded only from Windermere, there are now several sites for this North American gyrodactylid from Scotland. The distribution of these gyrodactylids has been strongly influenced by post-glacial recolonisation of the UK by their hosts. The euryhaline and holarctic *G. arcuatus* is ubiquitous, and also occurs in Iceland, suggesting survival in the periglacial environment and recolonisation via river mouths. *G. branchicus*, also representative of a holarctic group spread around northern coasts in the same way, but being limited to high salinity, has been unable to penetrate inland. The southern North Sea populations of this species may be isolated from northern populations, have a different ecology, and may be distinct. *G. gasterostei,* unable to disperse through sea water, has a distribution centered on western and central Europe; it reached the UK through the Rhine/Thames watershed connection c. 7000 years ago, and has spread within the UK via the 18th Century canal network. It is still rare in the North and West. *G. alexanderi* is only found in the North and West. Its status in Europe may be that of glacial relict, excluded by competition with the more recently evolved *G. gasterostei*.

TEMPORAL VARIATION IN BODY MASS, BROOD SIZE AND HOST POPULATION GROWTH RATES OF WILLOW PTARMIGAN - A ROLE FOR PARASITES?

By P. HOLMSTAD & A. SKORPING

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In birds, a number of studies have demonstrated a correlation between body mass and fitness. It is therefore important to understand what causes variation in body mass of birds. Parasites may affect host body mass, and many studies have found a negative relationship between host body mass and parasite abundance, but other studies have reported no or even positive correlations between body mass and parasite load. However, most field studies have been conducted over short time intervals relative to host life expectancy, and the bulk of these only account for single parasite species or smaller subsets of the parasite species present in the host population.

In willow ptarmigan, we have monitored changes in population densities biannually for 13 years at the island Kvaløya in Northern Norway, and examined host samples for all known, eucaryote parasites for the last 11 years. Since parasite intensities in willow ptarmigan tend to covary, we need to correlate measures of host fitness with variation in the whole infracommunity rather than with single parasite species. Our objectives were i) to investigate whether temporal changes in body mass occur in juvenile willow ptarmigan, ii) what parameters such changes in body mass were correlated with and iii) whether parasite abundance were correlated with changes in host reproduction. We found a significant temporal variation in body mass of juveniles that covaried between sexes, with mean body mass ranging from 452.4 grams (2001) up to 540.2 grams (1996) in juvenile hens, and from 502.1 grams (2001) up to 600.7 grams (1997) in juvenile cocks. Body mass of juveniles were higher during the increase and peak phase of density change in the host population. Between-year variation in body mass showed a negative correlation with parasite abundance. Host reproductive parameters like brood size and population growth rate also showed a significant, negative relationship with parasite abundance.

INTERACTIONS BETWEEN ADULT AND JUVENILE SALMON LICE ON THEIR HOSTS REDUCE THEIR VIRULENCE

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Fertilised salmon-lice females (*Lepeophtheirus salmonis*) may produce at least 11 sets of egg-strings on their host. Eggs are released to the water column and the larvae undergo two free-living stages before becoming infective copepodids. After attached

to their salmonid host, they develop through four immobile and two pre-adult stages. Salmon lice show a sudden increase in virulence on their host after appearance of the mobile preadult stages. Heavy larvae infections may after three weeks at 10° C cause either host death or premature migration to freshwater, where the lice are killed. Hence, lice on heavily infected hosts experience reduced fitness because increased host mortality limits the number of egg-clutches produced by adult female lice.

Infection parameters on wild fish indicate, however, that there is a negative relationship between the presence of adult lice and younger stages. Thus, there may be an interaction effect between adults and larvae on the host, reducing the negative impact of new infections.

Interactions between adult female salmon lice and younger stages were examined by experimentally infecting hatchery-reared post-smolts with adult females three days prior to standard infections with copepodids, three days post copepodid infection and finally copepodid-infections without presence of any adult females. Our results show that after eight days, intensity of chalimus larvae originating from the standard infection was significantly higher in the two tanks where no females were introduced. Moreover, the intensity was slightly but significantly higher in the two tanks where adult females were introduced three days after infection compared to the two tanks where females were introduced in advance. This indicates that differences are not due to changes in larval preferences of hosts already infected with adults. Finally, number of larvae on individual fish was slightly but significantly negatively correlated to number of females on the individual salmon smolts.

Although the mechanism remains unknown, adult lice are able to prolong host-survival and indirectly lifetime reproductive success, by reducing the presence of younger stages on their hosts.

PARASITE-INDUCED HOST CASTRATION AND ITS EFFECT ON HOST LIFETIME GROWTH

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Some studies show that parasites have a negative effect on host growth, while others show exactly the opposite. This discrepancy may be a matter of where in the growth curves the comparisons are made. Early in a host life, a parasite-induced host castration may for instance increase growth because more resources are available for somatic growth instead of gonadal development. On the other hand, at later stages of an infection, parasite biomass may be too large to allow further host growth, and growth rate of infected hosts may be lower as compared to uninfected ones. This study compares lifetime growth curves of healthy and infected *Daphnia magna*

(Cladocera: Crustacea). The pathogen used is the bacterium *Pasteuria ramosa*. Different correlations are performed between parasite-induced castration and host growth, and between parasite biomass and host growth.

INTER- AND INTRA-MORPH PATTERNS IN HELMINTH COMMUNITIES OF SYMPATRIC SALMONID MORPHS

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Infection patterns of trophic transmitted helminth parasites were compared with feeding ecology in sympatric morphs of salmonids (whitefish, *Coregonus lavaretus*, and Arctic charr, *Salvelinus alpinus*) from three lake systems in northern Norway. There were clear intermorphic differences in diet and habitat niches within each of the lake systems, which were associated with differences in trophic morphology and community structure of parasite infection. Within both whitefish and Arctic charr, one morph utilised a broad niche and the other morph had a narrow niche. The morphs with a broad habitat niche (generalistic: littoral and pelagic habitats) had a more diverse parasite community compared to the morph with a narrow niche (specialist: pelagic or deep-water habitat). Additionally a clear intramorphic pattern occurred as the generalistic morph with a broad diet and habitat niche typically utilised both the benthivore and zooplanktivore trophic niches. In all lakes, the species composition and intensities of helminths reflected the trophic diversification of salmonid ecotypes with respect to different habitat choice (generalistic *versus* specialist) and dietary specialisation (benthivore *versus* zooplanktivore feeding strategies within the generalistic morph). Zooplanktivore ecotypes typically had acquired parasites mainly from pelagic copepods, while benthivore feeders had the highest proportion of parasites with transmission stages from benthic organisms. The host feeding behaviour seems to be a major determinant of the helminth community structure, and helminths appear to be useful indicators of long-term trophic specialisation of salmonid ecotypes.

PATHOLOGICAL NOTES ON AVIAN BLOOD-FLUKE INFECTIONS

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Compared to the mammalian schistosomes, represented by the well-known genus *Schistosoma*, the genera that infect birds are usually thought to be of much less importance. However, the general paucity of reports dealing with clinical aspects of avian schistosome infections can, at least in part, be explained by the fact that these small

parasites, hidden within the blood vessels of the host, are often overlooked during gross necropsy. Recent studies on *Trichobilhazia* (the largest genus of the family Schistosomatidae) have shown that these parasites are able to cause a disease, which is comparable with human schistosomosis. Similar complications are expected for blood flukes belonging to other avian schistosome genera.

Previous work has mainly focused on symptoms occurring during the patent phase of infection, when eggs are disseminated to various organs of the final host, where granulomatous reactions develop around the eggs. However, adult *Trichobilharzia* worms also can cause lesions, because accumulation of worms may be accompanied by thrombi in veins with subsequent extensive haemorrhage and perivascular cell infiltrations around the vessels.

Recent investigation have elucidated that immature flukes may also cause severe tissue injuries. The affinity of immature nasal *T. regenti* for nervous tissue can cause severe injury due to the feeding, migration and establishment of the parasites in the CNS. The infection induces inflammatory reactions and pathological changes in the nervous tissue (e.g. eosinophilic meningitis).

Avian schistosomes have mainly been detected in Anseriformes but have also been found in the orders Podicipediformes, Ciconiiformes, Coraciiformes, Passeriformes, Galliformes and Columbiformes. Usually, birds are expected to acquire infections in the breeding areas. The parasites can also be transported to and from wintering areas of the birds, either as mature flukes within the host, or as larvae within snails, which are attached to the final host. Due to the host specificity of *Trichobilharzia* larvae introduced species require compatible snails for the establishment of the life cycle. When compatible snails are present, severe complications may arise, e.g. in bird keeping, in cases when effort is focusing on the protection of endangered species, in ponds or reservoirs which are located in parks or zoological gardens and also in natural water systems.

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BIOGEOGRAPHY OF PARASITES AND UNDERSTANDING OF THE SPECIES CATEGORY IN PARASITOLOGY

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Biogeography of the majority of groups of parasites has been insufficiently studied. However, data on the subject are important for the understanding of numerous problems in parasitology. The main aim of this study is to discuss some general issues

of the geographical distribution and taxonomy of several groups of parasites based on our long-term parasitological studies and the literature data.

The analysis of the distribution of helminths of mustelids in the Holarctic has revealed that a small numbers of helminths get into new ranges during the migration of their hosts. Immigrants obtain most of their parasites from the aboriginal host species in new ecosystems. Thus, we may approach the formation of new helminth species as an ecological process determined as a hostal radiation as distinct from the parallel evolution of hosts and parasites. Considering the available data, the latter makes a single, but important way of evolution for some groups of helminths. This conclusion is in agreement with the data of analysis of the helminths fauna of charadriiform birds in Alaska, showing that during the post-glacial period more significant changes occurred in the fauna of birds than in the fauna of helminths. Thus, the 'conservatism' of faunal helminth complexes may be regarded as a good marker of paleobiogeographical events.

Attempts to confirm the conclusions obtained during the biogeographical analysis of helminths with the data on the distribution of two groups of parasitic protists (species of Haemosporida of birds and *Eimeria* spp. of rodents) have revealed that the character of their distribution is different. Among the haemosporidians, the number of cosmopolitan species is much higher. It looks likely that the current distribution of bird haemosporidians in the Holarctic is a secondary phenomenon related to the adaptation of 'tropical species' to the northern conditions and their evolutionary rapid distribution in the middle and high latitudes. There are no cosmopolitan species of *Eimeria* of rodents, except a few parasites of synantropic and relatively recently acclimatised hosts.

The differences in the biogeography of helminths and protists can be explained by differences in their ecology and evolution as well as by unequal treatment of species category in the taxonomy of helminths and unicellular parasites, or by combination of both these factors.

DO PARASITES INFLUENCE REPRODUCTIVE EFFORT IN ATLANTIC COD?

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All organisms should optimise current reproductive effort in order to maximise lifetime reproductive output. As a consequence investment in reproduction should increase with age for iteroparous organisms, compared to the investment in other life history parameters, for example fat storage and muscle mass. If parasites reduce a host's future reproductive value, they should have a similar effect as age on current reproductive effort. Atlantic cod (*Gadus morhua* L.) can be a useful host to examine the effect of parasites on reproductive effort. Coastal cod do not undertake extensive

spawning migrations and a major part of reproductive effort can therefore be measured as weights of gonads relative to body mass. In the present study six hundred Atlantic cod were individually marked and caged for 19 months. During this period each cod was inspected for *Lernaeocera branchialis* (L.) at several intervals. At the end of the study period we counted the number of *Anisakis* sp. in each cod and recorded the weight of different organs. Age, sex and body condition all showed a significant influence on the relative mass of gonads. The most pronounced effect of parasite infections appeared to be indirect, through the influence on body condition. Variation in relative gonad mass will be discussed in the context of life history theory.

MORPHOLOGY, BIOLOGY AND DNA AS A BASIS FOR DESCRIPTIONS OF SPECIES IN *GYRODACTYLUS* (MONOGENEA)

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On a basis of morphology (mainly opisthaptoral hard part differences; in few cases improved by morphometric analyses) and biology (e.g. host, host organ, spreading capacity) even very similar *Gyrodactylus* species have been described. Anchors and ventral bars of two species can be very similar, while their marginal hook sickles show clear species differences e.g. *G. rarus* and *G. branchicus*. Sometimes such differences are overlooked, e.g. in the description of *G. truttae*. In other cases, the presence of seasonal variations may cause problems for the evaluation of borders between two similar forms. Combined morphological and genetical analyses can reveal whether or not the differences are on a species or an intraspecific level. During the last years I have been involved in interesting co-operations with colleagues working in the genetical field, mainly in Scotland, Belgium, Poland and Finland. Our combined studies revealed the presence of e.g. two very similar species - but described as one, and the presence of three morphologically very similar species on closely related goby species. One of the latter *Gyrodactylus* species was initially determined as a described similar species, while its biology and morphometrical analyses indicated a separarte species.

Very low genetic distance was observed between similar but morphologically and even morphometrically clear species. An example of a morphologically based grouping not fitting perfectly with the genetical information was revealed by a *Gyrodactylus* species parasitizing a goby host. From a morphological point of view, however, this species is closely related to/belonging to the same species group as *G. quadratidigitus*, parasitizing another goby host. Recently published DNA-results regarding the subgenus *G.* (*Limnonephrothus*), including the *G. wageneri*-group fit in the main with grouping on a morphological basis.

FASCIOLA HEPATICA: MORPHOLOGICAL ALTERATIONS FOLLOWING TREATMENT IN VIVO AND IN VITRO WITH NITROXYNIL

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This project was carried out to study the morphological effects of nitroxynil, a halogenated phenol drug, on the liver fluke *F.hepatica*. Male Sprague-Dawley rats were dosed orally with Nitroxynil at a concentration of 40 mg/kg and adult flukes were recovered after 24h, 48h and 72h. A 24h *in vitro* experiment was also carried out at a concentration of 100 μg/ml. Morphological changes to the flukes were monitored by means of SEM and TEM.

The SEM results demonstrated extensive disruption to the tegument. At 24h treatment *in vivo,* swelling and blebbing of the tegumental syncytium was observed. Similar disruption was recorded at 48h *in vivo,* along with more severe disruption, with stripping of the anterior apical plasma membrane and a single large swelling of the dorsal mid-body region. After 72h, some flukes had already disintegrated, with specimens exhibiting sloughing of the anterior tegumental syncytium and large holes through the body of the fluke. The disruption seen at 24h *in vitro* resembled that in 48h *in vivo.* The overall spread of disruption was similar *in vivo* and *in vitro*, with the dorsal surface more severely disrupted than the ventral surface and the anterior region of the fluke more than the posterior region.

TEM observations determined the internal changes in the tegumental syncytium and the gut induced by nitroxynil. In both tissues there was little evidence of disruption until 72h *in vivo* and 24h *in vitro.* At these time periods, there was pronounced swelling of the mitochondria and of the mucopolysaccharide masses surrounding the basal infolds in the tegument, also swelling of the tissue around the sub-tegumental muscle blocks. The only changes observed in the gut cells were swollen mitochondria and cisternae of granular endoplasmic reticulum.

The results confirm that nitroxynil, *in vivo* and *in vitro,* causes progressively severe morphological disruption with longer exposure periods. Nitroxynil is seen to affect both absorptive surfaces however; the tegument appears more disrupted than the gut even though the gut may be equally important as a route of entry for the drug. The tegument is the parasite's main protection against foreign compounds and, if disrupted, this would allow the drug access to other internal tissues, leading to more widespread damage.

ESTABLISHING HARVEST LOCATION FOR ATLANTIC COD: PLAYING SHERLOCK HOLMES WITH PARASITES

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Although parasites have been widely used for discrimination of fish stocks, few studies have attempted to use parasite clues to establish the harvest or spawning location of individual fish. The project CODTRACE under the 5FP aims to develop a number of biological markers, including parasites, to establish the harvest and spawning location of Atlantic cod, *Gadus morhua,* in European waters. To illustrate the difference between the 'stock discrimination' and 'traceability of harvest location' concepts, we use preliminary parasite information from the CODTRACE project. The dataset concerns 148 cod harvested in three basins: Baltic (N = 60), Irish (N = 60) and North (N = 28) Seas. These cod harboured 32 metazoan parasite species, totalling about 19,000 parasite specimens. A categorical principal component analysis of parasite abundances indicated some stock segregation of cod between the three basins. To evaluate the ability of parasite abundances to predict the harvest origin of each individual cod, we optimised two neural networks (Bayesian Network and Radial Basis Function) using the abundance of only four species: *Derogenes varicus, Anisakis simplex, Hystherothylacium aduncum* and *Contracaecum* sp. The Bayesian Network could correctly allocate to their harvest location 94 % and 97 % of cod in the training and test sets, respectively, whereas the corresponding percentages for the Radial Basis Function were 98 % and 99 %. We conclude that parasites show promise as biomarkers of the capture location of cod, at least at the coarse geographical scale considered. In terms of cost-efficiency, our study suggests that a set of few parasite species might be enough to produce legal evidence for harvest location of cod. However, the Sherlockian exercise will become more complex as more cod and additional basins are incorporated in the study, and probably it will require information from six to ten parasite species to obtain comparable classification success.

CHROMOSOME STUDIES IN DILEPIDID CESTODES

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Polyphyly of the classical Dilepididae *sensu lato* is evident. The diversity within the family Dilepididae *sensu* Bona (1994) is also considerable and it is likely to constitute a polyphyletic assemblage. It is evident that the dilepidids requires further evaluation. Chromosomal studies should be widely applicable to the problems of sort-

ing out groups of phyllogenetically related species. Unfortunately, chromosomal data for dilepidids are very limited.

Chromosome studies were initiated in the three species of dilepidid cestodes in order to obtain species-specific characteristics which could be used both in the identification of the species and in establishing subfamiliar groups. *Molluscotaenia crassiscolex* showed diploid number 2n = 12. The karyotype included five metacentric and one submetacentric chromosome pair (No 2). The chromosomes were small; the largest measured 3.26 μm and the smallest were 1.47 μm. They can be divided into two length groups: large elements of 1-3 pair, and smaller elements of 4-6 pair. *Anomotaenia bacilligera* revealed diploid number 2n = 16. The karyotype was composed of biarmed elements; six pairs were metacentric and two pairs (No 5 and 7) were meta-submetacentric. They decreased in size fairly gradually from 4.01 to 2.13 μm. A modal diploid number 2n = 18 has been determined in *Dilepis undula.* The only other karyological information available for dilepidid cestodes concerns the chromosome number of two species: *Liga brasiliensis*, 2n = 14, and *Choanotaenia* sp., 2n = 16.

Cestodes, in general, are fairly conservative karyotypically, with related species on generic and even family levels differing by a few chromosome rearrangements and possessing 'typical' karyotypes. Polymorphism of dilepidids, observed in morphological and ecological characters, is also obvious on karyological level.

ARE CHEWING LICE INTERMEDIATE HOSTS FOR THE CESTODE *HYMENOLEPIS MICROPS* IN WILLOW PTARMIGAN?

By D. PISTONE, P. HOLMSTAD, T. CIEPLINSKA, N.K. ELLINGSEN & A. SKORPING

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The cestode *Hymenolepis microps* is a common intestinal parasite in willow ptarmigan. *H. microps* is able to maintain high prevalences even at relatively low host densities in strongly seasonal arctic environments. This requires effective transmission routes. Members of the family Hymenolepididae normally require arthropod intermediate hosts, although there are exceptions with monoxenous life cycles. In the case of *H. microps*, the intermediate host(s) is not identified.

Willow ptarmigan consumes plant material throughout the year, and active consumption of invertebrates are confined to the first two-three weeks of their life, making transmission by accidental ingestion of infected invertebrates less likely. However, a typical feature of ptarmigan behavior is that they roost on top of their fecal pellets, in order to utilize the residual heat. This behavior may lead to entrapment of cestode eggs in ptarmigan feathers, which in turn may be ingested and undergo further development in chewing lice (Mallophaga). Ptarmigan do ingest chewing lice actively

while grooming, and when the insect is digested the cestode cysticercoids might be released in the intestines of the birds.

We applied standard histological techniques and light microscopy on two species of Mallophaga of willow ptarmigan, *Lagopeucus affinis* and *Goniodes lagopi*, examining them for larval and postlarval stages of the parasite. We have found structures that resemble the characteristic shape of a cysticercoid with a fully developed scolex invaginated into its body, in the abdomen of the chewing lice collected on hosts known to harbor infections of *H. microps*. These structures were not found in lice collected from uninfected hosts. This should support the hypothesis that Mallophaga might play a role as intermediate hosts in the life cycle of *H. microps*.

CERCARIAL DERMATITIS IN THE NETHERLANDS

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The interest in the occurrence of cercarial dermatitis or swimmers itch has increased in The Netherlands since the European Union envisaged and prepared a revised Directive of the European Parliament and of the Council concerning the Quality of Bathing Water. Swimmers itch has been described from The Netherlands since 1953 and has been present before that time. There appears to be an increase in the frequency of occurrence during the last 10 years, suggesting it to be an emerging disease, but records do not exist.

Infections with ocellate furcocercariae were established in *Lymnaea stagnalis*, snails from the *Radix ovata peregra* complex and *Stagnicola palustris* and the planorbid snail *Planorbis corneus.* The parasitic species involved in The Netherlands are *Trichobilharzia ocellata* and, because of this host diversity, most probably also *T. franki* and *T. regenti*. In 2002 information was obtained from 5 locations were swimmers itch occurred in The Netherlands. On these sites infections were observed in *Radix auricularia, R. ovata* and *L. stagnalis*. We obtained cercariae from *R. auricularia* from 2 sites and from *L. stagnalis* from one site. Species determination is presently undertaken by PCR.

The Health Council of the Netherlands focused the control of swimmers itch on the snails and proposed to that end the introduction of a safety chain, consisting of 5 steps in a circular sequence of control measures; pro-action, prevention, preparation, repression and aftercare/mitigation. These measures should be implemented for every recreational surface water in the country. In *pro-action* a risk profile is made in which, amongst others, the snail population is considered and bird breeding colonies recorded. Places can be assigned to a list of waters for recreational purposes or loose that assignment. In the *prevention* step snails are inspected for the presence of schistosomes. When ocellate furcocercariae are detected snails will be collected and removed from the location in a *repression* step. During *mitigation* local practitioners

can be warned and measures indicated to be taken as pro-action. Preventive measures should be taken as early in the chain as possible. Decision-making schedules for management measures were given for existing and newly made bathing water. At present efforts are made to provide provincial and public health authorities with directives to mitigate the problem through collaboration with site management.

ERYTHROCYTE ANION CHANNELS UP-REGULATED BY *PLASMODIUM FALCIPARUM*: A TARGET FOR FUTURE ANTIMALARIAL CHEMOTHERAPY.

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Until recently, electrophysiological studies on human RBCs have been difficult, and little is known of the anionic conductive pathways present in the RBC membrane. Anion permeability is particularly relevant in the context of malaria where a wealth of transport studies have demonstrated the importance of parasite-induced transport pathways for anions and other solutes in infected RBCs. The patch-clamp technique was used to investigate the membrane anionic conductance of human red blood cells (RBCs) infected by *Plasmodium falciparum.*

Unstimulated uninfected RBCs possessed negligible numbers of active anion channels. However, anion channels could be activated in the presence of PKA and ATP in the pipette solution or by membrane deformation. These channels displayed linear conductance (~15 pS), were blocked by known anion channel inhibitors and showed the permeability sequence I- > Br- > Cl-. In addition, an outwardly rectifying anion channel (~80 pS, outward conductance) was spontaneously active in less than 5% of excised patches. The host membrane of malaria-infected RBCs possessed spontaneously active anion channel activity, with identical conductances, pharmacology and selectivity to the linear conductance channel measured in stimulated uninfected RBCs.

The data are consistent with the presence of two endogenous anion channels in human RBCs, of which one (the linear conductance channel) is up-regulated by the malaria parasite *P. falciparum.* These anionic pathways are possible anti-malarial targets for selective inhibition, and routes for drug delivery.

THE DISPLACEMENT OF *BOOPHILUS DECOLORATUS* BY *BOOPHILUS MICROPLUS* IN THE SOUTPANSBERG REGION, LIMPOPO PROVINCE, SOUTH AFRICA

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In a survey in the Soutpansberg region of the Limpopo Province of South Africa conducted from May 1999 to December 2001, 25,987 *Boophilus* ticks were collected from cattle at 29 communal dip tanks and 5 commercial farms. Of these 1719 (6.6 %) were *Boophilus decoloratus* and 24,268 (93.4 %) *Boophilus microplus*, a tick which had not been reported from this area previously. *Boophilus microplus* was the most common *Boophilus* tick found on the communal cattle, whilst *Boophilus decoloratus* was the most common tick found on the commercial farms. Where *Boophilus microplus* and *Boophilus decoloratus* were found together, there was a tendency for *Boophilus microplus* to displace *Boophilus decoloratus* during the period of observation. The displacement was monitored at those dip tanks/farms where both species were found together during the survey. At these dip tanks/farms partial or total displacement of *Boophilus decoloratus* by *Boophilus microplus* occurred. The displacement was almost complete at the communal dip tanks, whilst on the commercial farms it was still in progress at the end of the survey. There have been earlier reports of a zone of sterile hybrids between *Boophilus decoloratus* and *Boophilus microplus*, which would slow down the spread of *Boophilus microplus* into new areas. The present study showed that reproductive interference was insufficient in preventing *Boophilus microplus* from spreading when the climatic conditions were favourable to *Boophilus microplus*, as the displacement in most of the area appeared to be rapid and complete. CLIMEX maps were used to show the potential distribution of the two ticks in the survey area in years with average as well as twice-average rainfall to simulate the heavy rainfall in recent years. The Ecoclimatic indices and the implication of their use are discussed.

EFFECTS OF *HAEMOPROTEUS* INFECTIONS ON THE LONGEVITY OF BITING MIDGES *CULICOIDES IMPUNCTATUS* (DIPTERA, CERATOPOGONIDAE)

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Haemosporidian blood parasites of the genus *Haemoproteus* (Haemosporida, Haemoproteidae) are common in birds all over the world. Some species have been re-

ported to cause serious diseases in avian hosts. However, little is known about the effects of *Haemoproteus* infections on vectors. Because studies on the effects of haemoproteids on the longevity of their vectors are uncommon, our objective was to follow the survival rate of the biting midges *Culicoides impunctatus* following experimental infection with *Haemoproteus belopolskyi, H. fringillae* and *H. lanii.*

The work was carried out at the Biological station of the Zoological Institute of the Russian Academy of Sciences on the Curonian Spit in the Baltic Sea in June 2001. Wild-caught females were experimentally infected by feeding them on naturally infected birds. One blackcap *Sylvia atricapilla* served as the donor of gametocytes of *H. belopolskyi* (intensity of parasitemia was 1.9%), one chaffinch *Fringilla coelebs* of *H. fringillae* (2.4%), and one red-backed shrike *Lanius collurio* of *H. lanii* (2.2%). A group of flies, which were fed on an uninfected bird, was used as a control.

Parasites were not detected in the control biting midges. For all groups of infected biting midges, ookinetes were seen in the stomach content 1-2 days post-feeding, oocysts were recorded in the midgut wall 3-4 and 5-6 days post-feeding, and sporozoites were found in the salivary glands 5-6 and 7-8 days post-feeding. The survival rate of the control group differed considerably from that of the infected groups. Non-infected biting midges lived longer than infected flies. There was a highly significant difference in the percentage of surviving flies between the control group and each infected group for all post-feeding periods ($P < 0.001$ for each infected group for all post-feeding periods). For days 1-2, 3-4, 5-6, and 7-8 post-feeding, the percentage of uninfected flies surviving exceeded that of infected flies by 2.1, 3.9, 4.6, and 4.4 times, respectively ($P < 0.001$ for each post-feeding period). The mortality rates of the infected flies were highest for days 1-2 and 3-4 post-feeding. This period corresponds to the period of time when ookinetes mature and penetrate the midgut wall and young oocysts develop. It is likely that this is the most dangerous period of life for the infected flies.

ITS rDNA REGION AS A MOLECULAR TOOL FOR *GYRODACTYLUS* SPECIES IDENTIFICATION AND DESCRIPTION

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Explicit, repeatable, unambiguous, and global species identification in the monogenean genus *Gyrodactylus* Nordman, 1832 is not easy to achieve. About 400 species have been described, but this represents a small proportion of the likely more than 20 000 total expected. The 'species' should be a category with a name, hypothesised to represent a more or less coherent evolutionary group. Delineating evolutionary groups (biological species of Mayr, 1963) is difficult simply because they are often gradual. With parasites, it is even more difficult and may further blur the taxonomic boundaries due to the scarcity of useful characters. The morphological discrimination of spe-

cies within the species-rich and diverse genus *Gyrodactylus* is mainly based on the opisthaptoral hard parts such as marginal hooks, ventral bars and anchors. These characters exhibit a range of intraspecific variation. Recent developments in microscopic techniques and data processing improve greatly resolution of morphological characters but the lack of dimensionality of morphological measures probably limits the usefulness of this approach. In this way it is possible to describe strains, but it may be not necessarily possible to classify them into meaningful groups. The large number of species and intraspecific morphological variations will always cause problems in identifying single *Gyrodactylus* specimens, especially when collected on unexpected hosts, or from previously unstudied localities. Therefore molecular methods have been introduced for distinguishing *Gyrodactylus* species. The most common sequence used is the sequence of internal transcribed spacers (ITS) region of the genomic ribosomal DNA (rDNA) repeat. It represents a part of the repeat, which encompasses small ends of 18S and 28S rRNA genes and full sequence of both spacers with centrally located 5.8S rRNA gene. The sequence was successfully used to separate 60 *Gyrodactylus* species but it seems that the question of resolution with respect to true or hypothetical species boundaries is still open. This presentation is a trial to evaluate the usefulness of the usage of ITS rDNA region for *Gyrodactylus* species delineation. Some consequences of our findings for the *Gyrodactylus* species concept will be also discussed.

SUBMITTED PAPERS - POSTER PRESENTATIONS

ALVEOLAR ECHINOCOCCOSIS AND TRICHINELLOSIS OF WILD CARNIVORES IN THE CENTRAL REGION OF RUSSIA

By O.N. ANDREYANOV, A.S. BESSONOV & I.A. ARKHIPOV

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With the aim to investigate species helminth composition and the rate of infection in carnivores in Ryazan Area (Central Russia) one examined fifty-one animals including 14 foxes *(Vulpes vulpes),* 8 raccoon dogs *(Nyctereutes procyonoides),* 29 forest martens *(Maries martes).* With account of possible *Echinococcus* infection small intestine contents were fixed and tested according to WHO recommendations. Muscles were tested on *Trichinella* using compression trichinelloscopy and artificial digestion methods. The recovered parasites were identified. Among dissected foxes there were *Alaria alata* - in 9 cases (64.29%), *Echinochasmus perfoliatus* - in 2 cases (14.24%), *Toxocara canis* - in 4 cases (28.57%), *Toxascaris leonina* - in 4 cases (28.57%), *Uncinaria stenocephala* - in 5 cases (35.71%), *Trichinella spiralis* - in 4 cases (28.57%) and *Macracanthorhynchus catulinus* - in 1 case (7.14%). In 8 tested raccoon dogs *A. alata* was recovered in 5 cases, *E. perfoliatus -in 2* cases, *T. spiralis* - in 1 case *and U. stenocephala* - in 1 case. In 29 tested martens *A.alata* was in 1 case (3.45%), *E. perfo-*

liatus - in 3 cases (10.34%) and *T. spiralis* - in 4 cases (13.79%). Among 9 helminth species found in wild carnivores only 2 species are dangerous for humans including *E. multilocularis* and *T. spirals. E. multilocularis* was registered in wild carnivores in the Central Region of Russia for the first time. This study was supported by INTAS grant 00-0685.

HABITAT SELECTION BY *CORYNOSOMA AUSTRALE* (ACANTHOCEPHALA) IN THE INTESTINE OF THE SOUTHERN FUR SEAL, *ARCTOCEPHALUS AUSTRALIS*

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We investigated the habitat selection by *Corynosoma australe* in the intestine of 28 Southern fur seals (>2 yrs old) harvested by authorized hunters in Uruguay in August 1990-1991. The intestines (mean length ± SD: 17.4 ± 4.4 m) were divided into 5 equal sections, their contents being flushed through a sieve 0.2 mm mesh. *C. australe* was the only helminth species found. All hosts were heavily infected (mean intensity ± SD: 2,561 ± 2,178; median: 1,557). Intestine length (IL) was a strong predictor of intensity (I) (best model: $I= 0.42\ IL^3 -52.2$, $r^2 = 0.746$). The percentage of juvenile females also was mainly accounted for by IL (linear relationship, partial r= 0.508). Apparently, seals are continuously infected in the winter and IL seems to be a good indicator of recruitment. Worms were found throughout the intestine, but sections 1-2 contained less than 5 % of the population. Intensity significantly differed between all sections; the concordance of differences across hosts indicated a strong site fidelity for the medium and, especially, posterior intestine. The distribution of males was significantly more anterior than that of females, so was the distribution of juvenile females compared with that of gravid females. The overall sex ratio was strongly biased against males, but the proportion of males significantly decreased from section 1 to 5. We interpret that cystacanths (with a sex ratio likely close to 1:1) excyst in the stomach, then pass along the anterior intestine to establish mainly in the medium-posterior intestine, where they develop into adults and copulate. Adult males would be lost disproportionately in the posterior intestine because of their shorter life span. The niche of gravid females significantly expanded with intensity; the position of their median worm also was negatively correlated with intensity, but the correlation actually resulted from a decrease of variability in position at higher intensities. The distribution of the reproductive population may spread and become more predictable mainly because there are more recruited individuals filling an apparently vast suitable habitat, i.e., the medium-posterior intestine.

HADWENIUS SPP. (DIGENEA: CAMPULIDAE) OFF PATAGONIA, ARGENTINA

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Digeneans of the family Campulidae infect marine mammals worldwide. Among the 8 genera described, the genus *Hadwenius* is the most diverse, with 8 species reported in the intestine of at least 16 odontocete species. However, *H. pontoporiae* in the franciscana, *Pontoporia blainvillei*, has been the only species identified at species level in the South Atlantic Ocean. In this study we report on species of *Hadwenius* found in 3 dolphin species from waters from Patagonia, Argentina, i.e., the dusky dolphin, *Lagenorhynchus obscurus* (n=12), the hourglass dolphin, *L. cruciger* (n=1), and Commerson's dolphin, *Cephalorhynchus commersonii* (n=5 from central Patagonia; n=3 from Tierra del Fuego, southern Patagonia). Specimens were stained in boracic carmine and iron acetocarmine and mounted in Canada balsam. This material was compared with vouchers deposited at the Marine Zoology Unit (ICBIBE), University of Valencia, Spain. Two species of *Hadwenius* were identified: *H. subtilis* from the dusky dolphin (prevalence: 52.17%; intensity: 42.6±51) and the hourglass dolphin (n=21), and *H. delamurei* from the Commerson's dolphin (prevalence: 55.55%, intensity: 5.2±7.36 central Patagonia; prevalence: 13.04%, intensity: 7±8.66 southern Patagonia). Although some morphological differences between our material and the vouchers were observed, we believe they may be attributable to intraspecific variability. These species had only been collected from odontocetes of the Northern Hemisphere: *H. subtilis* in long-finned pilot whales, *Globicephala melas*, killer whales, *Orcinus orca*, and white whales, *Delphinapterus leucas*, and *H. delamurei* in long-finned pilot whales and Risso's dolphins, *Grampus griseus*. The finding of these species in coastal cetaceans from Patagonia confirms the wide geographical and ecological distribution of *Hadwenius* species.

ALVEOLAR ECHINOCOCCOSIS AND HYDATIDOSIS IN ANIMALS AND HUMANS IN RUSSIA: CURRENT SITUATION

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According to literature data from the past 2-3 decades, alveolar echinococcosis in carnivores is mainly prevalent in the Far North of Russia (Nenets Autonomous District, Taimyr Autonomous District, Yakutia), in the Far East (Chukot Autonomous

District, Magadan, Kamchatka, Amur and Sakhalin Regions), but less in Siberia (Omsk Region), Povolzhje (Bashkiria, Mordovia) and in the South of Russia (Dagestan). *Echinococcus multilocularis* larvocysts can be found in the intermediate rodent hosts in the above regions. Among the final hosts most often infected by tapeworms is *Alopex lagopus* (25.44-76.7%) while *Vulpes vulpes* is infected far less (10.9-29.3%). *Canis lupus* (4.3%), *Canis familiaris* (2.3-2.8%) and *Nyctereutes procyonoides* (1.6%) are only rarely infected. The rodent species that are infected by *E. multilocularis* larvocysts in the North of Russia are *Lemmus sibiricus* (3.8-21.15%), *Dicrostonyx torquatus* (1.2%), *Microtus gregalis* (2.3%), *Mus musculus* (8.3%), *Clethrionomys glareolus* (6.5%), *Clethrionomys rutilus* (6.5-46.0%) and *Tamias sibiricus* (14.3%). In the Far East and Povolzhje, the following species are infected: *Ondatra zibethicus* (0.11-0.19%), *Clethrionomys rufocanus* (2.5%), *Microtus oeconomus* (up to 52%), *Microtus arvalis* (0.7-1.9%). The rate of hydatidoses infection among the population is quite low (in medical accounts these infections are usually not divided into cystic and alveolar ones). In 2000 there were 231 recorded patients (0.6 per 100 000 humans), in 2001-308 (0.8 per 100 000 humans). It is estimated that for human hydatidosis, 20-30% of the patients suffer from an alveolar infection while 70-80% of the patients suffer from a cystic one. According to the data on infection rates in animals, we suppose that most cases of human alveolar hydatidosis occur in the North-West (16 and 25 cases in 2000 and 2001, respectively), Privolzhsk (144 and 180 cases, respectively), Siberia (25 and 25 cases, respectively) and Far East (30 and 34 cases respectively) federal districts of Russia. This study was supported by INT AS grant 00-0685.

APLOPARAKSOIDAL CESTODES: ARE THEY MEMBERS OF THE SUBFAMILY HYMENOLEPIDINAE OR THE REPRESENTATIVES OF AN INDEPENDENT FAMILY?

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The taxonomic position of the so-called aploparaksoidal cestodes is rather vague. They are attributed either to the subfamilies Hymenolepidinae or Aploparaksinae (Hymenolepididae), or they are considered as a separate family Aploparaksidae. That is because of different attitudes towards the meaning of their morphological features in taxonomy. However, while studying the Aploparaksidae, we have come to a conclusion that they form a phylogeneticly isolated branch, which is equivalent to the Hymenolepididae, and for that reason they should be considered as a separate family of the Hymenolepidoidea. This conclusion is based on the following facts.

1. Intermediate hosts. In all life cycles studied by us (over 40 species), the intermediate hosts are species of the Annelida. In species of Hymenolepididae, the annelids have not been recorded as intermediate hosts. These differences can be consid-

ered as convincing evidence that the Aploparaksidae and Hymenolepididae belong to different phylogenetic branches.

2. Postembryonic development and morphology of metacestodes. We have found that polymorphism of the metacestodes is characteristic of the representatives of this taxon. It manifests by the evolutionary transformation of the cercomer (this structure is lost in the case of parasitism in vertebrates) into additional defensive structures. Six modifications of cysticercoid, which have not been found in the Hymenolepididae species, form a distinct evolutionary branch. Thus, there is no reason to doubt the monophyletic origin of the Aploparaksidae.

3. Final hosts and geographical distribution. The *Wardium* species parasitize only charadriiform birds, and they are considered to be cosmopolitan parasites. The *Aploparaksis* spp. also parasitize mainly charadriiforms, and *Monorcholepis* spp. parasitize only passeriforms. Species of both these genera are abundant only in the Holarctic. Based on that we may hypothesise that the *Wardium* is a primary unite of the group, which evolved before the glacial period. Presumably, species of *Aploparaksis* and *Monorcholepis* appeared in Beringia during the Pleistocene, and this group is expanding geographically at the present time.

Therefore, the species of Aploparaksidae are descendants of the ancestral branch of the Eucestoda, which is possibly more ancient group than the Hymenolepididae. At the same time, the recent Aploparaksidae is a young and evolutionary dynamic group, which is proved by the plasticity of metacestodes of the parasites as well as the range of their final hosts.

CERCARIAL DERMATITIS (SWIMMERS' ITCH) IN DENMARK, AN UPDATE ON RECENT CASES

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Cercarial dermatitis (swimmers itch) caused by cercariae of bird schistosomes (*Trichobilharzia* spp.) invading human skin has a widespread distribution in the World including Europe. It is not only of common occurrence in the southern part of Europe but has now been reported in all Nordic countries such as Norway, Iceland, Sweden, Finland and Denmark. The first Danish cases of human infection were reported in 1959 (from Hjørring, Jutland) but until recent years this zoonosis did not attract much interest. However, in the period from 1997 until 2002 a number of human cases have been reported from various counties in Zealand (Sjælland). All cases were registered following complaints from both adults and children after contact with lake water (both North Zealand and Central Zealand). Unconfirmed cases from Jutland were noted as well. Due to limited knowledge about this parasitosis in primary

health care institutions cases of incorrect diagnosing have occurred. The causative organisms, cercariae from *Lymnaea pereger*, were reported in Denmark already in 1932, but no detailed distribution map for *Trichobilharzia* is as yet available for Denmark. However, preliminary distribution studies have been initiated.

MORPHOLOGICAL AND DEVELOPMENTAL CHARACTERISTICS OF THREE GEOGRAPHICAL ISOLATES OF *ECHINOCOCCUS MULTILOCULARIS* AT LARVAL STAGE IN EXPERIMENTALLY INFECTED LABORATORY RODENTS

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We assessed some morphological and developmental characteristics of larval *Echinococcus multilocularis* (*EM*) in experimentally infected laboratory rodents. Three geographical isolates of *Em* from East Europe (EEI), Kazakhstan (KI) and Kamchatka (KamI) were used. The following characteristics were investigated: protoscolices (P) hook morphology, productivity of larvocysts (L) with respect to P and microscopical acephalocysts (MA), growth rate of L in cotton rats (CR) and white rats (WR), but also receptivity of WR to parental infection by P. The following parameters of P of each isolate were determined: total amount of hooks, total length (TL) and blade length (BL) of large and small hooks, and the ratio of BL to TL of large and small hooks. Quantitative parameters of hooks were investigated in 20 P of each isolate that developed in the abdominal cavity of CR. Productivity of L with respect to P and MA was determined by calculating them in 1 g of L from WR 4 months post infection. Growth rate of L was determined according to data of 4-10 passages in CR and WR (from 8 to 14 animals in each group). The investigation revealed statistically significant differences ($P < 0,001$) between EEI, KI and KamI for following parameters of P hooks: BL of large hooks ($12,6 \pm 0,04$; $11,8 \pm 0,05$ x $11,9 \pm 0,05$ mkm accordingly), BL of small hooks ($9,3 \pm 0,04$; $8,3 \pm 0,05$ x $8,4 \pm 0,05$ mkm accordingly), ratio of BL to TL of large ($49,7 \pm 0,17$; $46,4 \pm 0,20$ x $46,8 \pm 0,21$ % accordingly) and small ($42,0 \pm 0,18$; $38,0 \pm 0,21$ x $39,3 \pm 0,25$ % accordingly) hooks. The differences between KI and KamI for these parameters were statistically not significant ($P > 0,1$). Productivity of L of KI and KamI with respect to P was approximately on two orders below than such one of L of EEI and approximately on two orders above than such one with respect to MA. *Em* isolates had some differences in growth rate of L. The weight of L of EEI reached 80-120 g in CR after 10-14 months, in WR after 8-11 months; the weight of L of KI and KamI reached the same level in CR after 3-4 months, in WR - after 4-8 months of development. The receptivity of WR to parental infection by P of different isolates was following: WR were not receptive to infection

with P of KI and KamI, while receptivity of WR to infection with P of EEI was 100%. The results are evidence that there are differences between the three investigated geographical isolates of *Em* as to larval development in experimentally infected laboratory rodents. The work was supported by INTAS grant 00-0685.

INVESTIGATING THE GENETIC BASIC OF *GYRODACTYLUS SALARIS* RESISTANCE IN ATLANTIC SALMON (*SALMO SALAR*)

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Gyrodactylus salaris Malmberg, 1957, an ectoparasite living on the skin and fins of salmonids, has had a catastrophic effect on wild Atlantic salmon (*Salmo salar* L.) stocks in Norway. Understanding the immune response, and the basis for observed resistance in some salmon populations, will play an important part in controlling this parasite. An overview of the experimental approach taken to address these aspects, and some preliminary results obtained, will be presented. Development of molecular markers linked to resistance could be used to introduce heritable resistance to parasites into commercial salmon populations through marker assisted selection methods. Two types of family groups were produced, from Scottish salmon susceptible to *G. salaris* and from resistant Russian River Neva salmon, and will be used to elucidate the molecular basis for variation in resistance to *G. salaris,* and to study mechanisms involved in host-parasite interaction. Fish gene expression is being analysed; genes that are stimulated or suppressed during infection, and, particularly genes that show differences in expression between resistant and susceptible fish, are being characterised. QTL markers linked to resistance will be identified. A number of differentially expressed products from *G. salaris* infected and control salmon have been isolated using differential display and subtractive hybridisation techniques, two of which are presented here. The first contains regions highly similar to the human, and chicken homolog, of 14.7K-interacting protein 2 (FIP-2), thought to play a role in tumour necrosis factor α/nuclear factor-κb signaling. This gene was upregulated *in G. salaris* infected fish. The second product showed high similarities to a gene induced following VHSV infection of fish. The whole gene has not been fully characterised previously. This product was downregulated in *G. salaris* infected fish.

These proteins have not been described previously in a fish immune response to parasite infection, and contribute to understanding fish host/parasite interactions.

RAPID IDENTIFICATION OF *ACANTHAMOEBA* SPP IN ENVIRONMENTAL AND CLINICAL SAMPLES USING CYTOCHEMICAL MARKER FOR CELLULOSE

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The identification of *Acanthamoeba* spp. is primarily based on morphological features of the organism. Our objective was to develop a rapid method, which would allow for the specific identification of *Acanthamoeba* spp. both, in clinical material and in environmental samples.

23 strains of free-living amoebae isolated from keratitis cases and from different water sources by filtration and subsequent cultivation on non-nutrient agar were assigned to genera *Acanthamoeba*, *Naegleria* or *Hartmannella* using morphological taxonomic criteria. Corneal samples from human *Acanthamoeba*-keratitis and tissues from experimentally infected mice were fixed in formalin and for sectioning embedded in paraffin or snap frozen. Dimers of cellulose-binding domains of *Trichoderma reesei* cellulase (D-CBD) obtained as a recombinant protein, were coupled to the fluorescent dye using Alexa Fluor® 568 Protein Labelling Kit according to the method provided by the manufacturer. For staining with D-CBD conjugate, slides containing cysts collected from the agar plates or tissue sections were immersed with PBS and incubated with D-CBD for 30 min at room temperature, washed 3 times with PBS and mounted with Vectashield non-fading medium.

We showed that cellulose could be easily detected by immunofluorescence using conjugated D-CBD in the inner cyst wall of *Acanthamoeba* spp. The reference strains of *Acanthamoeba* spp. and all *Acanthamoeba* strains isolated from water and from keratitis patients gave positive reaction. All of *Naegleria* and *Hartmannella* isolates were negative in the test. Using the fluorescent conjugate of D-CBD parasites could be also demonstrated in mouse and human tissue sections.

The D-CBD conjugate for direct staining of *Acanthamoeba* cysts is an alternative, potentially useful diagnostic tool, which allows for rapid and specific demonstration of parasites in both tissue sections and environmental amoebae.

PARASITES OF FARMED JUVENILE ATLANTIC COD CAUGHT IN THE WILD IN ICELANDIC WATERS

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Experimental rearing of Atlantic cod (*Gadus morhua* L.) has recently started in Iceland. During the developing phase of mastering the start-feeding of cod juveniles from hatcheries, a simultaneous rearing of cod juveniles caught in the wild will be developed. Farming of these wild cod will inevitably lead to disease problems such as parasitic infections. In a recent survey of parasites in 0+ wild cod juveniles in Icelandic waters we found at least 26 parasitic species. Fifteen of these were already detected when the juveniles reached the age of six months.

The aim of the present study is to examine parasites of wild cod juveniles during experimental farming and evaluate the effect on the fish health.

Juvenile wild cod (average weight 4 g, approximately 6 months old) were caught in a fjord in NW-Iceland in the autumn of 2002. The fish are reared up to 100-200 g weight in shore based tanks and will be moved subsequently into sea cages in the summer of 2003. During the winter of 2002-2003 fish were sampled on several occasions for parasite examination. The studies are specifically aimed at detecting parasites or pathogens affecting the condition or causing death of the fish.

The following 17 parasites have been found: Protozoa; *Ichthyobodo* sp., *Loma* sp., *Trichodina cooperi*, *T. murmanica*, and an unidentified flagellate (in the urinary bladder). Myxosporea; *Myxidium* sp. and *Zschokkella* sp. Monogenea; *Gyrodactylus* sp. Digenea; *Brachyphallus crenatus*, *Derogenes varicus*, *Lepidapedon elongatum* and *Podocotyle atomon*. Cestoda; plerocercoid larva. Nematoda; *Anisakis simplex* and *Hysterothylacium aduncum*. Crustacea; *Caligus* sp. and *Clavella adunca*. Also X-cell tumours of pseudobranchs and other tumours of unknown aetiology were encountered. *Trichodina* spp., *Loma* sp. and possibly *Ichthyobodo* sp., together with the X-cell tumours, have caused disease problems of the farmed juveniles so far.

As expected, parasites having direct life cycles are most likely to cause problems in a "closed rearing environment". The severity of infections caused by certain parasites, such as *Loma* sp., was more pronounced than expected, and follow-up studies are planned.

GASTROINTESTINAL HELMINTHS OF CUVIER'S BEAKED WHALES, *ZIPHIUS CAVIROSTRIS*, FROM THE WESTERN MEDITERRANEAN

By M. FERNÁNDEZ, F.J. AZNAR, F.E. MONTERO & J.A. RAGA

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In this study, we report on the gastrointestinal helminths of 2 Cuvier's beaked whales, *Ziphius cavirostris* (1 female and 1 male) stranded in the Spanish Mediterranean in 1996, and provide, for the first time, information on intestinal parasites. Six helminth taxa were found. Thirty larvae Type II of *Anisakis* were collected from the stomach (17 specimens) and the intestine (13 specimens) of both hosts. *Anisakis* Type II includes two species that are undistinguishable at the larval stage, i.e., *A. physeteris* and *A. brevispiculata*, being *A. physeteris* the only one identified in Cuvier's beaked whales. Two Type I larvae (L_4) of *Anisakis* were collected from the intestine of the male. This morphotype includes 5 species, i.e., *A. simplex*, *A. pegreffi*, *A. simplex C*, *A. typica* and *A. ziphidarum*, which cannot be identified at the larval stage. All species, except *A. simplex*, have been reported from Cuvier's beaked whales. Ten mature specimens of two species of *Tetrabothrius* were found in the intestine of the female whale. Both species likely represent new taxa. The only species of *Tetrabothrius* that had been reported in beaked whales is *T. forsteri* in *Mesoplodon* spp. Two morphotypes of *Scolex pleuronectis*, were found in both hosts: small (especially in the mucosa of the terminal colon and rectum), and large (in the anal crypts). One specimen of the acanthocephalan *Bolbosoma vasculosum* was collected from the intestine of the male. Although *B. vasculosum* is known from many odontocetes including beaked whales, the specimens are invariably found as juveniles. Composition of the intestinal community fit predictions for oceanic cetaceans, and includes polymorphids and tetrabothriids similarly as for other beaked whales.

USE OF GENETIC ANALYSIS AND SCANNING ELECTRON MICROSCOPY TO INVESTIGATE RELATIONSHIPS WITHIN "*PYGMAEUS*" MICROPHALLIDS (TREMATODA: MICROPHALLIDAE).

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The "*pygmaeus*" group of microphallids, by definition, is composed of species in which the metacercariae develop inside daughter sporocysts without encystment. Four species occur on the shores of the North Atlantic and they are *Microphallus pyg-*

maeus, *M. piriformes*, *M. pseudopygmaeus* and *M. triangulatus*. These organisms are parasites in marine and coastal birds and their daughter sporocysts occur in littoral gastropod molluscs, mostly members of the genus *Littorina*. As metacercariae develop to a transmissible stage inside their sporocysts, the life cycles of these parasites are completed when infected molluscs are ingested by seabirds. The four species can be distinguished by the gross morphology of the adults and metacercariae, and the life cycles also display significant differences. This study presents results of genetic analyses of the "*pygmaeus*" group specimens from differing geographical regions and, in the case of *M. pygmaeus*, from differing molluscan hosts. It also compares scanning electron microscope images of spines on the ventral surface of metacercariae. Genetic analysis included Universally Primed PCR (UP-PCR), which enables amplification of DNA from any organism without previous knowledge of its DNA sequences by generating multiband profiles (fingerprints) by gel electrophoresis. Universal primers used had the following sequences: AA2 (16 mer): 5'- CTGCGACCCAGAGCGG-3' and L15/AS19 (15mer): 5'- GAGGGTGGCGGCTAG-3'. UP-PCR product cross hybridization assay facilitated investigation of overall sequence similarity (homology) of the UP-PCR products. Cross-hybridization of the UP-PCR products was applied to ascertain if all four taxa represent separate species. UP-PCR showed no apparent pattern between genetic diversity of the metacercariae and their geographic region or molluscan host species. UP-PCR cross-hybridization showed that *M. pseudopygmaeus* and *M. triangulatus* are genetically very similar, thus, these taxa represent one species complex. *M. pygmaeus* and *M. piriformes* genetically are well separated from each other and also from the pseudopygmaeus-triangulatus complex. SEM of ventral spines, and analyses of spine angles and number of teeth per spine, showed that all species differed significantly from one another. It was concluded that *M. piriformes* represents the original western member of the "*pygmaeus*" group. The other members of the group show a distinct preference for sea duck host final hosts. *M. pygmaeus* probably diverged from *M. piriformes* as it progressively specialised for ducks. *M. pseudopygmaeus* and *M. triangulatus* diverged from each other and the piriformes-pygmaeus ancestral line relatively recently. *M. pseudopygmaeus* specialised for adoption of a wide range of gastropod host species and *M. triangulatus* developed morpho-functional specialisation associated with final host exploitation.

HIGH PREVALENCE OF BLOOD PARASITES AND BREEDING SUCCESS OF HAWFINCH

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The hawfinch *Coccothraustes coccothraustes* is a Palearctic monogamous bird belonging to the Fringillidae. It breeds sparsely and spends most of its time in tree-top foliage. This makes it difficult to catch a representative number of this species for parasitological investigation during the breeding period, when most haematozoan in-

fections are patent. Since surveys of blood parasites of hawfinch are uncommon, our objective was to determine the identity, prevalence, and intensity of blood parasites in this bird.

From 1 May through 26 June in 1982-2001, 57 hawfinches were caught on the Curonian Spit in the Baltic Sea. Blood films were prepared and examined microscopically.

Overall prevalence of blood parasites was 100%. A least ten species of *Haemoproteus, Plasmodium, Leucocytozoon, Trypanosoma,* and microfilariae were recorded. The prevalence of *Plasmodium* spp. in this study exceeded 60%. Such a high prevalence of *Plasmodium* spp. has never been recorded in fringillid birds in Europe. The majority of the infections (86.0% of all positive birds) were mixed infections with parasites from two to five different genera present in each blood film. There was no significant difference in the prevalence of haematozoa between males and females or between young and adult birds. Intensity of *Haemoproteus* spp. infections varied from < 0.01% to 5.7%, and *Plasmodium* spp. from < 0.01% to 1.0%. All *Leucocytozoon* spp. infections were < 0.1%. Trypomastigotes of *Trypanosoma* spp. and larvae of microfilarae were uncommon. The high prevalence of blood infections in this long-term study showed that active transmission probably takes place each year, and that the hawfinch is an attractive host for the vectors.

The hawfinch does not breed on the Curonian Spit. According to ringing data, only a few hawfinches occur there during seasonal migrations in spring and autumn. However, tens of them have been caught at the study site during summer movements of unknown origin, with the majority of birds recorded between mid-May and mid-June each year. It is worth noting that all females had brood-patches. Furthermore, approximately half of the birds were migrating in pairs. These birds are likely to have been unsuccessful breeders migrating through the Curonian Spit. The high prevalence of blood parasites in these hawfinches suggests a possible causative relationship between the haematozoan infection and the lack of breeding success.

SYLVATIC SPECIES OF *TRICHINELLA* IN DOMESTIC PIG

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The extensive and intensive infection with *Trichinella nativa* and *T. britovi* in Estonian wildlife is documented. Only few cases of pig trichinellosis are registered. This study deals with a case of trichinellosis in domestic pig.

Muscle samples (á 10 grams, from 11 different muscles) from 9-month sow were examined using compression and artificial digestion methods. For identification of

Trichinella genotypes RAPD analysis was carried out in the Trichinella Reference Centre. To elucidate the transmission patterns the muscle samples of a cat and 18 brown rats from the same farm and of 21 fur animals from nearby farm were investigated as well.

Domestic pig was lightly infected with *T. britovi*, whereas intensity of infection was highest in the body of tongue (12,0 larvae per gram). Cat muscles were heavily infected (95 LPG in eye muscles, 90 in the masseter muscle, 85 in the temporal muscle and 80 in the diaphragm). Two brown rats were infected with *Trichinella* (prevalence 11,1%, average intensity 50 and 90 LPG). 55% of investigated blue foxes proved to be infected with an average intensity of 2—19 LPG. Larvae from the sow were identified as *T. britovi*, whereas those from the rats and blue foxes were found to be *T. spiralis*. The recovered larvae from a cat could not be genotyped due to degradation. *T. britovi* is widely distributed in red foxes and raccoon dogs in this area.

This was the first finding of *T. britovi* in domestic pig in Estonia. It is concluded that the source if infection for domestic pig were neither the skinned carcasses of blue foxes from the nearby farm nor rats. More likely the pig was infected with sylvatic species of *Trichinella* (*T. britovi*) via unboiled (undercooked) carcass of some wild carnivore.

THERAPEUTIC ACTIVITY OF NOCODAZOLE AT EXPERIMENTAL LARVAL ECHINOCOCCOSES. 1. EFFICACY OF INTRAMUSCULAR AND SUBDERMAL INJECTIONS OF NOCODAZOLE AT *ECHINOCOCCUS MULTILOCULARIS* INFECTION IN COTTON RATS

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We assessed the therapeutic activity of intramuscular (i/m) and subdermal (s/d) injections of nocodazole (N) (methyl [5-(2-thienylcarbonyl)-1H-benzimidazol-2-yl]-carbamate) at larval alveolar echinococcosis of cotton rats (CR) at a late stage of infection. Infection of 27 CR of both sex aged 1.5 months was carried out by intraperitoneal injection of microscopic acephalocysts with diameter of 50-300 mkm recovered from larvocysts (L) *E.multilocularis* (*Em*) of experimentally infected CR-donor. N (product of Aldrich Chemical Company, Inc., USA) used in the form of suspension in physiological solution. Treatment was started on day 38 post infection (PI). At that time 5 CR were autopsied to detect the mean initial mass of L *Em* per one animal (MIML) which was 3.25 g. 8 CR received N i/m at daily dose level of 0,125 g/kg following two regimens (first and second regimens – 5 and 3 injections accordingly) twice a week with interruptions between injections and regimens for 2-3 days and 2

weeks accordingly. 6 CR received N s/d at daily dose level of 0.20 g/kg at the same regimens. 8 untreated CR were left as control. All treated and control animals were autopsied on 82 day PI. Viability, mean mass of L (MML) and ratio of MML to mean body weight of host (MBWH) in percentage per one CR were detected at autopsy of all animals. The results showed that i/m and s/d injections of N at total dose level of 1,0 and 1,6 g/kg accordingly caused complete destruction of all L in all treated CR. MML was 2,47 and 2,84 g in CR received i/m and s/d injections of N accordingly and not exceeded MIML and 2,2% of MBWH. In control CR all L *Em* were alive and fertile. MML in animals of control group was 87.84 g (77,2% of MBWH). In 3 CR of control group mass of L varied in range of 133-147 g and exceeded MBWH. High efficacy of i/m and s/d injections of N-substance at alveolar echinococcosis of CR at late stage of aggressive experimental infection serve a base for perspectivity of development of convenient injection forms of N for clinical use. The work was supported by INTAS grant 00-0685.

THERAPEUTIC ACTIVITY OF NOCODAZOLE AT EXPERIMENTAL LARVAL ECHINOCOCCOSES. 2. EFFICACY OF SUBDERMAL INJECTIONS OF NOCODAZOLE AT *ECHINOCOCCUS GRANULOSUS* INFECTION IN WHITE MICE

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We assessed the therapeutic activity of subdermal (s/d) injections of nocodazole (N) at a late stadium of larval cystic hydatidosis in white mice. 55 outbred mice (females) were infected intraperitoneally with 2000 protoscolices obtained from larvocysts (L) *E.granulosus* of human origin. Treatment of animals was started on 230 days post infection (p.i.). By that time 12 mice were autopsied to detect mean initial mass of developed L (MIML) and percentage of MIML to mean body weight (BW) of host (MBWH) per single mouse. MIML was 4.86 g (14.7% of MBWH). 24 mice of the experimental group received suspension of N in physiological solution s/d (in different points) within 4 months twice a week at gradually increased dose level of 5-20 mg/kg of BW. 19 untreated mice served as control. All treated and surviving control animals were autopsied on day 443 p.i. Viability, degree of fertility, mean mass of revealed L (MML) and percentage of MML to MBWH per one mouse were detected at autopsy. The presence of N in s/d fatty cellular tissue of treated mice was recorded at autopsy. Results showed that in 19 (79.2%) treated animals all L (in 14 mice) or the overwhelming majority of L (in 5 mice) were perished and collapsed. MML in treated animals was 4.10 g (12.0% of MBWH) and did not exceed MIML (indicator of complete inhibition of parasite's growth). Mass of perished L in recovered mice reached

10.89 g (36.9% host's BW). N didn't reveal in sites of infections in 17 (70.8%) of the treated mice. All revealed L in control mice were alive. MML in animals of control group was 21.96 g (70.0% of MBWH). Seven control mice perished after 12-13 months p.i. in results of intensive invasion; mass L in these animals exceeded host's BW. Received data serve a base for perspectivity of development of convenient injection forms of N for chemotherapy of larval cestodes. The work was supported by INTAS grant 00-0685.

EVALUATION OF THE THERAPEUTIC ACTIVITY OF FLUSAMIDE AT EXPERIMENTAL MODEL OF INTESTINAL CESTODOSES

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The aim of investigation was evaluation of the therapeutic activity of original chemical agent flusamide (F) (N,S-containing heterocycle derivative) at mixed-infection with two intestinal cestodes - *Echinococcus multilocularis* (*Em*) and *Hymenolepis nana* (*Hn*) - using method of primary screening. Mixed-infection was reproduced in adult golden hamsters (GH) of both sex by means of introduction of strobilar *Em* and *Hn* at prepatent stage of development into stomach. 5 GH-donors of strobilar *Em* and *Hn* were dissected in 22 days post peroral infection by protoscolices of *Em* (East-European isolate) and activation of latent invasion with *Hn* at the medicinal immunosuppression background. 27 GH-recipients were immunosuppressed with single subdermally injection of hydrocortisone with the average dose of 0,13 g/kg of body weight on the day of infection. The doses of strobilar *Em* and *Hn* were about 5000 and 12000 specimens accordingly per one animal. F introduced once into stomach of GH at 6th day after transplantation of cestodes at dose levels of 0,27-0,50 g/kg of body weight. At the moment of initiation of treatment, adult strobilar *Em* and *Hn* prevalenced in intestine of GH. In test and control groups there were 6 and 21 GH respectively. All GH of test and control groups were dissected in 8 days post infection. The following parameters were determined: presence and quantity of strobilar *Em* и *Hn*, approximate ratio of quantity of mature cestodes to total number of population of corresponding parasite species in each GH. The results of investigation showed that F (at dose levels of 0,27-0,50 g/kg of body weight) is highly effective against prepatent and mature *Em* and *Hn* and its efficacy was 100%. The work was supported by INTAS grant 00-0685.

PARASITES OF BROWN TROUT (*SALMO TRUTTA*) AND ARCTIC CHARR (*SALVELINUS ALPINUS*) IN TWO ICELANDIC LAKES - PRELIMINARY RESULTS -

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Studies on the parasites of brown trout and arctic charr in two lakes, Elliðavatn and Hafravatn, near Reykjavík in Iceland started in 2002. Both lakes are of similar size (1.8 and 1.1 square km) and with in- and outflowing rivers. Elliðavatn has a clay-bottom and the maximum depth is less than 2m but Hafravatn has a stony shoreline and the maximum depth is 28 m. Other fish species found in these lakes are three-spined stickleback (*Gasterosteus aculeatus*), eel (*Anguilla anguilla*) and salmon (*Salmo salar*).

In autumn and spring during the next two years, trout and charr from these two lakes will be caught in order to survey their parasite fauna. The parasites found will be identified and their prevalence and intensities determined. So far, five trouts and five charrs from each lake, caught last autumn and early winter, have been examined.

Preliminary identification of species: *Capriniana* sp., *Trichodina* sp., *Hexamita* sp., *Dermocystidium* sp., *Chloromyxum* sp., *Myxobolus arcticus*, *M. cerebralis*, *M. neurobius*, *Diplostomum spathaceum*, *Apatemon* sp., *Crepidostomum* sp., *Phyllodistomum* sp., *Diphyllobothrium* sp. , *Eubothrium crassum*, *E. salvelini*, *Philonema* sp., *Pseudocapillaria* sp. and *Salmincola* sp. Photographs have been taken of all species found and will, together with specimens, be used for further- and control identification and for comparison with species found elsewhere.

The poster presents photographs of the parasite species found.

POPULATION DYNAMICS OF *ANISAKIS SIMPLEX* LARVAE IN NORWEGIAN SPRING SPAWNING HERRING - PRELIMINARY DATA FROM A LARGE SCALE STUDY

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Norwegian spring spawning (NSS) herring *(Clupea harengus)* is one of the most important marine resources in the Norwegian economic zone. A rather subjective, but none the less important, quality criterion is the number of encapsulated nematode larvae that may be encountered in herring viscera and flesh during handling or consumption. According to Norwegian quality regulations, visible parasites at spot

tests must be removed. Furthermore, in order to avoid anisakidosis, i.e. human infection with live anisakid larvae, pelagic fish products, including raw, lightly salted or marinated herring, have to be deep-frozen for at least 24 hours before market release. Thus, although not representing a direct human health hazard, visible nematode larvae in fish are unappealing and may greatly reduce the market value of the fish.

As the Norwegian fishery industry is aware of the nematode problem, a large-scale 3 year survey has recently been launched to map the occurrence and distribution of nematode larvae in the economically most important herring and mackerel stocks in the Norwegian economic zone. An initial test sample of freshly frozen adult NSS herring, caught in late february 2003 on the spawning grounds in north-western Norway (~ 62° N), were searched for nematode larvae. The sample comprised a total of 36 herring, 9 males and 9 females from each of two length/age groups (23-28 cm/3-5 years and 28-33/9-12 years, respectively). In addition to recording routine parasitological parameters, the total body length of every single larva, post-fixed in 96% ethanol, was measured to nearest 0.5 mm.

All nematode larvae outside the alimentary tract were *Anisakis simplex* L_3. The prevalences and abundances of infection were 78 and 6.2±6.05 and 100 and 34.8±23.05, respectively, for the smaller and longer herring groups. A significantly positive correlation was found between host body length-weight ratio and *Anisakis* abundance, and subsequently, between host age and parasite abundance.

These results agree with previous findings in both herring and other fish species. However, the results of this limited initial sample do not support the general assumption that nematode larval body length is positively correlated with host body size and age. Hopefully, the ongoing project will provide more comprehensive information on the population dynamics of anisakid nematode larvae in pelagic fish in Norwegian waters.

IDENTIFICATION OF EUROPEAN DIPLOZOIDS (DIPLOZOIDAE, MONOGENEA) BY RESTRICTION DIGESTION OF THE RIBOSOMAL RNA INTERNAL TRANSCRIBED SPACER (ITS2)

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The European diplozoid fauna includes around 18 species parasitising the gills of cyprinid fish exclusively. Species determination of diplozoids is based on morphology of the central hooks and attachment clamps and is difficult and demands great skill and experience. Moreover, attachment clamps grow gradually and species determination of diplozoids in different developmental stages is not always clear. The second

internal transcribed spacer (ITS2) of ribosomal DNA of diplozoid parasites was amplified, sequenced and restriction enzyme digestion was performed to reveal restriction fragment length polymorphism (RFLP). Genetic markers for 8 species of European diplozoids were developed. As those diplozoids are very closely related, it was very difficult to find suitable enzymes to reveal different patterns for each species. Only a combination of digestion with *Alu*I, *Hae*III, *Hinf*I, *Rsa*I and *Sph*I proved convenient to discriminate all species studied. Morphological analysis and measurements of the attachment apparatus of the studied species are also presented, together with comparison of the shape of trapeze spur and the anterior joining sclerites.

MOLECULAR PHYLOGENETIC ANALYSIS OF THE GENUS *GYRODACTYLUS* (PLATYHELMINTHES: MONOGENEA) INFERRED FROM rDNA ITS REGION: SUBGENERA VERSUS SPECIES GROUPS

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Analyses of the small subunit ribosomal RNA gene sequences of the representatives of major taxa of Monophisthocotylea was performed to identify the sister group of *Gyrodactylus*. Nuclear ribosomal DNA sequences from the complete internal transcribed spacer (ITS) region were used to infer phylogeny of thirty-seven *Gyrodactylus* species and *Gyrodactyloides bychowskii*, *Macrogyrodactylus polypteri* and *Gyrdicotylus gallieni*, using maximum likelihood, parsimony and Bayesian inference. The genus *Gyrodactylus* appeared to be a monophyletic group in all analyses. Within the genus, there were three major groups recognised by high bootstrap values and posterior probabilities; group of the *G.* (*Gyrodactylus*) subgenus species and group separating species of *G.* (*Mesonephrotus*) and *G.* (*Metanephrotus*) from those of *G.* (*Paranephrotus*), *G.* (*Neonephrotus*) and *G.* (*Limnonephrotus*). However, none of the six subgenera appeared to be monophyletic, and the most basal subgenus *G.* (*Gyrodactylus*) was paraphyletic. Characteristics of the excretory system of *Gyrodactylus* as presented by Malmberg (1970) do not seem to be informative enough to reveal subgenera within *Gyrodactylus.* Also, the characteristics are difficult to use as the excretory system is unknown in the majority of newly described species and we suggest abandoning existing subgenera as indicators of phylogeny. The grouping of species based mainly on the morphology of the ventral bar and marginal hooks seems to have sufficient power to inform us about relationships between the *Gyrodactylus* species. However, comprehensive revisions in some species groups are required.

EVIDENCE FOR PARASITE INDUCED SEX RATIO DISTORTION IN AN INTERTIDAL AMPHIPOD

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Microsporidia are unicellular endoparasites that infect a wide range of host species causing infections of varying severity. In some freshwater amphipods, microsporidia exclusively infect females and are transmitted transovarially to their offspring. By transforming male offspring into females (feminisation), the transmitting sex is positively selected for, leading to a strongly female biased offspring sex ratio. Populations of the intertidal amphipod *Corophium volutator* in the Bay of Fundy (Canada) also show strongly female biased offspring sex ratio. These mudshrimp are a key species providing an essential food source for migrating birds as well as a variety of fish species. Various hypotheses for sex ratio distortion, like male biased predation by shore birds, have been discounted because they were unable to provide sufficient explanation for similar sex ratio biases in juvenile amphipods that are not yet subjected to differential predation. In order to test for possible feminisation due to microsporidian infection, we sampled a strongly female biased mudshrimp population from the Bay of Fundy. Ovaries, testes, eggs, and embryos from *C. volutator* were used to amplify a region of the microsporidian SSU rDNA. We found an infection rate of 28% for females (eggs/embryos) and no infection in any of the males. Further studies to evidence microsporidian sex ratio distortion will include molecular and ultrastructural description of the parasite, comparing offspring sex ratios from infected and uninfected females, and exploring parasite prevalence in populations with different sex ratio biases.

EFFECTS OF NATURAL PRODUCTS AGAINST SWINE SARCOPTOSIS

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Herbal medicine is a growing field within alternative medicine. Many of the active ingredients in manufactured drugs are derived from plant compounds and have a wide range of use. It is believed that plants are more natural, less toxic and safer than chemical preparations. As many synthetic drugs may have negative impact on the environment, and parasite resistance may develop after repeated applications, the use of natural products has become more popular. In order to control swine mange mites *(Sarcoptes scabiei var. suis)* in pig farms, trials were carried out to measure the effect of several plant extracts on the infections.

About 150 sows showing clinical signs of swine sarcoptosis were used in the trials. Each trial group was allocated as untreated control or treated over the whole body twice, with a week interval. Scrapings of infested skin from each pig were examined microscopically to determine the number of live mites, their eggs and larvae present. Local plants used in our trials were: mugwort *(Artemisia vulgaris)* tancy *(Tanacetum vulgare)*, wormwood *(Artemisia absinthum)*, cow parsley (*Heracleum sosnowskyi*), garlic *(Allium sativum)* and juniper *(Juniperus communis)*. Essential medicinal ethereal oils were as follows: black pepper *(Piper nigrum)*, pennyroyal *(Mentha pylegium)*, eucalyptus *(Eucalyptus globulus)* and tea tree *(Melaleuca alternifolia)*.

All the tested plant products appeared to be lethal against swine mange mites. Insect reproductive inhibitors and repellents, extracted from certain plants, affected reproduction of parasites in all trial variants. Already in the first post-treatment examination, the number of mites was significantly lower in scrapings from treated pigs and a great improvement of pigs was noticed two weeks after treatments.

The most active extract of tested local plants proved to be cow parsley (*Heracleum sosnowskyi*). After treatments with 10% extract of seeds, approximately 70 - 90% of parasites died in 2 - 4 weeks. The extracts of tansy and wormwood could diminish the number of parasites by up to 60% in 2 - 4 weeks. The essential ethereal oil of tea tree diminished the number of mites approximately 85% after two weeks.

It became evident that plant extracts may be used in practice as alternatives to neurotoxic insecticides and therefore the plant components with insecticidal effects may play a major role in the control of swine ectoparasites.

EVALUATION OF AN ANALYSIS FOR DETECTING ANTIBODIES AGAINST *ANISAKIS*

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Cross reactivity of antibodies against different nematodes like *Trichinella*, *Anisakis*, *Ascaris* and *Toxocara* are well known. Trichinellosis and anisakiosis are known diseases in Greenland, whereas problems with *Ascaris* and *Toxocara* are not common because of the low soil temperature. Humans get trichinellosis from eating meat from infected wildlife (e.g. walrus and polar bear), whereas *Anisakis* is found in different fish species. The aim of this study was to develop a tool for evaluating infection with *Anisakis* in humans and the specific aim was to evaluate an *Anisakis* E/S antigen in

use for testing antibodies against *Anisakis* for cross reactivity between *Trichinella* and *Anisakis*.

Serum samples from six persons were tested for antibodies against *Anisakis*; all six were involved in a known outbreak of trichinellosis on the west coast of Greenland in 2001. Of the six four had antibodies against *Trichinella* and two were negative.

An *Anisakis* E/S antigen was made from larvae collected from herrings. We used an ELISA assay and a Western blot for confirmation both using an *Anisakis* E/S antigen and a *Trichinella* E/S antigen. Three bands on the Western blot, which were absent on Western blot using the E/S *Trichinella* antigen, were found to be specific for *Anisakis*.

Of the six persons four were found positive for antibodies against *Anisakis* in the ELISA assay and when tested in the Western blot three had specific bands that were not similar to the bands found in *Trichinella* positive persons. One person failed to have these three specific bands and was therefore not found to have anisakiosis.

The *Anisakis* E/S antigen can be used in an ELISA assay to screen a population for antibodies, but positive results must be tested afterwards in Western blot to rule out false positive results.

PARASITES OF THREE-SPINED STICKLEBACKS (*GASTEROSTEUS ACULEATUS*) IN A FRESHWATER AND A SALTWATER HABITAT IN ICELAND - PRELIMINARY RESULTS -

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Studies on the parasites of three-spined sticklebacks started in 2001 in the freshwater lake Elliðavatn and at the coast of Hvassahraun in a habitat of variable salinity, both localities near Reykjavík in Iceland. The study is a part of the international "Survey of stickleback parasites project" which started during the "International Biodiversity Observation Year (IBOY) 2001-2002", an initiative of DIVERSITAS.

In autumn and spring, sticklebacks from these two habitats will be caught in order to survey their parasite fauna. The parasites found will be identified and their prevalence and intensities determined. So far, 15 sticklebacks from each locality, caught in the autumn, have been examined.

Preliminary identification of species. Saltwater: *Trichodina domerguei, T. tenuidens, Gyrodactylus sp., Podocotyle atomon* and *Anisakis simplex*. Freshwater: *Dermocystidium* sp., *Trichodina domerguei, T. tenuidens, Apiosoma* sp., *Myxobilatus* sp., *Gyrodactylus sp., Diplostomum spathaceum, Apatemon gracilis*, an unidentified trematode larvae and *Schistocephalus solidus.* Photographs have been taken of all species found and will, together with specimens, be used for further- and control identification and for comparison with species found elsewhere.

The poster presents photographs of the parasite species found.

CERCARIA NOTOCOTYLIDAE SP. 13 DEBLOCK, 1980 (DIGENEA) SHED BY ICELANDIC MUDSNAILS (HYDROBIA VENTROSA) DEVELOPED TO MATURITY IN AN INFECTION EXPERIMENT

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The Icelandic bird fauna is characterized by relatively few breeding species, which usually occur in large populations. In recent years the authors have been investigating digeneans infecting coastal and marine birds (final hosts) and some snail species (intermediate hosts) occurring in brackish saltmarsh ponds and in the littoral zone in Iceland. In most cases the taxonomical status and the life cycle of the digeneans found has been described but sometimes parasites are found for which this information is lacking. Here, we present the first results of an infection experiment whereby a digenean larva was fed to a duck and the previously unknown adult stage could be obtained.

In autumn 2002 forty cercariae of the species *Cercaria Notocotylidae* sp. 13 Deblock, 1980 (type Yenchingensis), subgroup I (Galaktionov & Skirnisson, unpublished), shed from mudsnails (*Hydrobia ventrosa*) originating from a salt marsh pond in Melabakkar, SW Iceland were allowed to encyst to metacercariae on fragments of grass and susequently fed to an adult domestic duck (*Anas platyrhynchos* f. dom.). Fifteen days later the gasterointestinal tracht of the duck was searched for adult digeneans.

Eight adult digeneans of the genus *Notocotylus* were found in the caecum of the duck. At least 20% of the metacercaria had developed to the adult stage of the parasite.

The quite good recovery rate (20%) in the infection experiment suggests that anseriform birds are the natural final hosts of the species. Two ducks, *Anas platyrhinchos* and *Anas crecca,* and the whooper swan *Cygnus cygnus* are the most common bird species feeding upon vegetation in ponds on the Melabakkar saltmarsh where the life cycle is maintained under natural conditions. One or more of these bird species are regarded to be the most probable final hosts for this digenean. Work on the description of both the larval and adult stages has already started.

ITS 1 NUCLEAR rDNA SEQUENCES USED TO CLEAR THE LIFE CYCLE OF THE MORPHOLGICALLY DIFFERENT LARVAE AND ADULT RENICOLID (*RENICOLA*; DIGENEA) PARASITES FOUND IN ICELAND

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In recent years various studies have been carried out in Iceland on the occurrence of digenean larval stages in littoral snails and their adult stages in birds. Focusing on the renicolids, so far two larval species are known to occur; *Renicola thaidus* Stunkard, 1964 in dogwhelks (*Nucella lapillus*) and *Cercaria parvicaudata* Stunkard & Shaw, 1931 in littorins (*Littorina* saxatilis and *L. obtusata*). Furthermore, two adult renicolids (kidney flukes) have been found; *Renicola somateriae* Belopolskaya 1952 in common eiders (*Somateria mollissima*) and *Renicola* cf. *lari* in herring gulls (*Larus argentatus*) (Skirnisson, unpublished). The aim of this study was to use molecular methods to find out if base pairs sequences of the ITS1 region were matching in the above-mentioned larvae and adults in order to try to clear their uncertain life cycles.

In summer 2002 DNA was isolated from fresh cercariae of *Renicola thaidus* from *Nucella lapillus* (sampled in Grótta, Reykjavík) and *Cercaria parvicaudata* from *Littorina saxatilis* (sampled in the harbour of Grindavík, SW Iceland). Adult kidney flukes were obtained from birds sampled in the vicinity of Reykjavík; *Renicola. somateriae* from common eiders and *Renicola* cf. *lari* from herring gulls. To isolate DNA we used the DNeasy® kit from QUIAGEN. The ITS1 region of the rDNA was amplified using a digenean specific primer located 195 base pairs (bp) from the 3′ end of the 18S rDNA S20T2 (5′-GGT AAG TGC AAG TCA TAA GC-3′) and a universal primer located 35 bp from the 5′ end of the 5.8S rDNA 5.8S1 (5′-GCT GCG CTC TTC ATC GAC A-3′). Amplified PCR products were purified using the ExoSAP IT™ purification kit and were sequenced directly using either ALF Express or ABI Prism 310 Genetic Analyzer. In the case of *Renicola somateriae* we also cloned the ITS1 region into a pUC 19 phasmid of *E. coli* (DH5α).

Identical sequences were found in *Renicola thaidus* and *Renicola somateriae*. However, as no DNA could be isolated from *Cercaria parvicaudata* and *Renicola* cf. *lari* their sequences are still unknown.

The results indicate that the *Renicola thaidus* larvae in dogwhelks and *Renicola somateriae*, the kidney fluke of the common eider in Iceland, actually are two developmental stages of the same species.

TRANSMISSION OF *TOXOCARA CANIS* INFECTION: A PILOT STUDY IN ESTONIA

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Objectives. Roundworm of dogs, *Toxocara canis,* is ubiquitously distributed and probably the most common helminth species infecting dogs. A variety of transmission routes may cause new infections in dogs and other mammals including man (human visceral larva migrans infection). In female dogs dormant larvae become mobilised in the latter part of pregnancy thus ensuring almost hundred percent infection in puppies. Our preliminary study of 1087 dogs (age between 2 weeks and 12 years) in Estonia did show that 17,5% of them were shedding *T. canis* eggs with faeces. From 138 litters under study 132 (96%) were infected, although bitches were regularly wormed.

The aim of the study was to estimate the present state of contamination of children playgrounds and public parks with *T. canis* eggs in Tartu, the second largest town of Estonia, where the concentration of dogs is relatively high.

Material and methods. Two distinct places were taken under investigation: Tähtvere Park, where dog owners' from private houses of this district usually walk their dogs, and densely populated Anne district of apartment houses. From Tähtvere Park 71 faecal samples of dog origin were collected. In total, 45 sand box samples were collected from children playgrounds in Anne district. In 21 cases faeces was found from sand boxes and sampled for the study. In other cases ca 50 g of sand was sampled from each sand box. Simple flotation technique was used for detection of *Toxocara* spp. eggs. Autopsies were carried out on 11 stray dogs, 15 cats and 2 rats.

Results and conclusions. Eight percent (6 from 71) of faecal samples of dog origin collected from Tähtvere Park in Tartu were *Toxocara canis* - positive. We do assume that sand boxes in Anne district were contaminated mainly with cat faeces. Both, sand and faecal samples contained *Toxocara* spp. eggs. In total 18% of sand box samples (8 from 45) were positive. According to our limited preliminary data sand boxes on children playgrounds are more often contaminated with *Toxocara* spp. eggs than public parks. As the eggs of *Toxocara canis* and *T. mystax* are morphologically identical, further DNA identification is needed in order to draw accurate conclusions. Autopsy findings showed *Toxocara canis* adults in three dogs and *Toxocara mystax* in six cats. To further elucidate the possible sources of human infection also paratenic hosts (pigs, hen etc.) whose meat man consumes have to be taken into consideration.

PLATYHELMINTHES OF SHREW (SORICIDAE) AND MOLE (TALPIDAE) IN LITHUANIA

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Although the shrews (*Sorex araneus* L., *S. minutus* L.) and moles (*Talpa europaea* L.) are very common and widespread in Lithuania, their parasites are scarcely investigated. We examined 152 specimens of Soricidae and 66 specimens of Talpidae, collected in 1999-2002 in western and eastern Lithuania, and found 15 species of cestodes and 5 species of trematodes in internal organs. Three species were present only in larval stages (*Dilepis undula*, *Hepatocystis hepaticum*, *Polycercus paradoxa*). *Sorex araneus* had 17 species, *S. minutus* 10 species and *Talpa europaea* 3 species of platyhelminths. In total, 16 % of shrews (16.8 % of *S. araneus* and 13.5 % of *S. minutus*) were infected with cestodes and trematodes, 68,7 % (69,0 % and 67,6 %, respectively) - only cestodes and 2 % (1.8 % and 2,7 %) - only trematodes, 1.5 % of moles had cestodes and trematodes, 13.6 % had only cestodes and 6.1 % - only trematodes. The maximum intensity of cestodes in shrews was 1666 and in moles 17, maximum intensity of trematodes in shrews was 303 and 4 in moles.

The general prevalence of platyhelminths infection was 86.7 % in shrews and 18.2 % in moles. Differences in the diversity of the platyhelmiths species and in the levels of infectiousness between shrews and moles exist because of their different behaviour and nutrition.

OBSERVATIONS OF HYPERPARASITISM ON *GYRODACTYLUS SALA*RIS MALMBERG (MONOGENEA) INFECTING ATLANTIC *SALMON (SALMO SALAR)*

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There is a growing concern to investigate alternatives to rotenone to eradicate the devastating pathogen *G. salaris* on wild *Salmo salar* in Norway. One option is biological control by hyperparasitism. *G. salaris* infected salmon parr were electrofished in River Lierelva in four seasons and examined for hyperparasites. Two ectoparasitic hyperparasites were observed by use of electron microscope (SEM/TEM): (i) microcolonies of rod-shaped bacteria; (ii) isolated individuals of *Ichthyobodo necator*. The flagellate *I. necator* occurred only occasionally in contrast to the bacterial hyperparasitism which was present in all seasons, but at variable prevalence and density. No internal hyperparasites were observed. A laboratory population of bacteria infected *G. salaris* was established and used to study the influence of bacteria on *G. salaris* sur-

vival and reproduction. However, no significant affect was observed on the *G. salaris* metapopulation. As microcolonies of bacteria adhered anteriorly to the host's adhesive areas and around the censory structures, a possible bacterial influence on parasite transmission was tested. A single salmon with bacteria infected *G. salaris* was constrained with an uninfected naïve salmon for 24h. *G. salaris* transmitted in correlation with parasite intensity on the donor. Transfer does not seem to be impaired by bacteria infection. Bacteria were seldom adhered to fish epidermis in contrast to the surface of *G. salaris* which indicate a preference for adhesion on the parasite tegument. Eating wounds after *G. salaris* did not seem to be contaminated by bacteria which indicated that *G. salaris* was not a vector for a potential bacterial fish disease. However, a final conclusion awaits diagnosis of the bacteria. No connecting structures or junctional complexes between the bacteria and *G. salaris* tegument was observed, and no disrupted or dying host cells were seen below the point of bacteria adherance. The association between bacteria and host seems permanent once the bacteria has adhered. The vertically attached bacteria in tegument depressions could occationally represent a bacterial membrane-to-host cytoplasm contact but with unknown physiological effects. Parasitized *G. salaris* seemed biologically unaffected by the ectoparasitic bacteria, which suggests that it is not an agent for biological control of gyrodactylosis. A review of bacterial hyperparasitic infections of platyhelminths is presented.

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LARVAL DIGENEA IN THE INTERTIDAL SNAILS, *LITTORINA OBTUSATA* AND *L. SAXATILIS* FROM REYKJAVIK, ICELAND

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Abstract

The intertidal snails, *Littorina obtusata* and *L. saxatilis* (Gastropoda) from one locality in Iceland in 1996 were examined for the presence of larval Digenea (rediae, sporocysts, cercariae, metacercariae). Larval stages were found in 15% of *L. obtusata* and in 35% of *L. saxatilis*. Microphallidae of the *"pygmaeus"* group, unidentified Microphallidae and *Parvatrema homoeotecnum* were found in both snail species, but *Cryptocotyle lingua* and *Parapronocephalum symmetricum* were found only in *L. obtusata. Himasthla* sp. and *Paramonostomum chabaudi* (*Cercaria lebouri*) were found only in *L. saxatilis. P. chabaudi*, *P. homoeotecnum* and *P. symmetricum* had not been reported in previous studies in Iceland.

Introduction

Littorina spp. snails (Mollusca: Gastropoda), the so-called periwinkles, play an important role as the first intermediate hosts of digenean trematodes which use marine and coastal birds, specifically the common eider, *Somateria mollissima* and gulls (Laridae) as final hosts. *Littorina obtusata* (L.) and *L. saxatilis* (Olivi) are common marine snails around Iceland. A third species of the genus also occurring in Iceland is *L. fabalis* (Turton) (= *L. mariae*) (Ingólfsson, 1996). On the other hand, the common European periwinkle, *L. littorea* (L.) is not found in Iceland.

The only survey on larval Digenea in marine molluscs in Iceland prior to the present observations was undertaken by Sannia & James (1977), in which three species of Digenea in *Littorina* spp. were recorded. Recently, detailed studies were made by Galaktionov & Skírnisson (2000) on digeneans from six intertidal molluscan species, sampled in 1998 from different localities in SW Iceland, in which 15 digenean species in *Littorina* spp. were found. Furthermore, recent investigations on the occurrence of the digeneans, *Cryptocotyle lingua* and *Prosorhynchoides gracilescens* (Rudolphi, 1819) in Iceland included examinations

of marine molluscs (Eydal *et al*, 1994, 2000). Some studies have also been made on Digenea in birds in Iceland. Several species of adult Digenea were recorded by Brinkmann (1956). In recent years, Digenea in the common eider and five gull species (*Larus* spp.) have been a subject of investigation in Iceland and several species were recorded (Skírnisson & Jónsson 1996; Eydal, 1998).

The aim of this survey was to collect primary data on the occurrence and composition of larval Digenea (sporocysts and other larval forms) in *L. obtusata* and *L. saxatilis* from a coastal locality in Reykjavík, Iceland.

Materials and Methods

L. obtusata were sampled in October and December in 1996 and *L. saxatilis* in October of 1996, at one locality, on the southwest coast of Geldinganes at Eidisvík in greater Reykjavík, Iceland, a rocky shore, moderately exposed to open sea. During sampling, the shore was moderately abundant with both snail species. The snails were collected by hand at low tide throughout their vertical distribution. They were brought to the laboratory and kept in aerated seawater at 4°C until examination. One hundred snails of each species were examined, and their size ranged from small/medium to large. The soft parts were removed from the shell and the internal organs (the digestive gland and gonads), were shredded in seawater and examined under a stereo microscope for presence of digeneans. Digenean specimens were examined in vivo using a light microscope. The intensity of infection was estimated. An attempt was made to identify all digenean larvae present, to species or genera, based on the descriptions and keys of James (1968, 1969). No attempt was made to further differentiate microphallids referred to as Microphallidae of the *"pygmaeus"* group (Galaktionov, 1996a; Galaktionov & Skírnisson, 2000). The use of ecological terms is in accordance with Bush *et al*, 1997.

Results

Digenean larvae representing six families were identified (Table 1). The prevalence of digenean species in each

Table 1. Digenea families, and the representative larval species, genus or group of species, found in *Littorina obtusata* and *L. saxatilis* at Geldinganes, Reykjavík.

Microphallidae; Microphallids of the "*pygmaeus*" group
Echinostomatidae; *Himasthla* sp. Podlipayev, 1979
Gymnophallidae; *Parvatrema homoeotecnum* James, 1964
Heterophiidae; *Cryptocotyle lingua* (Creplin, 1825)
Notocotylidae; *Paramonostomum chabaudi* Van Strydonck, 1965 [1)]
Pronochephalidae; *Parapronocephalum symmetricum* (Belopolskaya, 1952)

[1)] Earlier known as *Cercaria lebouri* Stunkard, 1932 (see Evans *et al*, 1997)

Table 2. Prevalence of larval infections in *Littorina obtusata* (n = 100) and *L. saxatilis* (n = 100) at Geldinganes, Reykjavík.

	L. obtusata	**L. saxatilis**
Microphallidae of the "*pygmaeus*" group (s, m)	8%	22%
Unidentified Microphallidae (s, c, m)	2%	1%
Cryptocotyle lingua (r,c)	1%	-
Himasthla sp. (r)	-	1%
Paramonostomum chabaudi (r, c)	-	1%
Parapronocephalum symmetricum (r, c, m)	2%	-
Parvatrema homoeotecnum (g, c, m)	2%	9%
Unidentified larvae (s, c, m)	1%	8%

r = rediae
s = sporocysts
c = cercariae
m = metacercariae
g = germinal sacs (a term used by James (1964))

host is presented in Table 2. Most of the 15 infected *L. obtusata* snails, or 13 (87%), had single-species infections, but two (13%) had double infections. One snail was infected with both Microphallidae of the *"pygmaeus"* group and *P. symmetricum* and one was parasitized with an unidentified microphallid and an unidentified metacercaria. Most of the 35 infected *L. saxatilis* snails, or 28 (80%), had single-species infections, six (17%) had double infections, all of which were infected with Microphallidae of the *"pygmaeus"* group. Four were also infected with *P. homoeotecnum*, one with unidentified metacerariae and one with *P. chabaudi*. One (3%) had triple infections with Microphallidae of the *"pygmaeus"* group, *P. homoeotecnum*, and an unidentified larva.

In almost every infected snail of both species, the intensity of infection was high, the digestive glands (and sometimes the gonads) were almost completely replaced by digenean larvae. In the ovoviviparous species, *L. saxatilis*, it was observed that eggs, embryos and juvenile stages were absent in 67% of the infected females, whereas 84% of noninfected females contained numerous eggs, embryos and/or juveniles.

P. homoeotecnum larvae were located in the gonads, rather than in the digestive gland, while other digeneans occupied the digestive gland. In five out of nine cases (56%) of *P. homoeotecnum*-infected *L. saxatilis* individuals, the digestive gland was parasitized by microphallids of the *"pygmaeus"* group. The expected prevalence of coexisting infections in *L. saxatilis* with these two larvae is 9/100 x 22/100 x 100% = 2%, but the observed prevalence was considerably higher, or

5/100 x 100% = 5%. *L. saxatilis* individuals infected with *P. homoeotecnum* were small/medium sized, in contrast to the other digenean species which were usually found in larger snails.

Discussion

The Digenea larvae found utilize marine and coastal birds as definitive hosts. Microphallids of the *"pygmaeus"* group are common digeneans of eider ducks, whereas *C. lingua* and *Himasthla* sp. are typical parasites of gulls (Galaktionov & Bustnes, 1995). James, (1964) describes the definitive host for *P. homoeotecnum* as the oystercatcher, *Haematopus ostralegus* and according to Galaktionov & Bustnes (1996) the definitive hosts for *P. symmetricum* are waders (sandpipers, and oystercatcher). The definitive hosts for *P. chabaudi* appear to be oystercatchers (*H. ostralegus*) and mallards (*Anas platyrhynchos*), although other species may also be involved (Evans *et al*, 1997).

Adult microphallids of the *"pygmaeus"* group have been recorded from the common eider in Iceland (Skírnisson, 1996). Adult *C. lingua* have been recorded from the arctic fox (*Alopex lagopus* L.) and five gull species (*Larus* spp.) in Iceland and *Himasthla* sp. (*H. elongata* (Mehlis, 1831)) was found in two species of gulls (*Larus hyperboreus* and *L. argentatus*) in Iceland (Skírnisson *et al*, 1993; Eydal *et al*, 1998). *P. chabaudi*, *P. symmetricum* and *P. homoeotecnum* had neither been found in molluscs, nor birds in earlier studies in Iceland, but were later found in *Littorina* spp. by Galaktionov & Skírnisson (2000).

In most cases, unidentified larvae (Table 2) were sporocysts containing undifferentiated germinal balls or cercariae not identifiable, and a few were encysted metacercariae.

Sannia & James (1977) recorded the following Digenea in 341 *Littorina* spp. from the coast of Eyjafördur, North-Iceland: *Microphallus pygmaeus* (Levinsen, 1881) (belonging to Microphallidae of the *"pygmaeus"* group), in *Littorina obtusata*, *L. saxatilis* and *L. fabalis*, and with *Podocotyle atom*on (Rudolphi, 1802) and *Cercaria littorinae-saxatilis* VI Sannia & James, 1977 being found in *L. saxatilis.*

Galaktionov & Skírnisson (2000) identified 15 digenean larval species in their studies on *L. obtusata* (n = 622) and *L. saxatilis* (n = 899) from Skerjafjordur and Grindavik in SW-Iceland, of which the following were not identified in the present study: *M. pygmaeus*, *M. piriformes* Galaktionov, 1983, *M. pseudopygmaeus* Galaktionov, 1980 and *M. triangulatus* Galaktionov, 1984 (or all four species comprising the Microphallidae *"pygmaeus"* group), *M. similis* (Jägerskiöld, 1900), *Cercaria islandica* I Galaktionov & Skírnisson, 2000, *Cercaria parvicaudata* Stunkard & Shaw, 1931, *Cercaria littorinae obtusatae* Lebour, 1911, *Podocotyle atomon* and *Renicola* sp. On the other hand, *C. lingua, Himasthla* sp., *P. symmetricum, P. homoeotecnum* and *P. chabaudi* were identified in both studies.

Of the 15 species found by Galaktionov & Skírnisson (2000) all but

three (*M. triangulatus, Cercaria islandica* I, and *Cercaria littorinae obtusata* found in *L. obtusata*) were found in both *L. obtusata* and *L. saxatilis* snails.

Prevalence of the digeneans found by Galaktionov & Skírnisson (2000) ranged from 0.2% (for *P. chabaudi, P. symmetricum* and *Himasthla* sp.) to 7.0% (pooled results for Microphallidae of the *"pygmaeus"* group). In the present study, the range varied from 1.0% (*C. lingua* and *Himasthla* sp.) to 22.0% (Microphallidae of the *"pygmaeus"* group).

According to James (1969), double infections with *P. homoeotecnum* and *M. pygmaeus* (of the Microphallidae *"pygmaeus"* group) are relatively common in *L. saxatilis* and more prevalent than could be expected by chance alone. The results in the present study are in agreement with this. The occurrence of *P. homoeotecnum* in the smaller *L. saxatilis* snails is also in accordance with James (1964), who found this species only in young snails. According to Galaktionov & Skírnisson (2000), it is possible that *Littorina* spp. act as second intermediate hosts for this species, and as a consequence the first intermediate host (possibly a bivalve) is yet to be identified.

The effect by digenean larvae on the molluscan host has been studied by several researchers and castration of the host is well known (James, 1965; Lauckner, 1980; Granovitch 1992; Sokolova, 1995). In the present study, absence of juvenile stages in parasitized *L. saxatilis* females was much more common than in non-parasitized females.

In the past, studies on Digenea larval stages of littorinids in northern regions have been scarce (for review see Sannia & James, 1977; Galaktionov & Bustnes 1995; Granovitch & Johannesson, 2000). Recent publications reveal a substantial increase in research of these parasites, their complex life cycles, and impact on seabirds and coastal ecosystems (Galaktionov, 1996a and 1996b; Galaktionov & Skírnisson, 2000; Granovitch & Johannesson, 2000).

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INTESTINAL MACROPARASITES IN ANGLERFISH (*LOPHIUS PISCATORIUS* L.) FROM ICELANDIC WATERS

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Abstract

The digestive tracts (stomach and intestine) from 34 anglerfish (*Lophius piscatorius* L.) collected off south and southwest Iceland in 1995 were examined for macroparasites. At least 18 parasite species were found. These included Digenea: *Derogenes varicus, Lecithaster gibbosus, Otodistomum* sp., *Prosorhynchoides gracilescens, Stephanostomum* sp., *Steringophorus furciger, Zoogonoides viviparus*; Cestoda: *Grillotia* sp., tetraphyllid plerocercoids; Nematoda: *Anisakis simplex, Capillaria* sp., *Contracaecum* sp./*Phocascaris* sp., *Hysterothylacium aduncum, H. rigidum, Hysterothylacium* sp., *Pseudoterranova decipiens, Spinitectus* sp. and an unidentified nematode, and finally, Acanthocephala: *Echinorhynchus gadi*. Nine of the species had not previously been recorded from anglerfish.

Introduction

Anglerfish, *Lophius piscatorius* L., is distributed in the E-Atlantic from N-Norway and Iceland, and as far south as the Guinea Bay on the African coast. *L. piscatorius* is common around the British Isles and along the European coast and in the Mediterranean. In Icelandic waters it is mainly distributed in the relatively warm waters south and southwest of the island, but rarely off the north and east coasts (Jónsson, 1992). In the past, few systematic investigations have been conducted on metazoan parasites in anglerfish and most studies were confined to specific parasite species or a limited number of hosts (Brinkmann, 1957; Berland 1961; Køie 1993, 2000; Quinteiro *et al,* 1993).

Materials and Methods

Thirty-four anglerfish were collected from *Nephrops* trawl (station nos. 1-14) and bottom trawls (station nos. 15-18) south and southwest of Iceland at depths of 120-341 meters in March and May 1995 (Figure 1, Table 1). Fish weights and lengths were measured to the nearest gram and centimetre, respectively. Stomachs and intes

Figure 1. Locations of sampling stations (nos. 1-18).

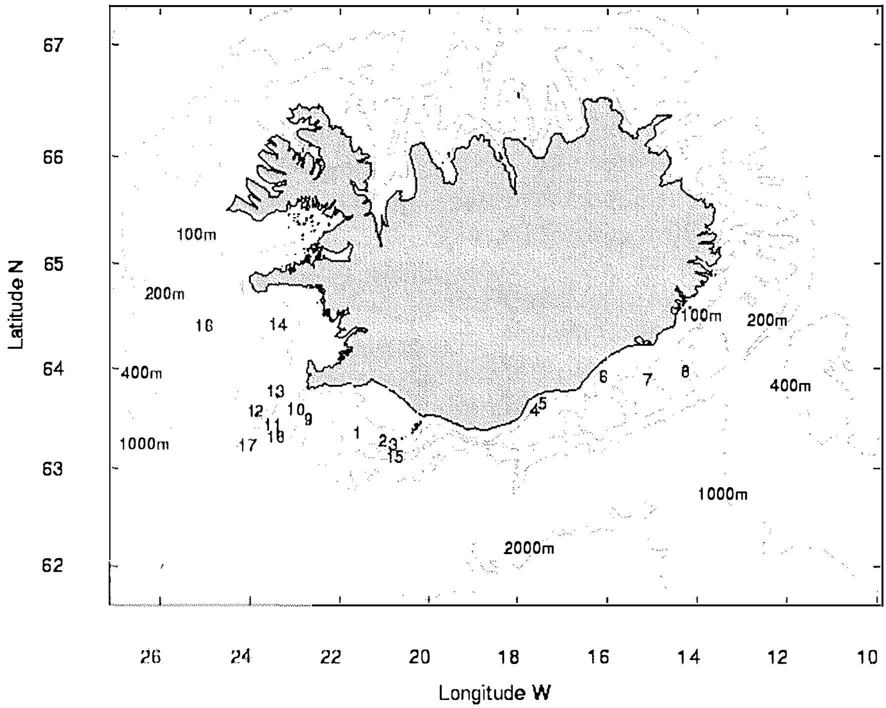

Table 1. Station list for sampled anglerfish (*Lophius piscatorius*).

Station no.	Station ID	Location Latitude N-Longitude W	Date	Depth (m)
1	D5-95-451	63°22' - 20°36'	06/05/95	150-168
2	D5-95-453	63°17' - 21°02'	06/05/95	156
3	D5-95-454	63°15' - 20°48'	06/05/95	158-170
4	D5-95-456	63°36' - 17°38'	07/05/95	120-148
5	D5-95-457	63°39' - 17°27'	07/05/95	130-148
6	D5-95-466	63°55' - 16°05'	08/05/95	160-168
7	D5-95-470	63°54' - 15°07'	09/05/95	152-170
8	D5-95-475	63°58' - 14°17'	10/05/95	200-224
9	D5-95-493	63°33' - 22°47'	16/05/95	178-200
10	D5-95-494	63°35' - 22°56'	17/05/95	146-166
11	D5-95-496	63°29' - 23°37'	17/05/95	188-198
12	D5-95-497	63°32' - 23°48'	17/05/95	190-192
13	D5-95-499	63°47' - 23°26'	18/05/95	143-154
14	D5-95-506	64°26' - 23°23'	19/05/95	160-164
15	TV-1-95-23	63°07' - 20°44'	05/03/95	280-290
16	TV-1-95-80	64°25' - 25°01'	10/03/95	204-230
17	TV-1-95-117	63°13' - 24°04'	13/03/95	245-267
18	TV-1-95-120	63°19' - 23°26'	14/03/95	326-341

tines were removed and frozen for later study in the laboratory. This investigation focused mainly on intestinal parasites. However, the stomach wall and stomach contents were also studied using a magnifying lens lamp. Intestinal contents (pyloric caeca/small intestine/ rectum) were washed in tap water in a 250μm-sieve and immediately fixed in 70% ethanol (denaturated with isopropyl alcohol). Whole samples or sub-samples were examined under a stereomicroscope. Glycerol or lacto-phenol was used as clearing agent for nematodes and acantocepalans prior to species identification using light microscopy. Digeneans and cestodes were serially dehydrated in 70%, 90%, 99% and 100% ethanol, stained in aceto-carmine, cleared in xylol and mounted in Canada balsam before species identification.

The 34 fish obtained for the study ranged from 21-119 cm in length and from 125g to 21.1kg in weight. Prevalence, mean intensity and median intensity were calculated for two length classes: 21-60 cm and 61-119 cm. Further grouping of the samples was considered impractical due to small sample size. Regression of parasite intensities to fish length was tested with simple least square regression (Splus, MathSoft, Inc.). Parasite numbers were transformed with natural logarithm in order to obtain normal distribution before regression analyses. Significance level was set at 5% ($p<0.05$). Ecological terminology in the text is adopted from Bush *et al* (1997).

Results and discussion

Stomach

Five parasite species were detected in the stomach wall or in the stomach contents (Table 2). *Otodistomum* sp. metacercariae were found encapsulated on the inner side of the stomach wall. Similar larvae are found in long rough dab (*Hippoglossoides platessoides* Fabr.) from Icelandic waters (Ólafsdóttir, 1999). Adult *O. veliporum* (Creplin, 1837) has been reported once from an unknown host off the south coast of Iceland (Brinkmann, 1956) and mature *O. cestoides* (van Beneden, 1871) worms have repeatedly been reported from rays (*Raja* spp.) in Icelandic waters (as *O. veliporum* by Rees (1953a); as *Otodistomum* sp. by Manger (1972); See also discussion by Gibson and Bray (1977). *Derogenes varicus* was the only parasite found in the lumen of the stomach. This species is widely distributed in a number of fish hosts through-

Table 2. Parasites detected in the stomach or stomach wall of anglerfish (*Lophius piscatorius*) taken off south and southwest Iceland in 1995.

DIGENEA:
Azygiidae
Otodistomum sp., metacercariae
Hemiuridae
Derogenes varicus (Müller, 1784) Looss, 1901 (sensu lato)
CESTODA:
Grillotiidae
Grillotia sp. plerocercoid
NEMATODA:
Anisakidae
Hysterothylacium rigidum (Rudolphi, 1809) Deardorff & Overstreet, 1981
Contracaecum sp./*Phocascaris* sp. larvae

out the world (Manter 1966, ref. in Køie, 1979) and has previously been reported (as *Derogenes* sp.) from anglerfish from waters of the Faroe Islands (Køie, 2000). *Grillotia* sp. plerocercoids were found encapsulated on the inner side of the stomach wall. *Grillotia erinaceus* (van Beneden, 1858) is common in the North Atlantic (Polyanskii, 1966; Lubieniecki, 1976; Margolis & Arthur, 1979) and the adult stage has previously been reported from starry ray (*Raja radiata* Donovan, 1808) off the north and east coasts of Iceland (Baer, 1962).

Fourth-stage and adult *Hysterothylacium rigidum* worms were found buried in ulcers in the stomach wall. Larvae have been reported from long rough dab in Iceland (Ólafsdóttir, 1999) and other fish are likely to serve as intermediate hosts (Køie, 1993). Anglerfish is, on the other hand, the only definitive host reported for *H. rigidum* (Berland, 1961; Køie, 1993; Petter & Cabaret, 1995). *Contracaecum* sp./*Phocascaris* sp. larvae were found encapsulated in the stomach wall. These larvae are found in a number of fish hosts and as mature worms in birds and marine mammals (Smith & Wootten, 1984).

Since the study focused mainly on intestinal parasites, and on the life cycle of *Prosorhynchoides gracilescens* (see Eydal *et al*, 1998, 2000), the stomach, and its contents, were only studied secondarily. In addition, large volumes and mucous consistency of stomach contents made thorough studies difficult.

Otodistomum sp. and *Grillotia* sp. have not previously been recorded from anglerfish.

Intestine

A total of 13 parasite species were found in the intestines (Table 3).

The specimens of *Stephanostomum* sp. were too poorly preserved for proper identification. The parasites, *S. cesticillum* (Mollin, 1858), *S. kovalevi* (Parukhin, 1968) and *S. lophii* Quinteiro, *et al*, 1993 have been reported from anglerfish (see Quinteiro *et al*, 1993), but none of these species have been found in Icelandic or adjacent waters. Adult *P. gracilescens* were found in all, but one of the fish. The larval stage is abundant in codfish where their distribution coincides with anglerfish in Icelandic waters (Eydal *et al*, 1998). The remaining digeneans are generalists in their choice of final hosts and have been reported from a large number of fish species in the North Atlantic (Polyanskii, 1966; Margolis & Arthur, 1979; McDonald & Margolis, 1995; Køie, 2000).

Tetraphyllid plerocercoids (sometimes referred to as "*Scolex pleuronectis*") and *E. gadi* are found in a large number of teleost fishes in the Atlantic Ocean (Polyanskii, 1966; Margolis & Arthur, 1979; Orts *et al*, 1988; McDonald & Margolis, 1995; Køie, 2000). Numerous reports also exist from Icelandic waters (Baer, 1962; Wesenberg-Lund, 1952; Ólafsdóttir, 1999).

Hysterothylacium sp. larvae were found encapsulated in the intestinal wall of two fish. These larvae were smaller than *H. aduncum,* which are typically encysted outside the digestive tract. The other Anisakid nematodes found in the intestines of anglerfish

Table 3. Prevalence (%) and intensity of parasites in the intestine of anglerfish (*Lophius piscatorius*) south and southwest off Iceland in 1995

		Fish length: 21-60 cm Sample size: n = 19				**Fish length: 61-119 cm Sample size: n = 15**			
	Station no.	**Preva-lence**	**-Intensity**			**Preva-lence**	**Intesity**		
			Mean	**Median**	**Range**		**Mean**	**Median**	**Range**
DIGENEA									
Acanthocolpidae *Stephanostomum* sp.	2,3,4,7	21	19.7	12.5	1-53	7	132	132	132
Bucephalidae *Prosorhynchoides gracilescens* (Rudolphi, 1819) Stunkard, 1976	All except 5	95	826.4	284	1-3908	100	2362.9	983	160-14390
Fellodistomidae *Steringophorus furciger* (Olsson, 1867) Odhner, 1905	3,6,9	0	-	-	-	20	20	26	3-31
Lesithasteridae *Lecithaster gibbosus* (Rudolphi, 1802) Lühe, 1901	3,11	11	2.5	2.5	1-4	0	-	-	-
Zoogonoidae *Zoogonoides viviparus* (Olsson, 1868) Odhner, 1902	1,2,3,4, 5,6,16	26	5.2	5	1-12	20	8	9	5-10
CESTODA									
Tetraphyllidea Tetraphyllid larvae	All except 2,4,5,8	68	58.1	49	2-160	87	128.9	51	2-600

NEMATODA									
Anisakidae *Hysterothylacium aduncum* (Rudolphi, 1809) Deardorff & Overstreet, 1981 (sensu lato) L3	All except 7	32	2.7	2	2-4	27	5.2	5	1-10
H. aduncum L4		53	8.7	5	1-40	27	6.7	4.5	1-17
H. aduncum L5 female		68	5.7	4	1-17	53	7.6	5	2-20
H. aduncum L5 male		37	3	2	1-9	27	2.5	2.5	1-4
Hysterothylacium sp. L3	2,6	5	4	4	4	7	5	5	5
Anisakis simplex (Rudolphi, 1845) Dujardin, 1845 (sensu lato) L3	4,6,8,9, 15, 18	11	1	1	1	33	9.2	5	1-20
Pseudoterranova decipiens (Krabbe, 1878) Gibson & Colin, 1982 (sensu lato) L3	4,18	5	1	1	1	13	5.5	5.5	1-10
Capillaridae *Capillaria* sp.	2,4,6,7, 10,12,13	37	5.7	5	3-12	13	11	11	10-12
Cystidicolidae *Spinitectus* sp.	2	5	4	4	4	0	-	-	-

have been reported from a number of fish species in Icelandic waters (Kreis, 1958; Hauksson, 1992). The prevalence and intensities of these nematodes are undoubtedly underestimated, as encapsulated larvae outside the digestive tract were not included in the study. *Spinitectus* sp. was found in one fish. The only species belonging to this genus and reported previously from Iceland is *S. oviflagellis* Fourment, 1883 found in long rough dab (Ólafsdóttir, 1999). *Capillaria* sp. were found in nine anglerfish. Nematodes of this genus are common parasites of gadoid fishes (Køie, 1993) and their presence in anglerfish is probably the result of a recent ingestion of a codfish.

P. gracilescens was by far the most common parasite in the present study (Table 3). The maximum intensity was estimated to be 14,390 individuals. Brinkmann (1957) observed 94% prevalence of *P. gracilescens* in anglerfish from Norwegian waters and stated that in one fish the parasite was found "in thousands". Much lower prevalence (1.5%) has been reported from anglerfish off the Spanish coast (Munoz *et al.*, 1989). *P. gracilescens* was the only species that increased significantly with fish length ($R^2 = 0.2614$, $F_{1,31} = 10.97$, $p<0,01$). Regression analyses of parasite intensity and fish length for other species did not reveal any significant relationship.

In addition to the *Stephanostomum* spp. mentioned above, few species previously reported from anglerfish were missing in the present study. *Prosorhynchus squamatus* Odhner, 1905 has been reported in anglerfish from waters of the Faroe Islands (Køie, 2000). This species has been reported in short-spined sea scorpion *Myoxocephalus scorpius* (L.) from Iceland (Brinkmann, 1956). *Bucephaloides meridionalis* was described as a new species in anglerfish from the eastern Atlantic by Gaevskaya & Aleshkina (1985). *Hemiuris levinseni* Odhner, 1905 has been reported from anglerfish caught off the Faroe Islands, (Køie, 2000). The species is a frequent parasite of gadoid fishes and may have been a parasite of prey.

L. gibbosus, Z. viviparus, S. furciger, Hysterothylacium sp., *Capillaria* sp., *Spinitectus* sp. and *E. gadi* have not been recorded earlier from anglerfish.

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should also be published on the SSP Home Page and thus be made available for everyone interested.

Plans, which consider irregular publishing of the SSP Bulletin in future years, mean that the Society will still need a responsible editor. From the beginning, Jorun Tharaldsen has been the Editor-in-chief. Together with a number of national editors, which have formed an Editorial Board, and several colleagues from the Board of the Society, she has done a great, unselfish job. At this turning point Nordic parasitologists want to thank Jorun Tharaldsen and all her collaborators for skilled work and we are glad to announce that Jorun Tharaldsen has agreed to continue as the editor of irregularly published SSP Bulletin issues at least until 2005.

Reykjavík, Iceland in May 2003

Karl Skirnisson

President of the Scandinavian Society for Parasitology

From the editor

Dear readers,

I would like to thank both the members and the board of the Scandinavian Society for Parasitology, and all those who have contributed to this journal since the journal was started in 1991. As the president, Karl Skirnisson has written above, times have changed a lot since that, communications and exchange of information via e-mail and the Internet, especially, have made great impact on our lives.

But all the advantages of these improved ways of communications have not replaced the need for personal contacts. Although the society consists of members from all the 5 Nordic countries, and quite a few others as well, we generally consist of very small, isolated groups, covering a great variety of subjects. The symposia have always been our most important meeting place, and hopefully that will continue as before. So, although this is the last regular issue, I will still be in charge of the issues of the Bulletin printed in connection with these for a while.

Oslo, Norway, May 2003-05-21

Jorun Tharaldsen
Editor

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