APPLIED ASPECTS OF MARINE PARASITOLOGY

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Horta 21-24 May 2006

Isabel Afonso-Dias, Gui Menezes, Ken MacKenzie & J.C. Eiras (Eds)

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ORGANIZING COMMITTEE AND EDITORS

Isabel Afonso-Dias
Centro de Ciências do Mar do Algarve (CCMAR)
FCMA, Universidade do Algarve
Campus de Gambelas
8005-139 Faro
Portugal
Phone +351 289 800 051
Fax +351 289 800 069
e-mail: ccmar@ualg.pt

Gui Menezes
Centro do IMAR da Universidade dos Açores (IMAR/DOP)
Department of Oceanography and Fisheries (DOP), Universidade dos Açores
Cais de Sta. Cruz,
9001-862 Horta
Portugal
Phone +351 292 200 400
Fax +351 292 200 411
e-mail: gui@uac.pt

Ken MacKenzie
School of Biological Sciences Zoology Building
University of Aberdeen,
Tillydrone Avenue
Aberdeen
Scotland, United Kingdom
Phone +441 224 272 861
Fax +441 224 272 396
e-mail: k.mackenzie@abdn.ac.uk

J.C. Eiras
Departamento de Zoologia e Antropologia
Faculdade de Ciências, Universidade do Porto
Pr. Gomes Teixeira
4099-002 Porto
Portugal
Phone: +351 223 401 513
Fax: +351 223 401 511
e-mail: jceiras@fc.up.pt

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EDITORIAL NOTES

The International workshop on "Applied Aspects of Marine Parasitology" was an initiative aiming to promote and improve the general knowledge about the current studies on fish parasites and its potential application in the regions of Azores and Algarve. Scientists studying marine parasites have become more holistic and more international in recent years. Therefore, this was a good opportunity to exchange information between colleagues and to promote interdisciplinarity in Marine Parasitology in Portugal.

The workshop was held at the Department of Oceanography and Fisheries (DOP), Horta, island of Faial in the Azores from 21 to 24 May 2006. The event took place in two different locations in Horta. The seminars were held in Centro do Mar (Sea Centre), in the old Whale Factory at Porto Pim, and the practical component took place in the laboratories of DOP. During the workshop laboratory exercises were carried out as well as lectures to cover essential methods, practical tips and different sampling approaches to study marine parasites under a different perspective.

The importance of this workshop was enhanced by undertaking a preliminary parasitological survey on some of the most important fish species captured around the Azores of which studies are scarce.

We would like to thank the sponsors who have generously contributed to finance the Workshop and to publish the Proceedings.

PREFACE
Klaus Rohde

Marine parasites are of immense ecological and economic importance. Almost all (if not all) groups of marine animals including the various invertebrates and, among the vertebrates, fish, marine birds and reptiles, are hosts to parasites, often with high prevalences and intensities of infection. Total global (marine and freshwater) aquaculture in 2004 was estimated to be worth more than US$ 55 billion. The commercial value of cultured salmonid fishes alone in 2001 was around US$ 3.84 billion (7.44 billion for all finfish). Disease, and much of it due to parasites, is the single most important factor threatening the aquaculture industry. Parasites led to the collapse of European flat oyster aquaculture in the years following 1979 and to devastating effects on oyster culture on the North American east coast over many years. Mass mortalities caused by parasites in the wild have not been well documented for marine vertebrates and invertebrates, due to the large spaces in the oceans involved and the difficulties in monitoring such effects, but there can be no doubt that parasites are responsible for mass mortalities of natural fish populations, and there is evidence for the involvement of parasites in beachings of whales. Introduction of a monogenean ectoparasite into the Aral Sea led to the total collapse of sturgeon and caviar fisheries there in the thirties. Economic losses may be via effects on the human psyche: reports on herring worms on German television some years ago induced many people to change their dietary habits away from fish, causing considerable losses to fishermen. Fish are routinely screened for parasite infections (many of them only of aesthetic significance), which causes considerable costs. However, the useful role of parasites must not be forgotten. They can be used in the study of host populations and migrations, and in pollution monitoring, because pollution affects the composition of parasite communities, and because some parasites store certain pollutants to a higher degree than their hosts.

For all these reasons, a workshop dealing with applied aspects of marine parasites is important. At such a workshop, experts from around the world can meet, exchange ideas and communicate recent findings to younger participants, that is, to the future of the discipline. The list of speakers and participants shows that both these objectives have been achieved at this workshop, which concentrated on fish parasites. Speakers included outstanding experts from Portugal, Canada, the United States, Germany, Britain, Norway, and Chile. Particularly welcome: the conference photo shows many young faces.

Seminars at the symposium ranged from disease trends in natural marine communities, the use of parasites in fish population studies, deep-sea parasites, parasites as indicators of environmental change, to parasites in mariculture, to mention only some. Of particular interest also: several seminars dealt with parasites in Azores and other Portuguese waters, and included a visit to the local fish market. There can be no doubt that a seminar on how to prepare whole mounts of parasites was welcomed especially by the younger participants.

The Proceedings of this workshop will be useful not only to people involved in local fisheries and parasite work, but also to the international community of parasitologists and to people associated with the fishing industry.

Klaus Rohde, Professor emeritus
e-mail: krohde@une.edu.au
School of Environmental Sciences & Natural Resources Management
University of New England
Armidale, Australia

LIST OF PARTICIPANTS

Afonso-Dias, Isabel
(ccmar@ualg.pt)
Centro de Ciências do Mar do Algarve (CCMAR), FCMA, Universidade do Algarve. Campus de Gambelas, 8005-139 Faro - Portugal

Alves, Inês Susana Pereira dos Santos
(alvin_@portugalmail.com)
Rua da Madalena, 55, Vairão, 4485-649 Vila do Conde - Portugal

Amaro, Bruno Eduardo Fortunato
(amarobruno@clix.pt)
Inspecção Regional das Actividades Económicas. Secretaria Regional da Economia - Serviço da Ilha do Faial, Rua do Pasteleiro, 92, 9900-069 Horta - Portugal

Berland, Bjørn
(Berland@bio.uib.no or berland@zoo.uib.no)
Biologisk institutt, Universitetet i Bergen, Allégt. 41, N-5007 Bergen - Norge/Norway

Branco, José
(branco@uac.pt)
Department of Oceanography and Fisheries, University of the Azores, Cais de Sta. Cruz, 9901-862 Horta - Portugal

Caira, Janine N.
(jcaira@uconnvm.uconn.edu)
University of Connecticut, Ecology and Evolutionary Biology, U-4375 N Eagleville, Storrs, CT 06268 - USA

Carrapato, Humberto
(h.carrapato@docapesca.pt)
Docapesca Portos e Lotas S.A., Rua General Gomes de Araújo - Bloco C - Pisos 3 e 4, 1350 - 355 Lisboa - Portugal

Carreiro, Eduarda Agnello M. de Vaz
(eduarda_agnello@hotmail.com)
Inspecção Regional das Actividades Económicas. Secretaria Regional da Economia, Av. Infante D. Henrique, 52, 9500-150 Ponta Delgada - Portugal

Costa, Graça
(gcosta@uma.pt)
Centro de Estudos da Macaronésia (CEM), Universidade da Madeira, Estação de Biologia Marinha do Funchal, Promenade Orla Marítima do Funchal, 9000-107 Funchal - Portugal

Eiras, Jorge
(jceiras@fc.up.pt)
Departamento de Zoologia e Antropologia, Faculdade de Ciências, Universidade do Porto, Pr. Gomes Teixeira, 4099-002 Porto - Portugal

Ferreira, Susana Margarida de Freitas
(smff@ci.uc.pt)
Imar - Inst. do Mar, Centro Interdisciplinar de Coimbra, Dept. de Zoologia, Universidade de Coimbra. Largo Marquês de Pombal, 3004-517 Coimbra - Portugal

George-Nascimento, Mario
(mgeorgen@ucsc.cl)
Universidad Catolica de la Santisima Concepcion, Fac. de Ciencias, Depart. Ecol. Costera, Casilla 297, Concepcion - Chile

Gomes, Jacinto José Carneiro
(jacinto.gomes@lniv.min-agricultura.pt)
Lab. Nacional de Investigação Veterinária, Estrada de Benfica, 701, 1549-011 Lisboa - Portugal

Hauton, Chris
(ch10@noc.soton.ac.uk)
University of Southampton School of Ocean and Earth Science, National Oceanography Centre, Southampton, Waterfront Campus, European Way, Southampton, Hants, S014 3ZH UK

Hemmingsen, Willy
(willy.hemmingsen@ib.uit.no)
Institutt for biologi, Universitetet i Tromsø, N-9037 Tromsø – Norway

Henriques, Andreia
(andreiahenriques@uac.pt)
IMAR Center of the University of the Azores, Department of Oceanography and Fisheries, University of the Azores, Cais de Sta. Cruz, 9901-862 Horta - Portugal

Isac, Radu
Direcção Regional do Desenvolvimento Agrário. Vinha Brava, 9701-861 Angra do Heroísmo - Portugal

Kadar, Eniko
(enikokadar@uac.pt)
IMAR Center of the University of the Azores, Department of Oceanography and Fisheries, University of the Azores, Cais de Sta. Cruz, 9901-862 Horta - Portugal

Klimpel, Sven
(Sven.Klimpel@uni-duesseldorf.de)
Heinrich-Heine-University Dusseldorf, Institute of Zoomorphology, Cell Biology and Parasitology, Universitatsstrasse 1, DE - 40225, Dusseldorf - Germany

Krug, Helena Maria de Noronha
(hkrug@uac.pt)
IMAR Center of the University of the Azores, Department of Oceanography and Fisheries, University of the Azores, Cais de Sta. Cruz, 9901-862 Horta - Portugal

Lafferty, Kevin D.
(lafferty@lifesci.ucsb.edu)
Marine Science Institute UC Santa Barbara, CA 93106 - USA

MacKenzie, Ken
(k.mackenzie@abdn.ac.uk)
School of Biological Sciences Zoology Building, University of Aberdeen, Tillydrone Avenue, Aberdeen, Scotland, United Kingdom

Marcogliese, David
(david.marcogliese@ec.gc.ca)
St. Lawrence Centre, Environment Canada 105 McGill, 7th Floor, Montreal, Quebec - Canada H2Y 2E7

Matos, Valentina
(val.matos@linus.uac.pt)
IMAR Center of the University of the Azores, Department of Oceanography and Fisheries, University of the Azores, Cais de Sta. Cruz, 9901-862 Horta – Portugal

Mealha, Sandra
Direcção Regional do Desenvolvimento Agrário. Vinha Brava, 9701-861 Angra do Heroísmo – Portugal

Menezes, Gui
(gui@uac.pt)
IMAR Center of the University of the Azores, Department of Oceanography and Fisheries, University of the Azores, Cais de Sta. Cruz, 9901-862 Horta - Portugal

Mesquita, Célia
(celia.mp.mesquita@azores.gov.pt)
Servigo de Desenvolvimento Agrario do Faial, Qt[a]. S. Lourengo, 9900-401 Horta, Faial - Portugal

Monteiro, Ana Marta
(monteiro.amarta@gmail.com)
IMAR Center of the University of the Azores, Department of Oceanography and Fisheries, University of the Azores, Cais de Sta. Cruz, 9901-862 Horta - Portugal

Monteiro, Luísa Margarida Pedrosa Santos
(luisamonteiro_@hotmail.com)
Direcção Geral de Veterinária, Rua Dr[a]. Cristina Torres, 3 rch - Esq., 3080-210 Figueira da Foz - Portugal

Monteiro, Luísa
(luisamonteiro_@hotmail.com)
Porto de Pesca, Docapesca - Divisão de Inspecção Higio-Sanitária, Cabedelo 3080 - 661 Figueira da Foz - Portugal

Morais, Isabel
Direcção Regional do Desenvolvimento Agrário, Vinha Brava, 9701-861 Angra do Heroísmo - Portugal

Pham, Christopher
(phamchristopher@uac.pt)
IMAR Center of the University of the Azores, Department of Oceanography and Fisheries, University of the Azores, Cais de Sta. Cruz, 9901-862 Horta - Portugal

Ramos, Paula
(pramos@ipimar.pt)
Instituto de Investigação das Pescas e do Mar (IPIMAR), Av. De Brasília, 1449-006 Lisboa - Portugal

Rosa, Alexandra
(xana@uac.pt)
IMAR Center of the University of the Azores, Department of Oceanography and Fisheries, University of the Azores, Cais de Sta. Cruz, 9901-862 Horta - Portugal

Santos, Maria João
(mjsantos@fc.up.pt)
Departamento de Zoologia e Antropologia, Faculdade de Ciências, Universidade do Porto, Pr. Gomes Teixeira, 4099-002 Porto - Portugal

Saraiva, Aurélia
(amsaraiv@fc.up.pt)
Departamento de Zoologia e Antropologia, Faculdade de Ciências, Universidade do Porto, Pr. Gomes Teixeira, 4099-002 Porto - Portugal

Severino, Ricardo Bruno de Araújo
(ricardoseverino@gmail.com)
Universidade do Algarve, FCMA, Campus de Gambelas, 8005-139 Faro - Portugal

Participant group photo (1 – Valentina Matos, 2 – José Branco, 3 – Isabel Morais, 4 – Christopher Pham, 5 – Luísa Monteiro, 6 – Ricardo Severino, 7 – Sandra Mealha, 8 – Susana Ferreira, 9 – Janine Caira, 10 – Gui Menezes, 11 – Isabel Afonso-Dias, 12 – Bruno Amaro, 13 – Inês Alves, 14 – Eduarda Carreiro, 15 – Jacinto Gomes, 16 – Célia Mesquita, 17 – Bjørn Berland, 18 – Alexandra Rosa, 19 – Kevin Lafferty, 20 – Willy Hemmingsen, 21 – Aurélia Saraiva, 22 – Maria João Santos, 23 – David Marcogliese, 24 – Paula Ramos, 25 – Ken MacKenzie, 26 – Helena Krug, 27 – Graça Costa, 28 – Jorge C. Eiras, 29 – Chris Houton, 30 – Mário George-Nascimento, 31 – Sven Klimpel, 32 – Eniko Kadar, 33 – Andreia Henriques).

EMERGING DISEASES IN MARICULTURE

WILLY HEMMINGSEN

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The continuing decline in stocks of most of the commercially important species of marine fish in the North Atlantic in recent years has led to a dramatic increase in marine fish farming in the Northern Hemisphere. Apart from the well-documented increase in salmon farming, species as Atlantic cod *Gadus morhua*, Atlantic halibut *Hippoglossus hippoglossus*, haddock *Melanogrammus aeglefinus* and wolffish *Anarhichas* spp., are of growing interest for fish farmers. To illustrate the potential, a recent report concluded that within 20 years, the value of farmed cod in Norway could equal that of today's salmon farming industry. With the development of intensive cultivation of these species and also based on previous experience, new disease problems are certain to appear.

The main limiting factor in the production of farmed fish is disease, whether it is the result of poor nutrition, adverse environmental conditions, or infections with microorganisms or metazoan parasites. Parasites can affect the production of cultured fish in different ways; spoiling the appearance of the product, posing a threat to human health or by causing direct disease and mortality of the stock. As pointed out in previous scientific work, prophylactic strategies in avoiding diseases are economically (and ecologically) preferable to addressing the problem when it first appears. Each mariculture site is unique regarding both intermediate and final hosts, which may create different parasite "suites" at every locality. The main threat to farmed fish from parasites comes from those with direct single-host life cycles, the transmission of which is favoured by the dense concentration of fish in an intensive farming situation.

For parasites with indirect life cycles both food falling through the nets and the site itself could attract possible intermediate hosts (birds, mammals, fish, gastropods etc.). This could elevate the levels of transmission stages, making these localities the focal points of parasite transmission.

Willy Hemmingsen (e-mail: willy.hemmingsen@ib.uit.no) - Institutt for biologi, Universitetet i Tromsø, N-9037 Tromsø – Norway.

PARASITES WITH DIRECT SINGLE-HOST LIFE CYCLES

The main threat to farmed fish from parasites comes from those with direct single-host life cycles, the transmission of which is favoured by the dense concentration of fish in an intensive farming situation. The most common and problematical disease agents are likely to be viruses, bacteria and fungi, but protozoan and metazoan parasites with direct life cycles can also cause serious problems wherever fish are held in dense populations. Under this heading we include most protozoans, all monogeneans and most crustaceans. The problems caused by metazoan ectoparasites are not only due to the direct effects of the parasites themselves, but also due to the fact that they can pave the way for secondary opportunistic infections of microorganisms (CUSACK & CONE 1986).

Marine myxozoans present a unique problem because, although many freshwater myxozoans have been shown to require an alternative invertebrate host in their life cycle (KENT et al. 2001) this has not been shown to be true of any marine myxozoan.

Protozoa

Amoebic gill disease due to *Neoparamoeba pemaquidensis* is well known to salmon farmers, but the disease has also been reported from turbot (DYKOVÁ et al. 1998), so we can predict that problems due to *Paramoeba* and *Neoparamoeba* spp. are likely to occur in other cultured marine fish. The disease is characterized by extensive hyperplasia and lamellar fusion of the gills.

The microsporidian *Loma branchialis* has been reported from several species of gadoid fish, most commonly from cod and haddock *Melanogrammus aeglefinus* (see LOM & DYKOVÁ 1992). The most common sites of infection are the gill filaments and pseudobranchs, but in heavy infections of cod the viscera, especially the heart and liver, were also observed to be infected (MURCHELANO et al. 1986). In the gills it causes clubbing of the lamellae with, presumably, respiratory impairment (MORRISON & SPRAGUE 1981). Another microsporidian, *Glugea stephani*, infects the intestinal wall of flatfish and, in heavy infections, the mesenteries and various internal organs (MCVICAR 1975; CANNING & LOM 1986).

There have been several reports of microsporidians of the genus *Pleistophora* infecting the musculature of cod (HEMMINGSEN & MACKENZIE 2001), including the description of *Pleistophora gadi* by POLYANSKY (1955). YOUNG (1969) reported an emaciated cod with ulcerated skin lesions with a *Pleistophora* infection of the musculature.

Some flagellate protozoans have invertebrates such as leeches and parasitic crustaceans as vectors and so are dealt with in the section below on parasites with complex indirect life cycles. Amongst those with direct life cycles is the well-known ectoparasitic pathogen of freshwater fish *Ichthyobodo necator*, which has been reported from wild and captive flatfish in the North Atlantic (BULLOCK & ROBERTSON 1982; CONE & WILES 1984) and so has the potential to be a serious pathogen of these fish in farm situations. Species in the related genus *Cryptobia* are also potentially serious pathogens of flatfish (NEWMAN 1978).

Ectoparasitic ciliates are amongst the most notorious pathogens of farmed fish, both freshwater and marine. Species of the genus *Trichodina* are the most frequently reported.

Two species of this genus — *T. murmanica* and *T. cooperi* — were reported by POYNTON & LOM (1989) to increase significantly on cod held in captivity. In flatfish, heavy *Trichodina* infections were reported from farmed plaice by MACKENZIE et al. (1976), and *Trichodina* infections were considered to be a major problem in turbot cultivation by MCVICAR (1978).

Endoparasitic ciliates can also be serious pathogens. DYKOVÁ & FIGUERAS (1994) reported heavy mortalities of turbot in a grow-out facility in Spain caused by concomitant infections of an unidentified histophagous ciliate and the microsporidian *Tetramicra brevifilum.* They considered that the ciliate played the dominant role in pathogenesis.

Myxosporea

Myxosporeans are already recognized as serious pathogens in mariculture (ALVAREZ-PELLITERO & SITJÀ-BOBADILLA 1993; MORAN et al 1999). The greatest problems in North Atlantic mariculture are likely to arise from members of the genera *Kudoa, Sphaerospora, Ceratomyxa,* and *Myxidium*. The most serious impact of *Kudoa* infections is not the pathogenesis, but the post-mortem spoilage effect. After death of the host fish, proteolytic enzymes released by the parasite cause muscle degeneration and liquefaction, so rendering the product unmarketable.

Helminths

The only helminths with direct life cycles to be considered are leeches (Hirudinea) and monogeneans.

Leeches are only likely to be pests of farmed fish held in cages close to the substrate, where these parasites lie in wait for their hosts, or they may be introduced with wild fish caught for stocking purposes. BARAHONA-FERNANDES & DINIS (1992) described problems with the leech *Platybdella soleae* on three species of sole caught as brood stock for a mariculture system. Leeches also serve as vectors for some blood protozoans (see below).

Monopisthocotylean monogeneans of the genus *Gyrodactylus* are common parasites of marine fish in the North Atlantic, with some fish species playing host to several species of site-specific gyrodactylids. For example, cod is host to six species of *Gyrodactylus* (see APPLEBY 1994). Two of these have caused disease in caged cod in Norway, with heavy *G. marinus* infections causing mortalities in large broodstock cod (SVENDSEN 1991).

Polyopisthocotylean monogeneans are mostly gill parasites and blood feeders. Species not considered at all pathogenic under natural conditions have the potential to cause anaemia, loss of condition and eventual death of fish held under conditions of high population density, as demonstrated by LONGSHAW (1996). Several species of the genus *Diclidophora* are host-specific for gadoid fish in the North Atlantic. Cod and haddock are exceptions, but saithe and whiting have *D. denticulata* and *D. merlangi* respectively. Experience with related species in Japanese mariculture suggests that these monogeneans could cause problems in North Atlantic mariculture.

Crustaceans

The problems caused by the parasitic copepod *Lepeophtheirus salmonis* in marine salmon farming are well documented (see PIKE 1989). Three other species of the genus *Lepeophtheirus* have the potential to cause similar problems in flatfish mariculture. BOXSHALL (1977) described the histopathology of *L. pectoralis* infection of the pectoral fin of flounder *Platichthys flesus. L. pectoralis* has been reported from a number of flatfish species (KABATA 1979). The other species are *L. hippoglossi*, best known as a parasite of Atlantic halibut, but also reported from other species of flatfish, and *L. thompsoni*, a parasite of turbot and brill *Scophthalmus rhombus* (see KABATA 1979). *Caligus elongatus* is also well-known as a pathogen of sea-cage salmon and has a much wider host range than *L. salmonis*.

PARASITES WITH COMPLEX INDIRECT LIFE CYCLES

Parasites with complex indirect life cycles tend to be adapted to low host densities. They use several hosts in their life cycles and usually include relatively long-lived 'resting' stages' in one of their intermediate hosts. Such parasites can persist in a population of farmed fish only if the other hosts necessary for completion of their life cycles are present in the immediate vicinity of the farm. Some protozoans, most helminths and a few crustaceans come into this category. Marine parasites tend to use transmission through intermediate hosts more than freshwater parasites, which rely more on active penetration of final hosts (MARCOGLIESE 1995).

Protozoa

Haematozoan protozoans with invertebrates such as leeches and parasitic crustaceans as vectors are already well known as disease agents in mariculture. Two species of the genus *Haemogregarina, H. sachai* and *H. simondi,* have been reported as pathogens of farmed flatfish, causing subcutaneous and ulcerated lesions (MCVICAR 1975; KIRMSE 1978, 1980, 1987). *Haemogregarina aeglefini,* a parasite of gadoid fish, and *Haemohormidium terraenovae,* a parasite of flatfish, are other potentially pathogenic species in mariculture. Infections with the latter species may have contributed to anaemia and mortalities of American plaice *Hippoglossoides platessoides* held in captivity (SIDDALL et al. 1994). Haemoflagellates have been reported to cause anaemia and death in some marine fish, particularly juveniles (KHAN 1985; BURRESON & ZWERNER 1984).

Helminths

Problems with helminth parasites with complex indirect life cycles in mariculture are likely to be mainly due to the spoilage caused by larval forms: digenean metacercariae, cestode plerocercoids and nematode larvae.

For digeneans the main culprits are likely to be species in the families Acanthocolpidae, Bucephalidae and Heterophyidae, all of which have fish as second intermediate hosts. The acanthocolpid species *Stephanostomum baccatum* and *S. tenue* are prime candidates as pests in mariculture. The first named is a common parasite of flatfish in the North Atlantic, forming prominent white cysts in the musculature (MACKENZIE & LIVERSIDGE 1975). The second provides a good example of how an apparently innocuous parasite under natural conditions can become a deadly pathogen when presented with the opportunity to infect a host species that it would be highly unlikely ever to come into contact with in nature. MCGLADDERY et al. (1990) described mass mortalities of maricultured rainbow trout *Oncorhyhchus mykiss* due to infections of *S. tenue* in the pericardial cavity. Digeneans of the family Bucephalidae have bivalve molluscs as first intermediate hosts and usually fish as second intermediate hosts. Several species in the North Atlantic have flatfishes as second intermediate hosts, with metacercariae encysting in the musculature (MACKENZIE & GIBSON 1970; MATTHEWS 1973). The best-known marine digenean of the family Heterophyidae is probably *Cryptocotyle lingua*. Farmed fish become infected with *C. lingua* when cages are sited close to rocky areas with large populations of the mollusc first intermediate host, the periwinkle *Littorina littorea* (see KRISTOFFERSEN 1991). The metacercariae of *C. lingua* encyst in subcutaneous, and sometimes deeper, tissues, causing the condition known as 'black spot'. Heavy infection could render a high proportion of fillets unmarketable. LYSNE et al. (1998) found that cod held in cages close to the surface became infected with significantly more cysts of *C. lingua* than those held in cages lower in the water column.

Adult digeneans usually infect the alimentary tract of fish and are generally benign unless they are present in exceptionally large numbers. Heavy infections are rare in cultured fish because natural feeding on the second intermediate hosts of digeneans is unlikely.

Cestode plerocercoids of the order Trypanorhyncha are frequently found encysted in the flesh of marine fish. RAE (1958) described the occurrence of plerocercoids of *Grillotia erinaceus* in the flesh of Atlantic halibut caught on certain grounds in the northeast Atlantic.

Larvae of anisakid nematodes not only form unsightly blemishes in farm fish fillets, but also constitute a human health hazard if eaten in undercooked or lightly pickled fish (MARGOLIS 1977; SMITH 1999). *Anisakis* spp. and *Pseudoterranova decipiens* have mammalian definitive hosts, cetaceans for *Anisakis* and pinnipeds for *P. decipiens*, and they are capable of infecting other mammals, including man. Farmed fish can acquire infections of ascaridoid nematodes by eating either infected invertebrate first intermediate hosts or infected fish. LEE (1988) found levels of infection with *P. decipiens* in farmed cod to be comparable to those in wild cod, but HEMMINGSEN et al. (1993) showed that the transmission of larval ascaridoid nematodes to caged cod could be broken through artificial feeding in the form of discarded cuttings from the codfillet industry which had been stored frozen at -30°C for at least 20 days before use. This treatment would have ensured that any nematodes present in the cuttings were killed, but feeding of fresh cuttings would be likely to pass on infections.

Adult acanthocephalans in the intestines of fish are not normally associated with severe pathology, but there have been reports of pathological changes in wild cod associated with *Echinorhynchus gadi*, a common parasite of gadoid fish (SHULMAN 1956; NORDENBERG 1963; WAWRZYNIAK & GRAWINSKI 1991). Farmed fish could become infected through eating infected amphipods and mysids.

Crustacea

The parasitic copepod *Lernaeocera branchialis* is probably the most serious metazoan pathogen of wild cod (HEMMINGSEN & MACKENZIE 2001). It also significantly affects the condition and fecundity of haddock (HISLOP & SHANKS 1981). While it is predominantly a parasite of gadoid fish, *L. branchialis* has a wide host range (KABATA 1979) and is therefore a potential threat to many species under consideration for mariculture in the North Atlantic. It is an unusual species among parasitic copepods in that it has an indirect life cycle involving an intermediate host on the gills of which the larvae develop until infective stages detach and swim off in search of suitable gadoid definitive hosts. Several teleost species, mostly flatfish but including lumpfish *Cyclopterus lumpus*, serve as intermediate hosts (KABATA 1979).

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INTERDISCIPLINARITY IN MARINE PARASITOLOGY

DAVID J. MARCOGLIESE

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Parasitology is unarguably interdisciplinary because it intersects phylogenetic and subjective fields within the biological sciences. Furthermore, parasitology provides a powerful tool that contributes greatly to our comprehension of contemporary environmental problems and management of natural biological resources, especially when used in conjunction with other disciplinary approaches. Examples incorporating parasitology in multidisciplinary and interdisciplinary research are provided for the evaluation of commercial fish stock structure, the effects of environmental pollution, and the oceanographic impacts of climate change. Finally, recommendations are made to include parasitology in a multidisciplinary context to better aid resource management and conservation needs.

David J. Marcogliese (e-mail: david.marcogliese@ec.gc.ca) - St. Lawrence Centre, Environment Canada 105 McGill, 7th Floor, Montreal, Quebec - Canada H2Y 2E7.

INTRODUCTION

Parasitology is uniquely different from other zoological disciplines. Unlike ornithology or herpetology, for example, parasitology is not taxon based. Parasitic organisms are phylogentically diverse, with representatives found in virtually all animal phyla (ROHDE 2005). Rather, parasitology is the study of a lifestyle. Yet we do not see other biological disciplines with similar foundations. There are no fields of study restricted exclusively to herbivores or predators. Typically, the trophic function of organisms (or their lifestyles) are considered in an ecological context.

By convention, when studying parasites, researchers must acquire knowledge of their hosts and host-parasite interactions. By this virtue alone, parasitology may be viewed as interdisciplinary in nature. However, the interdisciplinarity expands well beyond this basic fact. In addition to crossing phylogenetic disciplines, host-parasite interactions can be examined at numerous hierarchical levels of structural organization ranging from molecular and cellular through physiological and organismal to ecological, encompassing numerous subject areas such as morphology, histology, cytology, biochemistry, physiology, developmental biology, immunology, ecology, genetics and evolutionary biology, among others (CHANDLER 1946; Fig. 1). Traditionally, parasitology has been divided into subdisciplines, each of which is a discipline in its own right. For example, research articles in the *Journal of Parasitology* are categorised into sections which include Biochemistry and Physiology, Development, Ecology and Epidemiology, Ectoparasitology, Functional Morphology, Immunology, Invertebrate-Parasite Relationships, Life Cycle-Survey, Pathology, Systematics-Phylogeny, and Therapeutics-Diagnostics. Many of these sections are themselves biological disciplines not unique to parasitology. The essential point is that in studying parasites, researchers must acquire expertise in other disciplines (CHANDLER 1946); hence, the interdisciplinary nature of parasitology.

Over 50 years ago, CHANDLER (1946), in his Presidential Address to the American Society of Parasitologists (ASP), recognized the difficuly in studying parasites when he made the analogy between a parasitologist and an orchid: "A parasitologist… requires long and careful nurturing, he develops slowly; and he is himself a parasite in that he depends on many other scientists for material aid." In recent years, parasitology has been incorporated into programmes oriented around environmental and resource management. In the process, parasitologists are collaborating with colleagues from other disciplines and thus maximising the information and knowledge derived from their research. Parasitology, though, is not only dependent on other disciplines but it complements them greatly in enhancing the understanding of ecosystem structure and function and the management and conservation of natural resources (not to mention molecular and cellular interactions and physiological processes). Indeed, HOLMES (1991), embracing lateral thinking in his Presidential Address to the ASP suggested that parasitologists should be "leaders in the vertical integration of knowledge in biology".

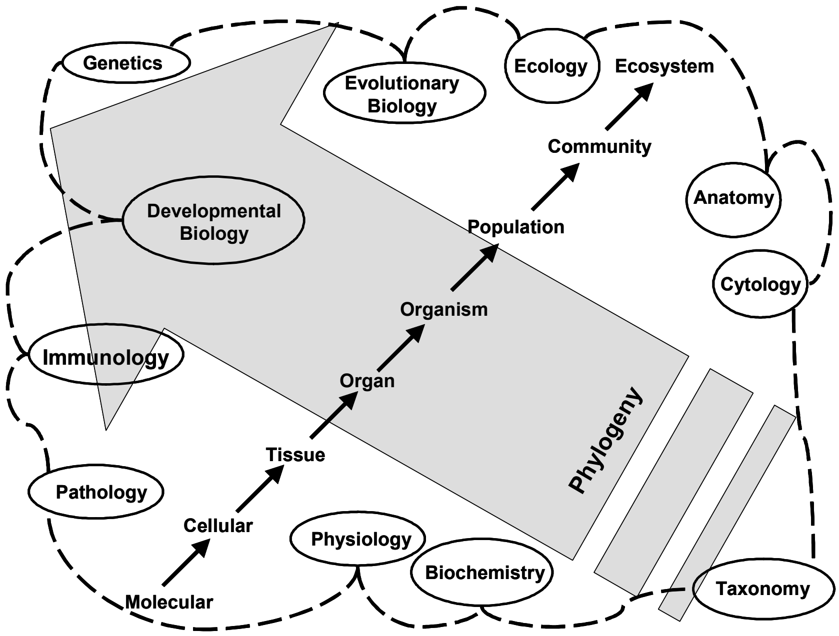

Figure 1. Schematic drawing representing the interdisciplinary nature of parasitology. Structural hierarchy is represented by the series of arrows running from the lower left to the upper right. Examples of the individual disciplines within parasitology are indicated within elipses along the periphery of the diagram. The large arrow running from the lower right to the upper left represents the phlogenetic diversity of parasitic organisms which crosses numerous higher taxa.

Parasitology clearly is a useful discipline that can contribute to our understanding of host diet, migration, population structure and phylogeny (WILLIAMS et al. 1992) and thus is of considerable value to resource managers. However, to be truly effective and contribute maximally to the development of scientific knowledge, parasitology can be most effective when used in conjunction with other concurrent research activities working toward similar goals (MARCOGLIESE 2004). Below, selected examples are provided of parasitological studies that I consider to be appropriately interdisciplinary and that explore new territory, embodying the interdisciplinarity of parasitology and its potential contributions to the management and conservation of biological resources. For the most part, I discuss applications to the marine environment and fisheries, but pertinent examples also will be drawn from freshwater systems, where recent advances may be applied equally to marine research.

FISH POPULATIONS AND STOCK STRUCTURE

Parasites effectively have been shown to be effective indicators of host migration, diet, population structure and phylogeny (WILLIAMS et al. 1992). For about half a century, scientists have used parasites as natural biological tags of fish host populations. This work has expanded to include macroinvertebrates and marine mammals (AZNAR et al. 1995; OLIVA & SÁNCHEZ 2005).

The most effective fisheries management strategy employs a variety of techniques to differentiate between and delineate fish stocks (BEGG & WALDMAN 1999). Potential sources of information include mark-recapture; catch data; otolith biochemistry; morphological measurements including meristics, morphometrics, and scale and otolith analyses; genetics, including variation in proteins, and mitochondrial and nuclear DNA; in addition to parasites (BEGG & WALDMAN 1999). However, few studies in the peer-reviewed literature actually use parasites in conjunction with any other of the preceding techniques (see references in BEGG & WALDMAN 1999).

Two recent studies that break this trend are particularly noteworthy. Four populations of winter flounder (*Pleuronectes americanus*) on the southwestern Scotian Shelf and northeastern Gulf of Maine in the Northwest Atlantic Ocean are clearly delineated by the use of parasites as biological tags (MCCLELLAND et al. 2005). These results were corroborated by comparisons of four microsatellite markers that identified significant genetic differences between all populations. In another study of seven flatfish species occurring sympatrically in the Northeast Atlantic Ocean off Portugal, MARQUES et al. (2006) demonstrated only low differentiation among samples from different areas using morphometric, meristic and parasitological information, although results did suggest ecological separation between certain areas along the coast. Conclusions derived from both of the above studies are reinforced because they were derived from multiple methods and disciplines. Indeed, without relying on complementary techniques, results from one approach can never be validated and stock determination cannot be reliably assessed (BEGG & WALDMAN 1999).

In a remarkably novel study, microsatellite genetic markers are compared among samples of steelhead trout (*Oncorhynchus mykiss*) and its digenean parasite, *Plagioporus shawi*, in the Northeast Pacific Ocean off Oregon (CRISCIONE et al. 2006). Surprisingly (or not!), the microsatellite markers of the parasite prove to be more effective than those of the host fish in differentiating among stocks of migrating fish. The authors attributed these results to the greater genetic structure among populations of *P. shawi* compared to the trout host, and suggested that this may be a common phenomenon in host-parasite systems.

ENVIRONMENTAL STUDIES

Numerous studies have examined the effects of environmental stress on parasite populations and communties, but few have combined parasitology with other fields of study in an interdisciplinary approach (MARCOGLIESE 2005). In a comprehensive study of parasites in relation to pollution, parasites, including metazoans and protists, were enumerated and various parasitological indices calculated in silver perch (*Bairdiella chrysura*) collected from numerous contaminated sites along the coast of Florida (LANDSBERG et al. 1998). Various somatic indices were measured for each fish. Habitats were characterized by measurements of standard water and sediment quality parameters in addition to a microtox assay. Ultimately, parasite species and parasitological indices were evaluated in relation to levels of organic and inorganic contaminants at each site. The authors concluded that parasites are more sensitive biomarkers of environmental stress than are morphological ones that reflected fish condition.

Extensive investigations incorporating metabolic, pathological and parasitological indices in fish were undertaken to monitor pollution in the North Sea, Red Sea and Mediterranean Sea (BROEG et al. 1999; DIAMANT et al. 1999). These studies were remarkable considering the breadth of expertise and amount of work required to obtain such a diverse array of information. By relying on a multidisciplinary approach, BROEG et al. (1999) were better able to interpret the variation in the data and differentiate between natural variation and pollution-mediated effects in the European flounder (*Platichthys flesus*) in the North Sea. Biochemical and histochemical techniques, in addition to the distribution of the ciliate *Trichodina* sp. and parasite species richness, distinguished among the study sites and earmarked a pollution gradient. Similarly, rabbitfish (*Siganus rivulatus*) collected from impacted and reference sites in the Mediterranean and the Red seas differed in their macroparasite fauna (gill monogeneans and gastrointestinal parasites) in addition to certain ecotoxicological biomarkers (DIAMANT et al. 1999).

While these studies are exemplary in their breadth and scope, they could be further improved if all the metabolic, pathological, parasitological and contaminant information were collected from the same individual fish. Presumably, the authors do possess this information but, admittedly, its analysis would be exceedingly complex. Indeed, BROEG et al. (1999) did report on the nature of the relationship between parasite species diversity and the intensity of *Trichodina* sp. with various biochemical and physiological biomarkers including liver colour, macrophage aggregate (MA) activity, lysozome stability and monooxygenase ethoxyresorufin o-deethylate (EROD) activity. Diversity correlated positively with MA activity and negatively with EROD activity while intensity of *Trichodina* sp. was positively related with liver colour and EROD activity and negatively with MA activity and lysozome stability.

Few parasitological studies focus on multiple measurements in individual fish, thus permitting more robust analyses of interdisciplinary parameters in a single environmental study. Pesticides, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) were measured in the livers of Mayan catfish (*Ariopsis assimilis*), as was the parasite fauna of each fish, in Chetumal Bay, Yucatan, Mexico (VIDAL-MARTÍNEZ et al. 2003). By using measurements in individual fish, the authors could test for associations between the contaminants and intensities of different species of parasites. With these protocols, it was possible to determine more effectively which species of parasite was sensitive to different pollutants, a relationship that is important to assist in the evaluation of the efficacy of a parasite as a bioindicator of environmental impact. Simply comparing the parasite fauna from sites with different levels of pollution does not permit equally robust conclusions. This approach also overrides the complicating factor of fish movement in and out of contaminated areas. VIDAL-MARTÍNEZ et al. (2003) subsequently discovered a significant negative correlation between DDT concentrations and the intensity of a digenean metacercaria, *Mesostephanus appendiculatoides*.

In a similar study, pesticides, PAHs, PCBs and heavy metals, as well as the parasite fauna, were determined in individual pink shrimp (*Farfantepenaeus duorarum*) from Campeche Sound, Yucatan (VIDAL-MARTÍNEZ et al. 2006). However, because the small size of the shrimp precluded both chemical and parasitological analyses from the same tissues, the authors cleverly sliced each shrimp in half longitudinally, using the right half for chemical analyses and the left half for histological analyses for parasites and symbionts. In this study, pesticides and PCBs were positively correlated with the number of symbionts. Approaches such as these are crucial in order to better comprehend the relationship between environmental pollution and parasitism.

While parasites may be good indicators of environmental stress (MACKENZIE et al. 1995), it cannot be overlooked that parasites themselves are natural biological stressors. Multiple stressors, be they natural or anthropogenic, may interact with each other, and indeed, have synergistic negative effects that are stronger than either one alone (SIH et al. 2004). This question has been considered only rarely for parasites and pollution (SURES 2004; MORLEY et al. 2006). Yellow perch (*Perca flavescens*) infected with larval nematodes (*Raphidascarias acus*) at a contaminated site in the St. Lawrence River, Canada, had higher levels of oxidative stress (a general indicator of fish health), as measured by lipid peroxidise in the liver, than did uninfected fish from the same site (Fig. 2; MARCOGLIESE et al. 2005). Both uninfected and infected fish from a reference site displayed the lowest levels of oxidative stress. Similarly, fish infected with >10 metacercariae of *Apophallus brevis* displayed greater oxidative stress than those with ≤10. Unlike *R. acus*, however, fish with >10 parasites at the reference site did display higher levels of oxidative stress than those with low infection intensities (Fig. 3). In another study, there was a significant positive relationship berween the number of pigmented macrophages in the spleen of spottail shiners (*Notrois hudsonius*) and the number of adult digeneans (*Plagioporus sinitsini*) in the gall bladder at polluted sites, but not a reference site, in the St. Lawrence River (THILAKARATNE et al. 2007). These studies demonstrate that parasites can have synergistic effects with contaminants on fish health and that parasites should be taken into account in environmental assessment of pollution impacts on naturally-occurring organisms. These types of results caution against relying solely toxicological studies on uninfected laboratory animals to determine environmental effects or attributing results from field studies solely to pollution. They also illustrate the fact that parasites may or may not have measurable effects under normal conditions; that is, the effects are context dependent and vary with species of parasite.

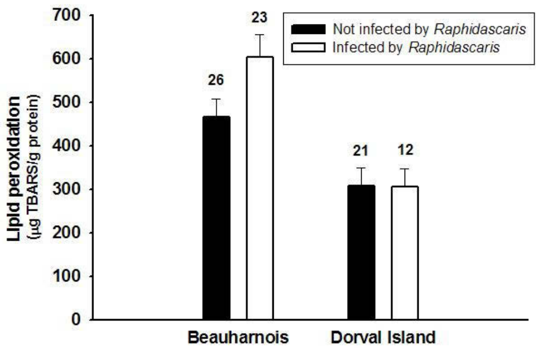

Figure 2. Measurements of oxidative stress (lipid peroxidation) in yellow perch (*Perca flavescens*) from a polluted site (Beauharnois) and a reference site (Dorval Island) in the St. Lawrence River. Open histograms indicate fish infected with the nematode *Raphidascaris acus*, while uninfected fish are represented by black histograms. Numbers above the histograms refers to the sample sizes. Published with permission from MARCOGLIESE et al. 2005.

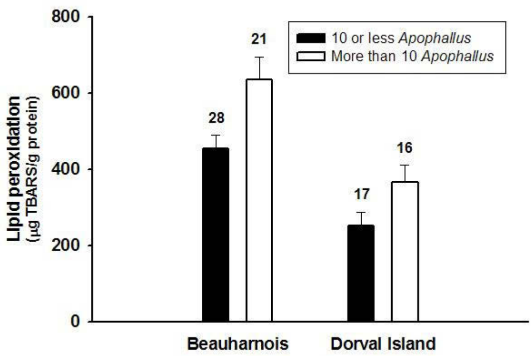

Figure 3. Measurements of oxidative stress (lipid peroxidation) in yellow perch (*Perca flavescens*) from a polluted site (Beauharnois) and a reference site (Dorval Island) in the St. Lawrence River. Open histograms indicate fish infected with >10 metacercariae of the trematode *Apophallus brevis*, while fish with ≤10 cysts are represented by black histograms. Numbers above the histograms refers to the sample sizes. Published with permission from MARCOGLIESE et al. 2005.

OCEANOGRAPHY AND CLIMATE CHANGE

Perhaps there is no pending perturbation that requires a comprehensive, robust and interdisciplinary approach more than that of climate change. The consequences of climate change for fisheries and the aquatic environment extend far beyond that of simple increased temperature. Climate change will affect precipitation, eutrophication, acidication, stratification, ultraviolet radiation, hydrology, oceanic currents, ice cover, freshwater and sea levels, the frequency of extreme climatic events, and host susceptibility and pathology (MARCOGLIESE 2001a).

The occurrence of anisakid nematodes in the flesh of commercial fishes is one of the most costly and prevalent parasitological problems in marine fisheries (MCCLELLAND 2002). The problems resulting from infections of sealworm (*Pseudoterranova* spp.) are primarily esthetic, while those associated with infections of whaleworm (*Anisakis* spp.) pose risks to human health from the consumption of raw or undercooked fish.

Between 1990 and 1992, an unusual decline in sealworm (*P. decipiens*) abundance was observed in all size classes of Atlantic cod (*Gadus morhua*) in the Gulf of St. Lawrence, eastern Canada. At the same time, abundance of another anisakid nematode, *Contracaecum osculatum*, increased in the same fish (BOILY & MARCOGLIESE 1995; MARCOGLIESE 2001b). Similarly, between 1988 and 1992 abundances of sealworm and *C. osculatum* decreased and increased, respectively, in grey seals (*Halichoerus grypus*) (MARCOGLIESE et al. 1996; MARCOGLIESE 2001b). Why these two nematodes displayed inverse patterns of abundance in both fish and seal hosts was difficult to explain given that the parasites have similar life cycles and seal populations were increasing.

Three hypotheses were proposed to explain the parasites' population dynamics (MARCOGLIESE et al. 1996; MARCOGLIESE 2001b). First, given that the groundfish fishery collapsed in the Gulf of St. Lawrence during the time of the study, seals during the earlier sampling period may have fed more on demersal prey such as cod, switching to pelagic prey such as capelin after the collapse. Sealworm has a benthic life cycle, but *C. osculatum* infects both demersal and pelagic fishes. Second, there may have been competition between the two parasite species inhabiting the stomachs of seals. Third, there may have been abiotic changes in the environment that differentially affected the two parasites.

The three hypotheses could be evaluated as a result of concurrent interdisciplinary studies in the Gulf of St. Lawrence (reviewed in MARCOGLIESE et al. 1996; MARCOGLIESE 2001b). First, diet information was available from the same seals. Grey seals actually fed on capelin more in 1988 than in 1992, while in 1992 they ingested more cod, thus refuting the diet change hypothesis. Furthermore, no evidence of competition between the parasites was apparent from distributional and fecundity data, thus refuting the competition hypothesis. Lastly, hydrographic changes occurred in the Gulf of St. Lawrence during the course of this study, whereby the Cold Intermediate Layer extended deeper and became colder. Most of the sediment surface in the southern Gulf of St. Lawrence now was covered by colder water. Sealworm eggs will not hatch at 0 ^{o} C, while those of *C. osculatum* do (MEASURES 1996, pers. comm.), thus explaining the unusual and opposite population dynamics of the two anisakid nematodes. No explanation would have been forthcoming had not diet studies be undertaken simultaneously with parasitology in the same seal samples, if oceanographic currents and conditions were not monitored in the same area, or if laboratory experiments were not completed on the same parasite species. It was subsequently determined that this decline in sealworm abundance extended beyond the Gulf of St. Lawrence onto the Scotian Shelf and the Gulf of Maine in American plaice (*Hippoglossoides platessoides*), but sealworm abundance since rebounded to and beyond previous high levels (MCCLELLAND & MARTELL 2001). Unquestionably, interdisciplinary approaches are required to evaluate effects of climatic changes in complex ecosystems, especially those subjected to intense resource exploitation.

RECOMMENDATIONS

Clearly there is much useful information for fisheries and environmental conservation to be gained from the study of parasites. Parasite species occurring in each individual host may be considered as information units that provides knowledge of host diet, migration, its role in the food web, stock structure and evolutionary history (MARCOGLIESE 2005). Incorporation of parasitology with other fields into joint interdisciplinary approaches to resource management will undoubtedly enhance knowledge of ecosystems under consideration and permit corroboration of results from single unidisciplinary studies. Indeed, integration of multiple disciplines has already proven both key and beneficial to our understanding of environmental problems such as those caused by pesticides (HAYES 2005). Thus, managers of exploited biological resources and resource conservation are encouraged to:

1) Promote studies by multidisciplinary teams in the same place at the same time to maximize the information obtained and our comprehension of ecosystem processes that occur there;

2) Maximize the use of each individual host in an interdisciplinary context (statistical analyses are much more robust if correlations and associations are achieved at the level of the individual organism rather than among samples); and
3) Take advantage of parasitological expertise and knowledge in the evaluation resources and development of management strategies.

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MARINE PARASITES AS BIOLOGICAL TAGS IN EUROPEAN WATERS: TWO SUCCESSFUL EU-FUNDED MULTIDISCIPLINARY PROJECTS

KEN MACKENZIE

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A historical account is given of the use of parasites as biological tags in population studies of marine fish in European waters, beginning in the 1960s with projects in the Barents and Black Seas and in the northeast Atlantic. From this period the classic studies by KABATA of myxosporean parasites infecting the gall bladders of whiting Merlangius merlangus and haddock Melanogrammus aeglefinus are selected as examples of successful and well-conducted studies. Since these early days most biological tag studies have been directed at herring Clupea harengus and cod Gadus morhua, reflecting the economic importance of these two species. The parasitology part of the multidisciplinary EU-funded WESTHER project which investigated the stock structure of herring to the west of the British Isles used some of the parasites selected as tags in an earlier study of herring recruitment in the North Sea and to the north and west of Scotland carried out by MACKENZIE in the 1970s and early 1980s. Another recent successful EU-funded multidisciplinary project, HOMSIR, which investigated the stock structure of Atlantic horse mackerel Trachurus trachurus throughout its geographical range, is also described.

Ken MacKenzie (e-mail: k.mackenzie@abdn.ac.uk) - School of Biological Sciences Zoology Building, University of Aberdeen, Tillydrone Avenue, Aberdeen, Scotland, UK

HISTORICAL ACCOUNT

The use of parasites as biological tags in population studies of marine fish in European waters dates back to the 1960s, when studies were carried out on Barents Sea cod *Gadus morhua* (see POLYANSKY & KULEMINA 1963), Black Sea anchovy *Engraulis encrasicolus* and horse mackerel *Trachurus mediterraneus pontius* (see NIKOLAEVA 1963; KOVALEVA 1965), Faroese haddock *Melanogrammus aeglefinus* (see KABATA 1963) and North Sea whiting *Merlangius merlangus* (see KABATA 1967). The fish species that have been the most frequent subjects of biological tag studies in European waters are cod *G. morhua* and Atlantic herring *Clupea harengus,* with seven studies each, reflecting the commercial importance of these two species. Horse mackerel *Trachurus* spp. have been the subjects of four studies, whiting *M. merlangus* of three, haddock *M. aeglefinus* and anchovy *E. encrasicolus* of two each, while single studies have investigated the stock structure of blue whiting, capelin, dogfish, garfish, mackerel, Norway pout and plaice.

Good examples of successful and well-conducted studies in European waters are the classic works of KABATA (1963, 1967) on the stock structure of whiting and haddock. KABATA used four genera of myxosporean gall-bladder parasites, *Ceratomyxa, Myxidium, Leptotheca* and *Sphaeromyxa,* to identify different stocks of their fish hosts based on variations in the geographical distributions of the parasites. These parasites are site-specific and easily identified by the characteristic shape and form of their spores, which means that large numbers of fish could be examined in a short period of time. KABATA concluded that two separate stocks of whiting occupied the northern and southern North Sea, based largely on the dominant prevalence of *Ceratomyxa* in the north and *Myxidium* in the south. He also showed that haddock on the Faroe Islands plateau formed a separate stock from those on the outlying banks, based on the presence or absence of *Myxidium* infections.

THE WESTHER PROJECT

www.clupea.net/westher

This multidisciplinary project was funded for three years from 1 January 2003 by the EU Commission within the *5th Framework Programme, Quality of Life and Management of Living Resources*. The full title of the project is "A Multidisciplinary Approach to the Identification of Herring (*Clupea harengus* L.) Stock Components West of the British Isles Using Biological Tags and Genetic Markers". The background to the study is that to the west of the British Isles there is a complex of local populations of herring, with little information on how discrete they are. This leaves them open to overexploitation, possibly resulting in loss of biodiversity and extinction. The objectives of WESTHER therefore were: (1) to determine the number of stock components present; (2) to determine affiliations of juvenile populations to adult spawning populations; (3) to determine the composition of feeding aggregations; and (4) to draw up improved guidelines for the conservation and management of biodiversity and stock preservation. The disciplines involved were host and parasite genetics, parasite tags, morphometrics (fish body and otoliths), meristics, otolith microstructure and otolith microchemistry, with each individual herring sampled being subjected to investigation by all of these methods. The partnership comprised six partners in three European countries – UK, Ireland and Germany – with the parasite tagging team consisting of six researchers based at the Universities of Aberdeen and Liverpool. Three different categories of herring samples were taken: juveniles, adult spawning and adult feeding aggregations. To comply with the requirements of other disciplines, only endoparasites were considered as potential tags. The parasitology team received the viscera of herring preserved in 90% ethanol and examined them under laboratory conditions.

Six biological tag studies had been carried out on herring in European waters prior to WESTHER (Table 1). One of these (MACKENZIE 1985) had a study area that overlapped that of WESTHER. The parasites selected for use in this study were the renicolid metacercariae *Cercaria pythionike* and *C. doricha* and the plerocercoid of the trypanorhynch cestode *Lacistorhynchus tenuis* (Fig. 1), all of which occur in the visceral cavity of herring. The same parasites were found to be useful tags again in WESTHER, supplemented by larval nematodes of the genus *Anisakis,* the most frequently used tags in earlier studies (Table 1).

Table 1
Biological tag studies on herring in European waters.

Study area	Author(s)	Parasite(s) used
Baltic Sea	REIMER (1970)	Digenea
	GRABDA (1974)	Nematodes (*Anisakis* spp.)
	GAEVSKAYA & SHAPIRO (1981)	Nematodes (*Anisakis* spp.), digeneans, cestodes
North Sea and west of British Isles	KÜHLMORGEN-HILLE (1983)	Nematodes (*Anisakis* spp.)
	LANG et al. (1990)	Nematodes (*Anisakis* spp.)
	MACKENZIE (1985)	Digeneans, cestodes
	WESTHER (2003-2006)	Digeneans, cestodes, nematodes (*Anisakis* spp.)

The features that make the renicolid metacercariae so useful as biological tags are that all infection occurs in the first summer of the herring's life, they have life spans in herring of many years, and have no associated pathology. This means that juvenile herring are effectively tagged on their nursery grounds and remain so for the rest of their lives, thereby enabling the tracing of samples of adult herring caught on spawning or feeding grounds to their nursery ground of origin. The cestode *L. tenuis* can also infect herring at an early age, but in this case further infections can occur later in life. Its value as a tag depends on its having a limited endemic area in the southern part of the study area of WESTHER. *Anisakis* larvae are cumulative with age in herring, and this fact must be taken into account when analysing their infection data. In combination with results from the other disciplines, analyses of the infection data and genetics of these parasites has resulted in a greater understanding of the stock structure and migrations of herring in the WESTHER study area and demonstrated the potential for further studies along similar lines (CROSS et al. *in press*, CAMPBELL et al. *in press*).

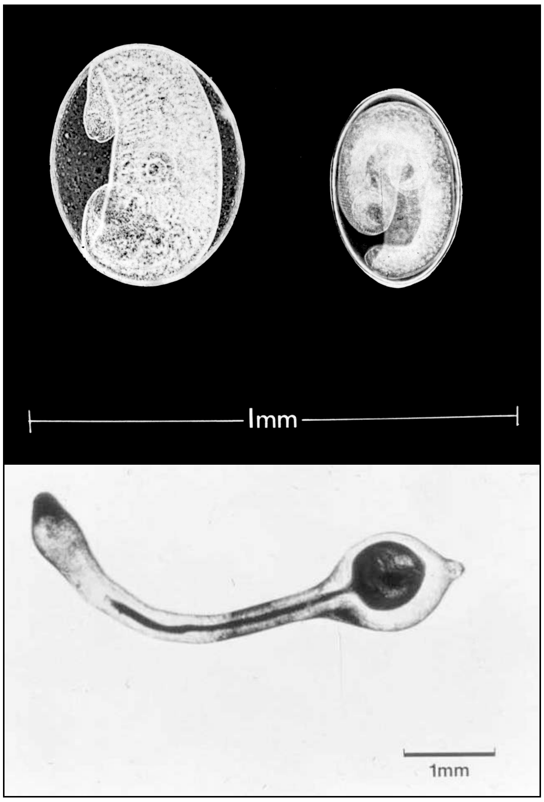

Figure 1. Parasites used as biological tags by MACKENZIE (1985) and in WESTHER. Up - *Cercaria doricha* and *C. pythionike*; Bottom – *Lacistorhynchus tenuis.*

THE HOMSIR PROJECT
www.homsir.com

This multidisciplinary project was funded for three years from 1 January 2000 by the EU Commission, also within the *5th Framework Programme, Quality of Life and Management of Living Resources*. The full title of the project is "A Multidisciplinary Approach Using Genetic Markers and Biological Tags in Horse Mackerel (*Trachurus trachurus*) Stock Structure". The objectives of HOMSIR were: (1) to provide information on the stock structure of horse mackerel, *Trachurus trachurus* (L.), throughout its entire geographical range; (2) to improve the management of this resource in European waters; and (3) to deliver an efficient multidisciplinary tool for fish stock identification. The disciplines involved were host and parasite genetics, parasite tags, artificial tags, morphometrics (fish body and otoliths), and life history traits. The partnership comprised 10 institutions in eight European countries – Spain, Portugal, UK, Germany, Greece, Italy, Norway and Ireland – with the parasitology team consisting of five researchers based in Aberdeen, Lisbon and Rome. Samples of horse mackerel were taken during their spawning season from 19 positions (Fig. 2), selected to cover the entire geographical range of the species. Fish were deep-frozen as soon as possible after capture and stored in individually labelled polythene bags for later examination. Both ecto- and endoparasites were therefore available and all were recorded. Samples of anisakid nematodes were preserved in ethanol, before they were completely defrosted as far as possible, for genetic studies.

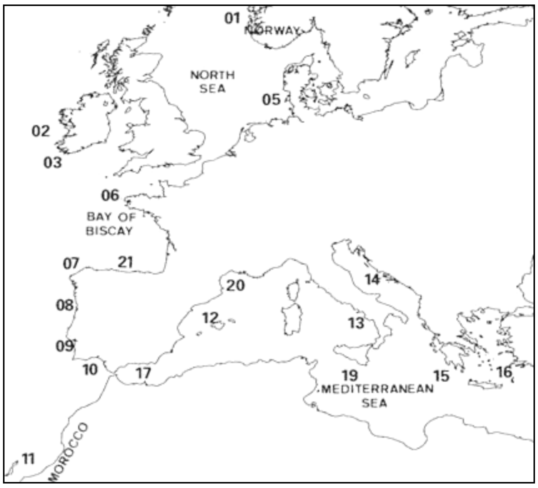

Figure 2. Sampling positions for horse mackerel in HOMSIR.

The Atlantic horse mackerel has a large and diverse parasite fauna (MACKENZIE et al. 2004), but most of the species are relatively rare, so that only a minority of the actual number of parasite species found could be used as tags. These included the anisakid nematode larvae *Anisakis* spp. and *Hysterothylacium aduncum,* the myxosporeans *Alataspora serenum* and *A. solomoni,* the monogeneans *Heteraxinoides atlanticus* and *Paradiplectanotrema trachuri,* the acanthocephalan *Rhadinorhynchus cadenati,* the digenean *Bathycreadium elongatum* (a new host record from horse mackerel), and the gill copepod *Lernanthropus trachuri.* In combination with results from the other disciplines, analyses of the parasite data confirmed the existence of three stocks of horse mackerel in Atlantic European waters and provided evidence that enabled the boundaries between two of these stocks to be redrawn, and also provided evidence of the existence of three stocks in the Mediterranean, where the stock structure had previously been unknown (CAMPBELL et al. 2004, MACKENZIE et al. *in press*, MATTIUCCI et al. 2008).

CONCLUSION

Parasites have been used successfully as biological tags for commercially important marine fish in European waters since the early 1960s. Recent studies have highlighted the value of the following new developments: (1) the multidisciplinary approach, which allows results from several methods to be compared, thereby strengthening confidence in the final result and conclusions; (2) the increasing use of multivariate statistical methods to analyse parasite infection data; and (3) the use of parasite genetics to identify sibling or cryptic species and subspecific populations of parasites indistinguishable by traditional morphological methods. The two projects featured in this paper – WESTHER and HOMSIR – amply illustrate the value of these approaches in studies of fish population structure. More such projects are urgently needed to provide information leading to the security and better management of more of our depleted fish populations in European waters.

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MARINE PARASITOLOGY IN PORTUGAL

GRAÇA COSTA

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The first relevant contributions to the field of Marine Parasitology in Portugal were made by CANDEIAS in 1952 and 1955. This author investigated the parasite composition of sardines, *Sardina pilchardus* and horse-mackerels, *Trachurus trachurus* and *T. picturatus*. From that period up to the present date, more than one hundred articles have been published on parasites of coastal and commercial fish and mollusc species. Most of the research in this field was pursued at the University of Porto, by EIRAS, AZEVEDO and co-workers, as well as several important contributions made by CARVALHO-VARELA and collaborators, at the University of Lisbon. Since 1994 parasitological studies were started in Madeira at the Centre for Macaronesian Studies (CEM). Parasitological research in Portugal has contributed to descriptions of more than 140 parasite species, some of them new species and new host records. Considering the importance of parasites, as accidental pathogens of man, leading to diverse allergic reactions, and the impact of parasites on both cultivated and wild fish, more attention should be given to this area by the Portuguese funding bodies.

Graça Costa (e-mail: gcosta@uma.pt) – Centro de Estudos da Macaronésia (CEM), Universidade da Madeira, Estação de Biologia Marinha do Funchal, Promenade Orla Marítima do Funchal, 9000-107 Funchal - Portugal

DEVELOPMENT OF RESEARCH IN MARINE PARASITOLOGY

Research on marine parasites, in Portugal, started in the early 1950's with the works of CANDEIAS on the parasites of sardines, *Sardina pilchardus* and horse-mackerel, *Trachurus trachurus* and *T. picturatus* (1952 and 1955) (see EIRAS 1990, 1998). PINTO (1956) studied the effect of the coccidian, *Eimeria sardinae* (Protozoa: Apicomplexa) in the testis of sardines. These early works in the field of fish parasitology have highlighted the potential of research in this area, in a country such as Portugal, with an extensive coastline and both historical and cultural tradition directed to the exploitation of the sea. Nevertheless in subsequent years only a few publications appeared authored by MACHADO-CRUZ (1959 up to 1982 in EIRAS 1990). It was during the seventies (from 1970 to 1979) that marine parasitology research became a real focus of interest. In this decade, several studies were published on the parasites of commercial fish species, landed at the fish market in Lisbon, by CARVALHO-VARELA and co-workers. These studies reported the occurrence of helminths, in particular larval nematodes (*Anisakis*, *Hysterothylacium* and *Contracaecum*), acanthocephalans, digeneans, monogeneans and postlarvae of cestodes (RODRIGUES et al. 1972, 1973, 1975a, b, c).

Research on fish parasites increased during the eighties, at the Faculty of Sciences, University of Porto, with the studies conducted by EIRAS and co-workers, on both marine and freshwater fish parasites (see EIRAS 1990). This author published several articles on the occurrence of the apicomplexan, *Haemogregarina bigemina*, parasite of the blood cells of coastal fishes (EIRAS 1987; EIRAS & DAVIES 1991), and on parasitic copepods, (*Lernaeocera lusci*), nematodes and cestodes (see EIRAS 1990; EIRAS & REGO 1987).

In the same period AZEVEDO, at the Institute of Biomedical Sciences (ICBAS) began his studies on parasites of fish and molluscs. Many interesting papers started to appear on the ultrastructure and life cycles of protozoan parasites, from several different phyla (Microspora, Myxozoa, Ascetospora and Apicomplexa). Some new species were described, such as: *Abelspora portucalensis* and *Haplosporidium lusitanicum* (Ascetospora) (Azevedo 1984, 1987).

Since 1990 up to the present, contributions were made to the field of Marine Parasitology by Eiras, Azevedo, Saraiva, Cruz, Costa, Casal, Santos & Russell-Pinto on marine and freshwater parasites (Eiras & Davies 1991; Azevedo & Cachola 1992; Casal & Azevedo 1995; Santos & Eiras 1995; Saraiva & Eiras 1996; Azevedo & Corral 1997; Cruz & Davies 1998; Russell-Pinto & Bowers 1998). These studies focused on the identification and morphological study of parasites, life cycles, pathology and infection dynamics. Many of the fish species studied were commercial ones (horse mackerel, *Trachurus trachurus*, sardines, *Sardina pilchardus*, mackerels, *Scomber scombrus* and *S. japonicus*, gadoids, *Trisopterus luscus*), with the parasites eliciting important pathological effects, impairing host survival or reducing their commercial value. Santos (1996, 1997) investigated the parasitofauna of wild seabass, *Dicentrarchus labrax*, reporting a number of parasitic agents, including protozoans and helminths.

In 1994 studies of marine fish parasites were started in Madeira, at the Centre for Macaronesian Studies - CEM (former CCBG) (see Costa et al. 1996). Research has been done mainly on helminths, including the nematodes, acanthocephalans, cestodes, monogeneans and digeneans. Most studies were done on commercial fish species (black-scabbard fish, *Aphanopus carbo*, chub mackerel, *Scomber japonicus*, oceanic horse-mackerel, *Trachurus picturatus*, blackspot seabream *Pagellus bogaraveo*) although coastal fish are occasionally subjected to parasitological surveys (see Costa & Biscoito 2003).

Recently Afonso-Dias at the University of Algarve, conducted parasitological surveys in angler fish, *Lophius piscatorius* and *L. budegassa*, hosts of a rich parasite fauna comprising myxosporeans, digeneans, cestodes, nematodes, acanthocephalans, hirudineans, copepodes and isopodes (see Afonso-Dias & MacKenzie 2004). Figure 1 summarizes the research done on marine parasites from 1950 up to the present date.

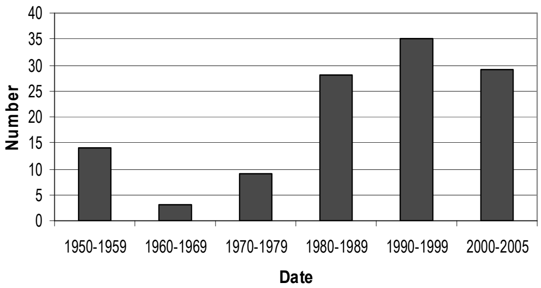

Figure 1. Summary of publications by Portuguese authors from 1950 to 2005.

IMPORTANCE OF MARINE FISH PARASITES

Parasites can be deleterious to their fish hosts, leading to reduction of growth and reproduction capacity, mortalities and increased vulnerability to predation (Bakke & MacKenzie 1993; Longshaw 1996; Kritsky & Heckman 2002). The impact of the parasites on the fish hosts can be estimated by the histopathological effects on the host, by a reduction of the commercial value of the fish, or by a long term survey where the researcher will notice a decline in the occurrence of heavily infected fish (Rohde 1993).

Silva & Eiras (2003) investigated the occurrence of anisakid larvae in the viscera and muscles of several commercial fish species in Portugal. They found high prevalence and mean intensities with *Anisakis* sp. in horse-mackerel, *Trachurus trachurus* (95.6% and 12.7 respectively), mackerel, *Scomber scombrus* (75.9% and 6.2 respectively), whiting, *Merluccius merluccius* (100% and 51.3 respectively) and blue whiting, *Micromesistius poutassou* (93.8% and 14.3 respectively). These infestations with anisakid larvae reduced the commercial value of the fish. In addition, anisakids (and other helminth parasites) can affect human health, as these larvae induce lesions in man, when ingesting raw or poorly cooked infected fish, and may be responsible for allergic reactions. These aspects are well documented in the works of UBEIRA et al. (2000) and AUDICANA et al. (2002).

Parasites can also have a negative impact in aquaculture, as caged fish can become infected with a diverse range of these creatures, both protozoan and helminth parasites (CARVALHO-VARELA & CUNHA-FERREIRA 1987; HEMMINGSEN et al. 1993; SITJA-BOBADILLA et al. 1995; MACKENZIE & HEMMINGSEN 2003). Myxosporeans are important parasites in cultured fish (DIAMANT & WAJSBROT 1997; COSTA et al. 1998) although other parasites, such as the nematodes, and monogeneans, are of economic importance, due to their adverse effects in fish cultures (FAISAL & IMAM 1990; CRUZ & SILVA et al. 1997). In Portugal, two of the cultivated fish species, *Dicentrarchus labrax* and *Sparus aurata,* are infected with a number of different parasites (CRUZ & SILVA et al. 1997; COSTA et al. 1998). Some of the parasite species are highly pathogenic, and of concern, as for example the monogeneans *Lamellodiscus ignoratus* and *Microcotyle* sp., which infect the gills, causing impairment of respiratory capacity of infected fish. Monogeneans are common in gilthead sea bream, *Sparus aurata*, in mainland Portugal (as well as in the Mediterranean) but were not observed in Madeiran aquaculture (COSTA et al. 1998). The investigations by SANTOS (1996, 1997) on the parasites of wild sea bass, *D. labrax*, highlighted the potential threats to the cultivation of the species, as adult fish are often recruited for intensive culture, as breeders, and juveniles in semi and extensive cultures.

Parasites are excellent living organisms to be used as models in evolutionary studies, can be used as indicators of ecosystem health, biodiversity and differentiation of fish stocks, migrations and feeding habits (HALMETOJA et al. 2000, WHITEFIELD & ELLIOT 2002). In a review by MACKENZIE (2002) the importance of parasites as biological tags is emphasized, presenting a list of criteria to be observed when choosing the appropriated parasites to be used as tags. The Atlantic horse mackerel *Trachurus trachurus* is a good example of a fish species, where the contribution of studies of its parasites, have helped to differentiate stocks from distinct Atlantic regions, including Portugal (see MACKENZIE et al. 2004).

FISH PARASITOLOGY IN MADEIRA

Research on marine parasites, in Madeira, started with a survey of the deep-water fish, *Aphanopus carbo*, in 1994 (COSTA et al. 1996). This fish species, subjected to a daily fishing activity in the island and highly appreciated in many culinary dishes, is infected by several parasites, namely: *Ceratomyxa tenuispora*, (Myxosporean) infecting the gall-bladder, postlarvae of cestodes (Eucestoda: Trypanorhyncha) found infecting the stomach of this fish host, in particular, *Nybelinia lingualis, N. yamagutii, Tentacularia coryphaenae* and *Sphyriocephalus tergestinus* (COSTA et al. 1996, 2003). Cystacanths of the acanthocephalan, *Bolbosoma vasculosum* are common in the visceral cavity of *A. carbo*, oceanic horse-mackerel, *T. picturatus* and chub mackerel, *S. japonicus* (COSTA et al. 2000). Presently research concentrates on further characterization of the helminth parasites of *S. japonicus* at morphological and molecular level.

CONCLUSIONS

In spite of the Portuguese geographic situation, with an extensive coastline and two main insular systems (Madeira and Azores archipelagos), its tradition in the exploitation of marine resources and regular inclusion of fish and seafood in their diet, the research in marine parasitology is not so substantial as in other European countries (e.g. France, UK, Norway). This situation could be due to the poor funding of this field of research by research grant bodies. Portuguese parasitologists often work with very tight budgets and small teams. This has constrained the possibilities of diverse research in the field, and the establishment of efficient networks between scientists. Aspects of applied parasitology, such as the use of parasites as biological tags, the use of parasites as indicators of ecosystem health, the use of parasites as models in studies of heavy metals and pollutant accumulations, as well as the application of molecular tools to the study of fish parasites, have been poorly developed in Portugal and should be improved.

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MARINE PARASITES AS INDICATORS OF POLLUTION

KEN MACKENZIE

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The main reason for choosing parasites as indicators is simply that more than half of the animal species currently known are parasitic, which provides a wider choice than free-living species. Add to this the fact that in metazoan parasites with complex life cycles the different developmental stages have widely differing biological requirements, thereby further increasing the number of potential indicators. Many parasites also have delicate free-living transmission stages that are highly sensitive to environmental change. This paper summarises the use of parasites as indicators of environmental change and pollution in the marine environment and the reported effects of different type of pollution, including hydrocarbons, heavy metals, pulp and paper mill effluents, eutrophication and mariculture. The general picture to emerge is that ectoparasites with direct single-host life cycles tend to increase in numbers with increasing levels of pollution, while endoparasites with more complex life cycles tend to decrease. This very much oversimplifies the true picture, however, and it is becoming clear that the relationship between pollution and parasite varies considerably depending on the nature and level of pollutant and the taxonomic group of parasite selected.

Ken MacKenzie (e-mail: k.mackenzie@abdn.ac.uk) - School of Biological Sciences Zoology Building, University of Aberdeen, Tillydrone Avenue, Aberdeen, Scotland, UK

INTRODUCTION

Good biological indicators to monitor the effects of pollutants on aquatic organisms must either be exceptionally sensitive or exceptionally resistant to environmental change so that significant changes in their numbers can be used as a warning of deteriorating conditions before the majority of organisms are seriously affected. Recent years have seen a knowledge explosion on the use of parasites as indicators of pollution in the marine environment (WILLIAMS & MACKENZIE 2003). So why choose parasites as indicators? Here are some good reasons.

- There are more parasitic than free-living species on earth (WINDSOR 1998), and parasitic organisms show enormous biological diversity, reflecting adaptations to the parasitic way of life in different types of hosts and in diverse environments.
- In metazoan parasites with complex life cycles the different developmental stages have widely different biological requirements, so that each stage must be assessed separately, almost as a different species, thereby greatly increasing the number of potential indicators.
- Many parasites have delicate short-lived free-living transmission stages that are highly sensitive to environmental change. These stages represent weak links in parasite life cycles and can be adversely affected by even minor environmental changes.

Many parasites are extremely sensitive to environmental change, while others are more resistant than their hosts. As a general rule, infections with endoparasites, most of which are helminths with complex indirect life cycles, tend to decrease, while infections with ectoparasites with direct single-host life cycles tend to increase, with increasing levels of pollutant. However, this is an oversimplification as there are enormous variations in the responses of different parasite species to different types of pollutant. The effect of a pollutant on an endoparasite can be direct or indirect – direct by parasites in the alimentary tract coming into direct contact with the ingested pollutant or indirect through adverse effects on other essential hosts in the life cycle of the parasite. This is also true for ectoparasites, which can be directly affected by contact with the pollutant in the external environment, or indirectly by suppression of the host's immune system, leading to increased rate of transmission and population growth of the parasite. The difference in susceptibility of host and parasite to the pollutant determines the exetent of change in the host-parasite relationship.

EFFECTS OF DIFFERENT POLLUTANTS ON MARINE HOST-PARASITE RELATIONSHIPS

Hydrocarbons

Some examples of the effects of hydrocarbon pollution on marine fish and molluscs are given in Table 1. Variations in the response of parasites to different forms of hydrocarbon pollution and different methods of experimental presentation is well illustrated by the contrasting responses of the ectoparasitic monogeneans *Gyrodactylus* spp. in the studies of KHAN & KICENIUK (1988) and MARCOGLIESE et al. (1998).

Heavy metals

Endoparasitic helminths, particularly acanthocephalans and cestodes, have been shown to accumulate heavy metals in their tissues at orders of magnitude greater than their hosts (SURES 2004). Most of these studies have been on freshwater host-parasite sytems, but marine acanthocephalans have also been shown to act in the same way. One recent study led the authors to suggest that these parasites can be useful for detecting low levels of heavy metal pollution in relatively pristine and remote regions such as the Antarctic (SURES & REIMANN 2003). Another recent study suggested a similar use of cestodes of sharks in another remote environment – the ocean depths – where elasmobranch fish are a major component of the fauna (MALEK et al. *in press*). On the other hand, uptake of mercury in nematode and isopod parasites of a crustacean and a fish were found to be lower in the parasites than in their hosts, and uptake was also lower in unparasitized hosts (BERGEY et al. 2002).

The combined effects of heavy metals and digenean parasites on a marine gastropod mollusc showed that parasitized molluscs consistently had lower levels of iron and copper, but higher concentrations of zinc, than uninfected ones (CROSS et al. 2003). This was thought to be the result of pathological effects of digenean infection on the mollusc hepatopancreas, which stores heavy metals. Heavy metals have also been shown to affect the viability and swimming performance of the free-living stages of helminth parasites. The horizontal swimming rate and longevity of cercariae of the digenean *Cryptocotyle lingua* were significantly reduced in an environment contaminated with heavy metals (CROSS et al. 2001).

Pulp and paper mill effluents

Some examples of the effects of these effluents on marine fish are given in Table 2, showing the different responses of parasites of different taxonomic groups to the same pollutant.

Eutrophication

The Baltic Sea provides some good examples of the effects of eutrophication on marine parasites (Table 3), the major effect over long periods being impoverishment of parasite faunas and a reduction in parasite biodiversity related to the associated oxygen depletion of the marine environment.

Mariculture

The presence of fish farms and fish processing plants can change the parasite faunas of local populations of marine organisms. Higher prevalences of three larval digeneans were found in intertidal molluscs *Littorina* spp. near such complexes than in those at control sites in the southern Barents Sea (BUSTNES & GALAKTIONOV 1999). The final hosts of the digeneans are seagulls (*Larus* spp.) and the differences between sites was attributed to the greater concentration of

Table 1
Examples of effects of hydrocarbon pollution on marine host-parasite relationships

Parasite(s)	**Host(s)**	**Location**	**Effect(s)**	**Reference**
Gyrodactylus *sp. (Monogenea)*	*Gadus morhua (Pisces)*	Newfoundland	Increase in infection in fish exposed to water-soluble hydrocarbons	KHAN & KICENIUK (1988)
Perkinsus marinus (Protozoa)	*Crassostrea virginica* (Mollusca)	Chesapeake Bay, USA	Increased susceptibility to infection	CHU & HALE (1994)
Trichodina sp. (Protozoa) *Gyrodactylus* sp. Gastrointestinal digeneans	*Hippoglossoides platessoides* (Pisces)	St. Lawrence estuary, Canada	Increase in *Trichodina* and decrease in *Gyrodactylus* and digenean infections in fish exposed to contaminated sediments	MARCOGLIESE et al. (1998)
Bucephalus-like sp. (Digenea) Gill ciliates (Protozoa)	*Bathymodiolus* sp. (Mollusca)	Gulf of Mexico	Unusually high infections near petroleum seeps	POWELL et al. (1999)
Gyrodactylus sp.	*Ammodytes hexapterus* (Pisces)	Alaska	Significantly heavier infections in fish exposed to contaminated sediments	MOLES & WADE (2001)
Trichodina spp. *Gyrodactylus pleuronecti* *Steringophorus furciger (Digenea)*	*Pleuronectes americanus* (Pisces)	Newfoundland	Infections increased to a threshold level then declined at higher cocentrations of pollutants Infection declined progressively as pollutant concentration increased	KHAN & PAYNE (2004)

Table 2
Examples of effects of pulp and paper mill effluents on marine host-parasite relationships

Parasite(s)	**Host(s)**	**Location**	**Effect(s)**	**Reference**
Glugea stephani (Protozoa) *Cryptocotyle lingua* (Digenea) *Echinorhynchus gadi* (Acanthocephala) Anisakid nematode larvae	*Pleuronectes americanus* (Pisces)	Newfoundland	Infections significantly higher at a polluted site for all parasites except *E. gadi,* for which it was lower. Differences in infection levels between clean and polluted sites suggest host immunosuppression due to pollutants.	BARKER et al. (1994)
Trichodina spp. (Protozoa)	*Myoxocephalus* spp. (Pisces)	Newfoundland	Infections significantly higher at polluted sites	KHAN et al. (1994)
E. gadi *Cryptobia* sp. (Protozoa)	*P. americanus*	Newfoundland	Significantly lower infections at contaminated site – indirect effects on intermediate hosts? Significantly higher levels at contaminated site	KHAN & PAYNE (1997)
Glugea stephani (Protozoa)	*P. americanus*	Newfoundland	Infections more extensive in tissues of fish at polluted sites	KHAN (2004)

Table 3
Examples of effects of eutrophication on marine host-parasite relationships

Parasite(s)	Host(s)	Location	Effect(s)	Reference
Many spp. of Protozoa & Metazoa	Many spp. of fish and molluscs	Baltic Sea	Major changes in infection levels over a 40-year period related to increased eutrophication.	REIMER (1995)
Metazoan parasite fauna	4 spp.of Gobiidae (Pisces)	Baltic Sea	Differences in parasite communities between sites related to different levels of oxygen saturation. Infection levels generally increased with increasing eutrophication.	ZANDER & KESTING (1996)
Trichodina spp.	*Platichthys flesus* (Pisces)	Baltic Sea	Seasonal changes in infection linked to eutrophication and changes in bacterial biomass.	PALM & DOBBERSTEIN (1999)
Metazoan parasite faunas	Fish, crustaceans & molluscs	Baltic Sea	Impoverishment of parasite faunas over an 18-year period due to increasing eutrophication.	KESTING & ZANDER (2000)

gulls attracted to the industrial sites, leading to increased transmission of the parasites. In the Eastern Mediterranean and Red Sea, the poorest and least diverse parasite communities in the rabbitfish *Siganus rivulatus* were found in samples taken close to mariculture sites (DIAMANT et al. 1999). These differences were attributed to eutrophication around mariculture sites together with a decline in the availability of natural food and an increase in the availability of artificial food falling through the farm cages.

Domestic sewage

No evidence was found of any effect of sewage sludge on the gastrointestinal helminth fauna of two species of flatfish in Scottish coastal waters (SIDDALL et al. 1994), but in Los Angeles Harbour, USA, infections of the white croaker *Genyonemus lineatus* (Pisces) with the larval nematode *Anisakis* sp. were significantly lower at a site close to the outfall of a sewage treatment plant than at a control site (HOGUE & PENG 2003), possibly as a result of the adverse effects of the discharge on the nematode's invertebrate intermediate hosts. In a coastal region of the Black Sea, levels of infection of the crustacean *Palaemon elegans* with three parasites - a fungus, a protozoan and a helminth – were significantly higher in an area where domestic sewage was discharged (TKACHUK & MODVINOVA 1999).

Mixed chemical pollutants

No evidence was found of any effect of mixed industrial waste on levels of infection with a larval helminth in blue mussels (Mollusca) along the Swedish coast of the Skagerrak (SVÄRDH & JOHANNESSON 2002). In the Black Sea, the reduced populations of benthic invertebrates in estuaries polluted by industrial, domestic and agricultural waste resulted in increased feeding by gobies on planktonic invertebrates, leading to increased infection with cestode larvae (KVACH 2001). No relationship between the presence of mixed chemical pollutants and infections with endoparasitic helminths were found in pink shrimps in Campeche Bay, Mexico, but a negative correlation were found between the pollutants and numbers of ectoparasitic protozoan symbionts (VIDAL-MARTINEZ et al. 2006).

CONCLUSION

Given the wide variations described above in the responses of different parasites to both the same and different types of pollutant, it is pertinent to ask the question: "Is it worth pursuing the use of parasites as indicators of marine pollution?" I believe that the answer is yes – but we require much more information on the effects of different concentrations and different methods of exposure of both single and mixed pollutants on selected indicator parasites, so that a directory of such effects can be compiled. As in other ecosystems, parasites can be used as indicators of the health or degradation of our seas – healthy ecosystems have healthy parasites (MARCOGLIESE 2005). At our present state of knowledge probably the most convincing use of parasites as indicators of marine pollution is the use of gastrointestinal helminths, particularly acanthocephalans and cestodes, as indicators of heavy metal pollution in remote and relatively pristine environments such as the polar regions and the deep ocean.

In the course of preparing this review it became obvious that certain parasites keep cropping up as useful indicators. Most prominent amongst these are the ciliate protozoans *Trichodina* spp., the monogeneans *Gyrodactylus* spp. and the digenean *Cryptocotyle lingua*. These are common and widespread marine parasites with proven abilities to respond quickly to anthropogenic change and are excellent models for further research.

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METHODOLOGY TO PREPARE WHOLE MOUNTS: SHORTCUTS

BJØRN BERLAND

BERLAND, B. 2008. Methodology to prepare whole mounts: shortcuts. Pp. 33-35 in AFONSO-DIAS, I., G. MENEZES, K. MACKENZIE & J.C. EIRAS (Eds) 2008. Proceedings of the International Workshop on Marine Parasitology: Applied Aspects of Marine Parasitology. *Arquipélago*. Life and Marine Sciences. Supplement 6: xiv + 49 pp. ISSN 0873-4704.

Any organism or tissue whose fine structures are to be studied by histology, or transmission electron microscopy (TEM), require fixation fluids with correct ion balance, pH, osmolarity and pure chemicals that react with the tissues and "lock them" so they will resist changes during the subsequent preparation processes. Parasites in and on fish, as well as other hosts, need to be identified to genus and species based on their morphology. The very small parasites, such as protozoans, require special techniques, but the metazoans, the helminths – monogenans, trematodes, cestodes, nematodes, acanthocophalans and crustaceans, should be collected alive, cleaned of mucus and fixed as life-like as possible. Sometimes one is forced to search for parasites in frozen fish (BERLAND 2006), also for them the methods described below apply. The specimens so collected and fixed may be cleared, stained and mounted as temporary or permanent total or whole mounts.

Bjørn Berland (e-mail: Berland@bio.uib.no or berland@zoo.uib.no) - Biologisk institutt, Universitetet i Bergen, Allégt. 41, N-5007 Bergen - Norway.

COLLECTING

Parasites from fish, and other vertebrates, should be collected as soon as possible after their hosts' death. Endoparasites should be placed in ca 1 % NaCl saline, or in seawater diluted with fresh water to ca 1 % salinity. Parasites from sharks and skates can be placed in seawater. It is practical to cut plastic tubing, or even bamboo, into short cylinders and close one end with fine plankton mesh to make strainers. Specimens can also be collected in saline in marmalade jars, and by shaking vigorously clean the specimens of mucus. The collected clean specimens should absolutely first be studied alive. The specimens from stomach, intestine, viscera, muscles etc. should be kept in separate vials both when alive and during fixation and later conservation.

The live worms in water or saline are transparent, their internal organs and structures are visible by microscopy. However, when they are killed by heat, or when dehydrated in strong alcohols, they become milky white – *opaque*. When we boil or fry an egg the coagulating egg white similarly becomes hard and opaque. The crucial question is – can opaque egg white and fixed specimens become transparent again?

FIXATION

To fix means to make firm. Ideally, collected specimens should be fixed in lifelike positions. Strong ethanol can be used as fixing fluid, but is mainly used for conserving specimens killed and fixed in other fixatives. Live specimens can be killed and fixed by heat in very hot water or ethanol, or formaldehyde solution. Many specimens can jointly be fixed *in bulk*, or one or a few live worms are placed neatly between two glass slides, or between slide and cover glass, as *press preparations*. The glasses are held together by dollops of Vaseline, Plasticine or even a chewing gum, and the fixing fluid is run between the glasses. When fixing is considered complete the specimens are transferred to 70-80 % ethanol for conservation, those fixed between glasses retain their flat shape.

REFRACTIVE INDEX

Small aquatic unpigmented organisms, such as plankton, are almost completely transparent – the refractive indices of their bodies are close to that of water – distilled water 1.33, sea water 1.434. When heated or fixed in standard fixatives, including alcohols, their refractive indices increase to 1.50 and above. When fixed specimens are placed in a fluid with refractive index in the range 1.50 - 1.56 they become so transparent that internal structures "disappear". In order to see these structures they have to be stained - this is the essence of histology. However, small opaque fixed organisms can be made sufficiently transparent in fluids of intermediate refractive index – about 1.45. Examples of such are glycerol, lactic acid and lactophenol. The necessary condition is that they are miscible with some water and that they penetrate the specimens completely. Thin sections and entire small organisms to be mounted in resins, such as Canada balsam (refractive index ca 1.54), must be dehydrated completely, and via xylene or toluene as intermedia, transferred to the resin.

FIXATIVES

Formalin is a saturated solution, approximately 40 %, of the gas formaldehyde in water. Full strength formalin is never used, but diluted with water 1 + 9 gives a 4 % formaldehyde solution (= 10 % formalin). A 2-4 % formaldehyde solution is a general fixative for tissues and parasites. Used cold the live worms wriggle and die coiled or contorted in it, but used hot they die instantly, becoming opaque. Hot 70-80 % ethanol (Looss' fluid) is excellent for nematodes and many other parasites, but is hardly possible to use under field conditions. In a laboratory live worms, in a little water, can be killed in seconds in a microwave oven; note that they instantly become opaque. Doing field work at sea I had to use cold 70 % ethanol, in which nematodes, and other parasites, die contorted or coiled. By mistake, I once fixed nematodes in pure glacial acetic acid (100 %), with unexpected and excellent results (BERLAND 1961). I later added some formalin (5 ml) to the glacial acetic acid (95 ml); this modified fixative was recommended, and named Berland's fluid, by GIBSON (1979). The acid penetrates rapidly into the specimens, making them uncoil and stretch, and remarkably transparent. Note that calcareous granules in some tapeworm larvae may react with the acid, producing gas bubbles. I have used glacial acetic acid and Berland's fluid routinely for many years for many helminths. Specimens should be fixed for a few minutes only before transfer to 70-80 % ethanol.

STAINING

Carmine and a few other dyes are taken up differently by the specimens' organs. This is like developing a colour film. In the platyhelminths the ovary, testes, eggs and other structures take various shades of red, blue, brown and yellow (metachromasy). As in the textile industry metal salts are used as mordants, serving as "glues" between the fibers and the dye; biological stains are based on the same principle. A good general carmine stain is Mayer's carmalum, which I have modified by dissolving the carmine in DMSO (BERLAND 2005). I have for years used a home-dyeing textile stain, Dylon Mexican Red, to stain fish parasites. A few grains of the dye powder in water, ethanol or lactophenol stain the specimens very quickly a bright red. Specimens are easily overstained [can be washed out by textile stain remover – di-Na-thiodinite].

CLEARING AND WATER TOLERANT MOUNTING MEDIA

Fixed specimens can be cleared, without staining, and mounted in the clearing fluid, as *temporary wet mounts* on slides. Fixed specimens can be cleared in glycerol (refractive index ca. 1.45), either in several steps of increasing strength, or in glycerol-ethanol left uncovered to let the alcohol evaporate; the specimens are left in nearly pure glycerol. Lactic acid, refractive index ca 1.44, also clears well. Lactophenol, being composed of equal parts (by weight) of lactic acid, phenol, glycerol and water, clears specimens very rapidly, and they remain soft and pliable. These three fluids are miscible with water and alcohols, so there is no need to dehydrate the specimens. For microscopy, the specimens are mounted as temporary wet mounts by placing them, in the clearing fluid, on slides with cover glass. Specimens stained in a textile stain can also be cleared in these media. The clearing media can after study be washed out in alcohol and are returned to 70-80 % ethanol, or glycerol-ethanol, for storage.

Specimens cleared in glycerol or lactophenol can be mounted as *permanent mounts* in glycerol jelly/glycerol gelatin on slides. The glycerol-jelly should be kept cool in closed vials, and a lump of it placed on a slide, heated gently and one or more specimens are placed and arranged in the molten jelly, add cover glass. The jelly sets when cooled, and as some of the water evaporates the jelly becomes firm in a day or two. The cover glass edges can be sealed with resin or varnish.

DEHYDRATING AND MOUNTING IN MEDIA INTOLERANT OF WATER

Specimens to be mounted in Canada balsam or a resin, should be stained. For whole mounts carmines , such as Mayer's carmalum, are good general stains. After staining specimens must be dehydrated completely. Standard procedure is to remove water in an ethanol "ladder": 30, 50, 70, 96 and 100 % (which may take hours for large specimens). From the last step, 100 %, the next step is an aromatic intermedium - usually benzene, toluene or xylene - which is miscible with the mounting medium, a resin or Canada balsam. The usual aromatic intermedia make specimens hard and brittle, and so do many alternative "oils" such as turpentine, eucalyptus oil and others. Bulky brittle parasites may break under gentle cover glass pressure. Benzene and its derivatives are health hazards, an alternative may be a citrus peel oil, or benzyl alcohol (see below) - one has to try which alternatives work well.

All manual and handbooks recommend ethanol as dehydrant. In many countries ethanol is heavily taxed, and 96 % and 100 % may be expensive and difficult to obtain outside research establishments. In cold countries 2-propanol (= iso-propyl-alcohol), sold in petrol stations as "condens remover", is a cheap and available alternative.

Glacial acetic acid, being water-free, is a potential dehydrant. It is weakly dissociated and when no water is present pH has no meaning. Specimens fixed in glacial acetic acid or Berland's fluid have already "survived" in it, so in principle glacial acetic acid can be used as dehydrant. I have for years used glacial acetic acid to dehydrate specimens, which remain soft, pliable and clear. It is a cheap excellent organic solvent, miscible with many "lab fluids". It smells strongly, but acetic acid (not glacial) is used in the kitchen.

Glycerol and phenol are alcohols, and as such they are miscible with water, and as we have seen, they are used to clear biological specimens. The aromatic benzyl alcohol – $C_6H_5{\cdot}CH_2OH$ – has been used in histology, but is largely "forgotten". It is water-clear, smells nicely (jasmine, used in perfumes), evaporates slowly and has refractive index 1.54. Dehydrated specimens transferred to it remain soft and pliable, and can be mounted at leisure in Canada balsam or other mounting media. Because of its high refractive index it clears very strongly, and being an alcohol it removes last traces of water. I have used it for many years with excellent results.

Nearly one hundred years ago P. MAYER (1920) in Germany used benzyl alcohol to clear specimens - he called it "liquid balsam". It is time to give it a renaissance.

Recently I have been visiting fellow at a university in Malaysia. Teaching practical methods in the study of fish parasites, I translated, and expanded, a manual I wrote many years ago in Norwegian, into English; this was published in Malaysia in 2005 (BERLAND 2005).

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INFECTIOUS DISEASE TRENDS IN NATURAL MARINE COMMUNITIES

KEVIN LAFFERTY

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Many factors (climate warming, pollution, harvesting, and introduced species) can contribute to disease outbreaks in marine life. Concomitant increases in each of these makes it difficult to attribute recent changes in disease occurrence or severity to any one factor. The diversity of patterns suggests there are many ways that environmental change can interact with disease in the ocean. For example, the increase in disease of Caribbean coral is postulated to be a result of climate change and introduction of terrestrial pathogens. Indirect evidence shows that (a) warming increased disease in turtles; (b) protection, pollution, and terrestrial pathogens increased mammal disease; (c) aquaculture increased disease in molluscs; and (d) release from over fished predators increased sea urchin disease. In contrast, fishing and pollution may have reduced disease in fishes. Fishing can affect infectious disease in several ways, (1) reduction of a stock can result in decreased transmission, (2) reduction in biological diversity can result in a reduction in parasite diversity, (3) release from predators can increase prey population density which can favour disease outbreaks.

Kevin Lafferty (e-mail: lafferty@lifesci.ucsb.edu) - Marine Science Institute UC Santa Barbara, CA 93106, USA

THE USE OF MARINE PARASITES IN FISH POPULATION STUDIES

MÁRIO GEORGE- NASCIMENTO

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Faced with the challenge of another review on parasites in population studies of marine fish, I have sampled recent and old studies in order to look for patterns of spatial and temporal sampling schemes, as well as, on ways data have been analysed. A central aspect to clearly have in mind is the perspective of what a population of fish means to the research scientist. I illustrate this mainly with studies on merluccids, deep sea and intertidal fish species from the southern cone of South America. My conclusions are that methodological approaches should be improved by framing a minimum sampling scheme, as well as by defining sampling sites and times, and also if random effects in ANOVAs should or should not be considered. To do this, variability of parasite populations needs to be systematically described at different spatial and temporal scales.

Mario George-Nascimento (e-mail: mgeorgen@ucsc.cl) – Universidad Catolica de la Santisima Concepcion, Fac. de Ciencias, Depart. Ecol. Costera, Casilla 297, Concepcion – Chile.

LABORATORY PROCEDURES TO STUDY FISH PARASITOLOGY

MARIA JOÃO SANTOS, AURÉLIA SARAIVA & BJØRN BERLAND

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The study of fish parasitology can be viewed in two different ways, from the point of view of the host or from that of the parasite. Both approaches present practical problems, which one should be aware of. The first one to be considered is the correct identification, either of the host (fish) or of the parasites. While the former is quite easily dealt with, the latter is not an easy task and can, sometimes, be very difficult to solve. The enormous parasite diversity (each species with its particular features), the presence of different larval stages in the same species, associated with their wide range of sizes (as a rule very small) can make this step in the study of fish parasitology a very difficult issue. During this section an overview of the most important parasite taxa and their diversity is given. Other problems, related with the availability of material, of either fish or parasite, as well as the need for special conditions for some parasite studies, are addressed.

The observation of histological slides, where some pathological phenomena can be detected, is a strong adjuvant of presumptive diagnostic of many diseases, namely those of parasitological aetiology. The purpose of this laboratory session is to provide basic information on histological techniques; in which both features will be considered, soft tissue histology and the main histopathology phenomena that might occur in tissue/organs usually involved in parasitological fish diseases.

The collection and preservation of animals or parts of them is in principal whole mount preparation, and is often very useful. This presentation includes a short overview of materials for research, including the collection and preservation of parasites. However, before embarking on fixing, staining, clearing and mounting, the importance of refractive index must be dealt with. Some examples of fluids in microscopy techniques, and their refractive indices were given.

Maria João Santos & Aurélia Saraiva (e-mail: mjsantos@fc.up.pt) - Departamento de Zoologia e Antropologia, Faculdade de Ciências, Universidade do Porto, Pr. Gomes Teixeira, 4099-002 Porto – Portugal; Bjørn Berland - Biologisk institutt, Universitetet i Bergen, Allégt. 41, N-5007 Bergen - Norway.

METAZOAN PARASITES AS INDICATORS OF ELASMOBRANCH BIOLOGY

JANINE N. CAIRA

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Metazoan parasites have the potential to provide information on the following aspects of the biology of elasmobranchs: 1) Feeding Biology and diet shifts, 2) Predators, 3) Stock discrimination, 4) Geographic variation, 5) Specific identity, 6) Phylogenetic relationships, 7) Geographic origins, and 8) Migrations. In selecting parasite taxa to consider as sources of the above information, parasite diversity, host specificity, and life-cycle complexity (direct or indirect cycle) are of greatest concern. Most appropriate as indicators of diet, diet shifts and predators, are parasites that are polyxenous, relatively host specific, and reasonably diverse. Thus, adult and larval cestodes provide the most promise; digeneans and nematodes have potential, but their much lower diversity limits their utility to some extent. Host stocks (i.e, populations), geographically variable groups (i.e., subspecies), and species comprise a continuum, with members differing essentially only in degree of genetic divergence. As a consequence, the parasites of utility for exploring such aspects of host biology are similar, but the detail of data required increases with decreasing genetic divergence. So, whereas presence/absence data may suffice for determinations of specific identity, prevalence and/or abundance data may be required to detect differences among host stocks. Monogeneans, copepods, and adult cestodes have utility at all levels of genetic divergence; leeches, isopods, adults and larval nematodes and digeneans have also been used to good effect in stock discrimination. The latter suite of taxa is similarly useful for investigations of geographic migrations. In this case, the collection of more fine scale data such as prevalence and/or abundance is also recommended. The parasites of greatest utility as indicators of elasmobranch phylogenetic relationships and origins are those that are most host specific. As a result, monogeneans, copepods and adult cestodes are key. At present the number of studies that have employed metazoan parasites as indicators of any aspect of elasmobranch biology are limited, however the potential remains great.

Janine N. Caira (e-mail: jcaira@uconnvm.uconn.edu) - University of Connecticut, Ecology and Evolutionary Biology, U-4375 N Eagleville, Storrs, CT 06268 – USA.

PARASITES AS CAUSE OF REJECTION IN PORTUGUESE FISH MARKETS

LUISA MONTEIRO

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The presence of parasites influences the wholesomeness and the quality of fishery products, representing a potential risk for the consumer. An integrated approach is necessary to assure food security from primary production into commercialization. Each batch of fishing products must be presented to the hygio-sanitary inspection at the moment of landing before going into the fish market, to check if they are suitable for human consumption. This inspection consists of an organoleptic evaluation conducted by sampling, which also includes detection of visible parasites. According to Regulation (EC) n: 2074/2005 (5 December), the presence of visible parasites is interpreted as a parasite or group of parasites which possess dimension, colour or are clearly distinguishable from the texture of the fish tissues. The fish is examined just by visual inspection, the only mean of observation at the inspector's disposal. If the fish or any other fish products are not in good condition then it will not be allowed to be sold at the fish market.

In accordance with the Governmental order n: 559/76, 7 of September, fish is classified and must be removed if it: A) is altered (adulterated, damaged or corrupted), B) is repugnant (parasites, tumours, abnormal smells), C) shows excessive slimness, D) is suspected of carrying microorganisms or toxic substances, and E) others. The fishery products must be frozen to = -20 ºC during at least 24 hours (Reg. (EC) n: 853/2004, 29 of April) if they are to be consumed raw. This treatment is also mandatory for fishery products originating from herring, mackerel, sprat and wild salmons from the Atlantic and Pacific that have been subject to a process of cold smoking during which the internal temperature did not exceed 60 ºC, or if they have been marinated and/or salted with an insufficient treatment to destroy nematode larvae. The knowledge and skills that we have with fish parasitosis anomalies is constrained by the skill process of inspection at Figueira da Foz fish market, with a similar proceedure at the other fish markets. In principle fish rejection with parasitic etiology takes place there, with the observation of external parasites, by macroscopic examination, but rejection also happens because of a repugnant aspect by colour alteration of, by muscular consistency modification or malformations, or if they present excessive slimness suggesting, by certainty, that these are manifestations associated with parasitic infestation. In the case of rejection, in which eventually the etiologic agent is not identified, the need to resort to objective approaches becomes itself imperative with the respective gathering of samples and subsequent realization of laboratory analyses for proof of the alterations that determined the sanitary decision. So in the cases of parasitized fresh fish, obtained in the course of the service of Hygio-Sanitary inspection in the Fish market and those of fish frozen during the work I carry out of evaluation of the frozen fish quality in the Portuguese Institute of Oncology of Coimbra - Francisco Gentil, that I present shows immediate recognition, which suits the organoleptic examination, of the following:

- Case 1. Parasitism by Microsporida (*Spraguea* spp.)
- Case 2. Parasitism by nematodes *Anisakis* spp.
- Case 3. Parasitism by nematodes eggs of *Huffmanella* spp.
- Case 4. Parasitism by plerocercoid larvae of *Gymnorhynchus gigas*

- Case 5. Parasitism by *Bothriocephalus* spp.cestodes
- Case 6. Parasitism by copepods (*Lernaeocera* spp.)
- Case 7. Parasitism by copepods (*Nicothoe astaci*)
- Case 8. Parasitism by copepods (*Sphyrion lumpi*)
- Case 9. Parasitism by isopods (*Anilocra physodes*)
- Case 10. Parasitism by isopods Bopyridae family

Luísa Margarida Pedrosa Santos Monteiro (e-mail: luisamonteiro_@hotmail.com) - Direcção Geral de Veterinária, Rua Drª. Cristina Torres, 3 rch - Esq., 3080-210 Figueira da Foz – Portugal.

FISH QUALITY REGULATION AND LEGISLATION

PAULA RAMOS

RAMOS, P. 2008. Fish quality regulation and legislation. Pp. 47 in AFONSO-DIAS, I., G. MENEZES, K. MACKENZIE & J.C. EIRAS (Eds) 2008. Proceedings of the International Workshop on Marine Parasitology: Applied Aspects of Marine Parasitology. *Arquipélago*. Life and Marine Sciences. Supplement 6: xiv + 49 pp. ISSN 0873-4704.

Today, consumers are committed to quality. In order to maintain market confidence, the EU established criteria and strict standards of quality. Migrant larvae of several nematode genera may reach humans by ingestion of infected fish when they are consumed raw or not adequately cooked. Current Portuguese / European Union regulations on food fish and products (Portaria n: 559/76 de 7 de Setembro; Regulamento (CE) n.: 853 /2004 do Parlamento Europeu e do Conselho de 29 de Abril de 2004; Regulamento (CE) n.: 2074/2005 da Comissão de 5 de Dezembro de 2005) require the visual examination of the fish, extraction of the visible parasites and the rejection of the heavily parasitized fish. All fish that are to be consumed raw or almost raw must be subjected to a freezing process (- 20 ºC for at least 24 hours in all parts of the fish). This also applies to fish products that are heated to a temperature of less than 60 ºC. As far as salted fish is concerned, the process must be sufficient to destroy the larvae of nematodes. This should reduce infection rates and, if Anisakis simplex infection predisposes an individual to Anisakis related allergic responses, there should also be a reduction in the prevalence of allergic reaction to ingested worm material in fish. A simple but effective control measure is to inform the general public about A. simplex infection and the risk of eating raw or undercooked fish. Nevertheless, such measures might not be adequate considering the thermostability of the allergens involved in such allergic reactions.

Paula Ramos (e-mail: pramos@ipimar.pt) - Instituto de Investigação das Pescas e do Mar (IPIMAR), Av. De Brasília, 1449-006 Lisboa – Portugal.

VISIT TO THE FISH MARKET

ISABEL AFONSO-DIAS

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The visit to Horta Fish market aimed to show to all participants the real conditions available to fish inspection and to motivate some discussion on ways to obtain fresh fish samples for more thorough parasitic surveys. Most Portuguese fish markets have good facilities and the Horta fish market is no exception; the place is ample, very clean and quite modern; the atmosphere is good and it is often crowded with buyers and fishermen selling their catch. Fish boxes are unloaded from the boats moored at the harbour and carried into the market. Fish boxes separated by boat queue on a conveyor belt that transport them towards the potential buyers. The fish landed is very fresh and it does not have to wait too long to be sold in the fish auction. The main fish species observed during the visit were wreckfish or stone bass (*Polyprion americanus* (Schneider, 1801)) and red sea bream or blackspot seabream (*Pagellus bogaraveo* (Brünnich, 1768)). Other species were also present, like conger eel (*Conger conger*, (Artedi, 1738) Linnaeus, 1758), yellowmouth barracuda (*Sphyraena viridensis* Cuvier, 1829), fork-beard (*Phycis phycis*, (Linnaeus, 1766)) and bluemouth rockfish (*Helicolenus dactylopterus* (Delaroche, 1809)). The fish arrives in the fish market selected by species and in some cases by sizes; large specimens of blackspot seabream fetch a higher price and have different common names than their smaller congeners).

Isabel Afonso-Dias (e-mail: ccmar@ualg.pt) - Centro de Ciências do Mar do Algarve (CCMAR), FCMA, Universidade do Algarve. Campus de Gambelas, 8005-139 Faro – Portugal.

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