ECOLOGICAL MONITORING AND ASSESSMENT NETWORK

(EMAN) PROTOCOLS FOR MEASURING BIODIVERSITY: PARASITES OF FISHES IN FRESH WATER

by

David J. Marcogliese
Environment Canada, St. Lawrence Centre,
Montreal, Quebec, Canada H2Y 2E7

and

Parasitology Module Steering Committee
Parasitology Section, Canadian Society of Zoologists

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Introduction

Parasitism reflects a lifestyle whereby one or more individual organisms (the parasite) lives in close obligate association in or on another (the host), and derives benefit such as nutrition at the host's expense, usually without killing the host. Parasites belong to many different phylogenetically distinct taxa, and as such, display a variety of life histories and body forms. Virtually every species of free-living organism has parasites. Indeed, there may be more species of parasitic organisms than of free-living ones (Price 1980). Thus, parasites contribute significantly to biodiversity simply in terms of the number and variety of species in existence.

Many parasites possess complex life cycles in that they have larval stages that infect intermediate hosts, where growth or development occurs, and definitive hosts, where maturation and sexual reproduction occurs. Transmission between these hosts in a life cycle may be through free-living infective stages or via predation by one host on the previous host in the life cycle. Because of their complex life cycles, parasites are indicative of many different aspects of their hosts' biology, such as host diet, migration, recruitment, population distinctness, and phylogeny (Williams et al. 1992). They also may be good indicators of environmental contaminants and stress (Sures et al. 1994; MacKenzie et al. 1995). Different parasites have a variety of intermediate hosts and often depend on trophic interactions for transmission, so parasites within a vertebrate host may be excellent indicators of food-web structure and biodiversity (Marcogliese and Cone 1996, 1997). Moreover, parasites may be important in regulating the abundance of host populations through parasite-induced mortality of heavily infected hosts (Anderson and May 1979; May and Anderson 1979).

Parasites can be divided into microparasites and macroparasites on the basis of size. The microparasites include viruses, bacteria, fungi, protozoans, and myxozoans. Surveys for microparasites generally include only Protozoa and Myxozoa. Macroparasites are larger multicellular organisms mainly comprised of the helminths and arthropods. Helminths include Monogenea, Trematoda (flukes), Cestoda (tapeworms), Nematoda (roundworms), and Acanthocephala (thorny-headed worms). Arthropod parasites of vertebrates in fresh water are represented mainly by the Copepoda. Table 1 provides the numbers and general characteristics of known parasites in Canadian freshwater fish.

Endoparasites are those sequestered in internal organs or cavities of a host, and ectoparasites are those found on external surfaces such as skin or gills. It is impossible to complete a parasite survey to find most endoparasites without killing the host.

Any sampling program for parasites first requires a sampling program for members of the host population. Methods for collecting free-living organisms of various taxa are outlined in other sections. During any sampling effort for parasites, care should be taken that members of the host population within any particular category (*e.g.*, age, size) are collected at random. Although this guide is aimed principally at parasites of fishes, it also may be applied to other vertebrate host groups (e.g. amphibians).

Two national parasite surveys of aquatic organisms are currently underway. One is a survey of yellow perch, *Perca flavescens* (Mitchill), parasites across Canada, coordinated by Dr. David Cone, Biology Department, St. Mary's University, Halifax, Nova Scotia B3H 3C3. The other is a survey of parasites of threespined sticklebacks, *Gasterosteus aculeatus* L., and other sticklebacks from fresh, brackish, and marine waters in Canada, coordinated by this author.

Abiotic Factors

Many parasites have free-living stages (eggs and/or larvae) or are exposed to the external environment (ectoparasites), so their distribution and abundance can be modified by environmental conditions as for any other organism. In general, abiotic information to be collected will be the same as that stipulated for the host organism (e.g. temperature, depth, water quality, etc.).

Sampling Procedures

Collection of host organisms - All organisms to be examined for any particular survey should come from the same habitat, and should not be pooled across habitats. Twenty-30 organisms are required for a general parasite survey. They should come from the average age or size class for the population, if possible. For best results, analysis of data by age, size, sex, or season, requires 30 host animals in each class. Samples of 25-30 fish permit detection of parasites if the prevalence is 10% or more. Detection of rare parasites requires greater sample size (des Clers 1994).

Preferably, host organisms should be examined fresh for parasites. Alternatively, organisms should be frozen as soon as possible after capture. Hosts fixed in preservative are of little use for parasitological examinations. Fish can be euthanized by pithing if small, a blow on the head if large, by cervical dislocation, or by an overdose of anesthetic such as tricaine methanesulfonate (MS 222). All hosts should be individually bagged to prevent loss of ectoparasites and labeled with collection data (date, sampling site, collector). Host organisms returned to the laboratory alive should be examined relatively quickly (within a few hours). Otherwise, parasites with direct life cycles may spread between hosts or increase on infected hosts. Note also that hosts kept in captivity for prolonged periods may lose many parasites. Loss of ectoparasites also may occur with certain methods of capture, such as gill-netting.

Parasite surveys should be done twice annually (spring-early summer and late summer) because parasite populations can fluctuate seasonally. If only one survey is possible, July is recommended for Canadian waters.

Equipment - Field collecting gear and instruments for physicochemical measurements will be the same as those used for free-living organisms (Table 2). Other equipment required includes bags for host organisms, containers or coolers for hosts, and ice for temporary specimen storage. Also required are waterproof field note books, pencils, waterproof labels, and ties for bags.

In the laboratory, required equipment includes a measuring board or ruler, balance, filleting knife, fine dissecting tools, Petri plates, beakers, squeeze bottles, Pasteur pipettes, vials, fixatives (ethanol, formaldehyde, alcohol-formalin-acetic acid [AFA]), mechanical counters, microscope slides and coverslips, biological stains (Schneider's acetocarmine), clearing agents (glycerol, xylene), mounting media (Canada balsam is best for long-term storage, Permount for "quick and dirty" work), pencils and/or alcohol-proof markers, diamond-point pencils for etching into slides, fine paint brushes, and self-adhesive labels (not lick-and-stick). For a list of suppliers, see Table 2. To see recipes for standard fixatives, preservatives, and stains, see Table 3. A stereomicroscope is essential for examination of host organs and tissue for macroparasites (helminths and arthropods). A compound microscope is required for examination for microparasites (protozoans and myxozoans).

Laboratory procedures for macroparasites - The following protocol for examining fish is adapted from Arthur and Albert (1994):

- Record host species, date caught, site sampled, method of collection, name of collector, name of examiner.
- Measure and weigh fish.
- Rinse external surface; collect rinse and examine with stereomicroscope for ectoparasites.
- Examine external surface using stereomicroscope.
- Remove gills, rinse. Examine each gill arch individually and the rinse with stereomicroscope.
- Rinse buccal cavity; examine rinse with stereomicroscope.
- Remove, dissect, and examine eyes (humor, retina, lens) with stereomicroscope.

1. Remove otoliths, fins, or scales for aging, if required.
2. Remove fins and examine with stereomicroscope.
3. Open body cavity ventrally; record sex.
4. Examine cavity and surface of internal organs (heart, liver, spleen, gall bladder, digestive tract, gonads, kidney, urinary bladder) for parasites. Then separate organs into Petri dishes with water.
5. Separate stomach, pyloric caeca, and intestine. Open longitudinally and examine for parasites with stereomicroscope. For extensive gut contents, rinse into beakers, mix with sodium bicarbonate (one spoonful per litre) to remove mucus, and allow parasites to settle. Decant and examine residue with stereomicroscope.
6. Cut organs and tissue (wall of stomach, pyloric caeca, intestine, liver, spleen, kidney, heart and large blood vessels, gonads, gall bladder, urinary bladder, brain) into smaller pieces, compress between glass plates, and examine with stereomicroscope.
7. Rinse the body cavity; and examine rinse with stereomicroscope.
8. Thin-slice musculature and inspect for parasites.
9. Record number of parasites of each species and their location in the host on data sheet.

Treatent, fixation, and preservation of macroparasites — Details can be found in Ash and Orihel (1991). Ideally, all live parasites should be fixed in hot or warm fixatives to kill them rapidly and at the same time avoid muscular contractions by the parasite, which then distorts their shape when fixed.

For living, small monogeneans firmly attached to the gills, freeze some tissue with parasite attached overnight in water or 0.7% saline solution. The parasite will detach from the tissue and relax. It can then be thawed, retrieved, and fixed in 10% buffered formalin (Table 3).

Other helminths (cestodes, trematodes, acanthocephalans) should be heat-fixed in 70% ethanol, or relaxed in tap water (if alive) and fixed in 10% buffered formalin or AFA.

Nematodes should be fixed in hot (not boiling) 70% ethanol with 5% glycerol (Table 3). Berland's fluid (Berland 1982) may also be used for nematodes and platyhelminths.

Encysted parasites can be removed from their cysts by careful dissection with fine needles or forceps, or gentle pressure with a coverslip on a slide. If these techniques fail, place the cyst in 0.5% trypsin and heat to 37-40°C. Encysted acanthocephalans found in the viscera can be placed in tap or distilled water in the refrigerator overnight to stimulate eversion of the proboscis. Fix in 70% ethanol, 10% buffered formalin, or AFA (Table 3).

Arthropods may be anesthetized in carbon dioxide bubbled through water. They can be fixed in 70% ethanol.

Leeches must be narcotized to avoid contracting when fixed. Carbon dioxide bubbled through water can be used to anesthetize leeches, after which they can be fixed in 10% buffered formalin.

Each parasite species or type from each organ should be placed in a separate vial and labelled with host species and host number, geographic locality, date of capture, location in host, fixative used, and date of examination. Formalin- or AFA-fixed specimens should be transferred to 70% ethanol after 1-7 d, and definitely for a few days prior to staining. Parasites can be handled and transferred using pipettes or fine paint brushes; care must be taken not to puncture them with sharp instruments.

Monogeneans, trematodes, cestodes, and acanthocephalans should be stained in acetocarmine and mounted on permanent slides. Acanthocephalans should be pricked in a few places with a fine needle prior to staining. Canada balsam is the best mounting medium for permanent museum storage, but the less-expensive Permount or Eukitt can also be used for routine work. Nematodes should be cleared by evaporation in glycerol in 70% ethanol, letting the alcohol evaporate in the case of small worms, or gradually reducing the alcohol content and increasing the glycerol content of the mixture with large worms (>1 cm) (Table 3). They may be examined as temporary mounts in

glycerol, or semi-permanent mounts in glycerine jelly.

Arthropod parasites can be examined whole. If necessary, mouthparts and other appendages can be removed and examined on temporary mounts. Organisms or appendages can be cleared if required by mounting in lactophenol.
Laboratory procedures for microparasites - Blood smears can be made from fresh fish only. The smear should be made on a microscope slide, allowed to air dry, fixed in 95% methyl alcohol for 3-5 min, and stained in Giemsa for 20 min.

Smears of liver, spleen, kidney, gonads, intestine, muscle, brain, and scrapings of the urinary and gall bladders should also be made on microscope slides, and fixed in 95% methyl alcohol.

Smears are examined for a fixed number of microscope fields (e.g. 10) or a fixed period of time (e.g. 5 min) with a compound microscope at 400X. The presence of parasites is recorded, and photographed for a permanent record.

Taxonomic Aids and Keys to Species

Identification of many parasite species may require consultation of original descriptions in the primary literature. However, for most common groups, identification to genus and often to species can be done through the synthetic keys listed in Table 4. Example specimens of parasites and hosts should always be retained to confirm identifications. Voucher specimens should be deposited in the permanent collection of a recognized museum, for future reference and use.

Data Analysis

The most common measurements of parasite population levels in hosts are prevalence, mean abundance, and mean intensity (Bush et al. 1997). Prevalence refers to the percentages of organisms infected by a particular species of parasite. Mean abundance refers to the number of parasites of a given species per host examined, infected and uninfected. Mean intensity is the mean number of parasites of a given species per infected host.

The scale of observation in parasitology is also important. A parasite population in an individual host is an infrapopulation, whereas that in a host population is a component population. All the parasites of a given species in an ecosystem compose the suprapopulation. Within an individual host, all the parasites found compose an infracommunity, and within a host population, a component community. All the parasites found in an ecosystem form the compound community (see Esch et al. 1990).

For microparasites, data are usually presented as prevalence. Quantitative data on infections can be presented as numbers per unit microscopic counts.

There are numerous measures of diversity used in parasitology, including species

richness, the Shannon-Wiener Index, Simpson's Index, Berger-Parker Index, and Brillouin Index (see Magurran 1988). Species richness is the simplest measure of diversity, and is recommended at all scales. The Brillouin Index is recommended for measurements of infracommunity diversity because it is an appropriate index for fully censused communities (as in an individual host). For component communities, the Shannon-Wiener Index

($H' = - \sum p_i \ln p_i$, where p is the proportion of species i in the community)

is recommended because it is less biased toward dominant species than other indices. However, all indices are fraught with problems of bias and interpretation. The user should become familiar with these problems, and choose the index most appropriate for the goals of the study.

It is often important to statistically determine whether parasite populations or communities differ from one site to another, or from one time period to another. To do so requires quantitative, replicated measurements. In the case of parasites, each fish host within a species can be considered a replicate, just as a quadrat on the forest floor or a grab sample of the benthos is a replicate. Replication is especially important if parasites are to be used as indicators of host population structure, environmental quality, biodiversity, or for monitoring disease and other problems resulting from parasitic infections.

Parasite distributions are almost always aggregated or overdispersed, meaning that most of the parasites in a population will be found in a small number of hosts, and most potential hosts may be lightly infected or uninfected (there are exceptions!). Thus, prior to any statistical analyses, data must be tested for normality. If the distributions are not normal, an appropriate transformation must be used to normalize the data, before parametric statistics can be applied. If the data cannot be normalized, nonparametric statistics should be used. The investigator should become familiar with the limitations of the analysis used and the consequences of violating assumptions inherent in various statistical procedures (Underwood 1981).

Quality Assessment/Quality Control (QA/QC)

A QA/QC plan is required for any successful monitoring program. It gives results credibility and helps structure the monitoring program. Ways to aid in structuring the program include field notes, sample collection forms, sample processing forms, procedures for verifying taxonomic identifications, data screening, and database management. A sample data sheet is shown in Appendix I. Questionable or uncertain identifications should be verified by an expert. For establishing sampling guidelines to ensure that results are meaningful, see Green (1979) or des Clers (1994). Approximately 20% of program resources should be dedicated to QA/QC.

Volunteer and Non-Specialist Involvement

People do not require a great deal of training to do adequate dissections to recover parasites. University undergraduate classes can acquire a lot of valuable data. However, people with some expertise or experience are required for many, but not all, identifications.

Volunteers and volunteer networks can contribute cursory observations of large parasites. In fresh water, these include some arthropods on gills and skin of fish. Copepods on gills are sometimes visible with the naked eye; they are white or cream-coloured and often have two egg sacs. The fish louse, *Argulus*, is quite large, and can be seen crawling on the body surface. Leeches also can be easily seen on the external surface. Certain digenean metacercariae form blackspot and can be seen on the skin or in the flesh of fish. As adults, these parasites infect birds or mammals. The large yellow grub *Clinostomum* is found in pustule-like capsules just under the skin of fish, often in the head or tail region. The huge, ribbon-like plerocercoids of the cestodes *Ligula* and *Schistocephalus* can be found in the body cavities of cyprinids and sticklebacks, respectively. These are tapeworms that use birds as definitive hosts. A large redish nematode, *Eustrongylides*, also can be seen in the body cavity of fish. This nematode is a larval stage, and its adults infect birds. Other tapeworm plerocercoids (e.g. *Diphyllobothrium*, *Triaenophorus*) are clearly visible as cysts on the viscera or in the musculature of fish. Adult cestodes such as *Proteocephalus* and acanthocephalans can be seen easily if the intestine is cut open.

Non-specialist volunteers should categorize the common macroparasite groups. Flatworms include digeneans (or flukes) and cestodes (or tapeworms). These are flat, and the cestodes are long and segmented, the digeneans short and rounded. Long narrow worm-like organisms are nematodes (or roundworms). Tubular animals with a spiny "head"-like portion are acanthocephalans (or thorny-headed worms). Crustaceans are external parasites with hard body parts. Databases collected by volunteer groups should be maintained independently from those of experts until verification of species is assured.

Materials and Suppliers

See sections on other organisms for field sampling and collecting equipment. Standard biological supply houses can provide most of the laboratory materials required. A list of suppliers is provided in Table 2.

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Experts to Contact for More Information

Dr. Martin Adamson
Department of Zoology
University of British Columbia
Vancouver, British Columbia V6T 1Z4
Tel: 604-822-3374
Fax: 604-822-2416
Email: damson@heth.bcu.ubc.ca

Dr. David K. Cone
Department of Biology
St. Mary's University
Halifax, Nova Scotia B3H 3C3
Tel: 902-420-5644
Fax: 902-420-5261
Email: cone@husky1.stmarys.ca

Dr. Terry Dick
Department of Zoology
University of Manitoba
Winnipeg, Manitoba R3T 2N2
Tel: 204-474-9896
Fax: 204-269-7431
Email: tadick@cc.umanitoba.ca

Dr. Cam Goater
Department of Biological Sciences
University of Lethbridge
Lethbridge, Alberta T1K 3M4
Tel: 780-329-2752
Fax: 780-329-2082
Email: goatcp@hg.uleth.ca

Dr. Tim Goater
Department of Biology
Malaspina University-College
Nanaimo, British Columbia V9R 5S5
Tel; 604-753-3245 ext. 2325
Fax: 604-755-8749
Email: goatert@mala.bc.ca

Dr. Murray Lankester
Department of Biology
Lakehead University
Thunder Bay, Ontario P7B 5E1
Tel: 807-343-8528
Fax: 807-346-7796
Email: mlankest@gale.lakeheadu.ca

Dr. David J. Marcogliese
Environment Canada
St. Lawrence Centre
105 McGill St., 7th Floor
Montreal, Quebec H2Y 2E7
Tel: 514-283-6499
Fax: 614-496-7398
Email: david.marcogliese@ec.gc.ca

Dr. Dan McLaughlin
Biology Department
Concordia University
1455 de Maisonneuve Blvd. W.
Montreal, Quebec H3G 1M8
Tel: 514-848-3409
Fax: 514-848-2881
Email: mcljd@alcor.concordia.ca

Dr. Lena Measures
Department of Fisheries and Oceans
Maurice Lamontagne Institute
PO Box 1000
Mont-Joli, Quebec G5H 3K6

Tel: 418-775-0571
Fax: 418-775-0740
Email: measuresl@dfo-mpo.gc.ca

Dr. Allen W. Shostak
Department of Biological Sciences
University of Alberta
Edmonton, Alberta T6G 2E9

Tel: 780-492-1293
Fax: 780-492-9234
Email: al.shostak@ualberta.ca

N.B.: The Directory of Parasitologists in Canada may be consulted at:

http://www.biology.ualberta.ca/parasites/indexen/directoryi.htm

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Table 1. Numbers and general characteristics of known parasite species infecting Canadian freshwater fish (Margolis and Arthur 1979; McDonald and Margolis 1995). Note that examination of the Canadian fish fauna for parasites is incomplete, and new Canadian host records and new parasite species continue to be found on a regular basis. The taxonomy of protists follows that of Roberts and Janovy (1996), and that of metazoans follows McDonald and Margolis (1995), with the exception of the Myxozoa, which are now regarded as a metazoan phylum.

Parasite taxa	Number of species	General characteristics
Kingdom Protista		Unicellular
Subkingdom Protozoa		
Phylum Sarcomastigophora	15	Single type of nucleus; flagella and/or pseudopodia
Phylum Apicomplexa	35	Exclusively parasitic; apical complex; micropore(s)
Phylum Microspora	8	Intracellular parasites; unicellular spores; polar filament
Phylum Ciliophora	23	Simple or compound cilia; subpellicular infraciliature; 2 types of nuclei
Uncertain status	1	
Kingdom Animalia		Multicellular
Subkingdom Eumetazoa		
Phylum Myxozoa	113	Polar capsules; valves; found in internal organs
Phylum Cnidaria	1	Hydrozoans; parasitic forms rare
Phylum Platyhelminthes		Flatworms
Class Trematoda		Exclusively parasitic; short to oblong, rounded
Subclass Aspidogastrea	1	Huge septate ventral sucker; in gut of fish
Subclass Digenea	113	Usually 2 cuplike muscular suckers; incomplete digestive system; adults primarily internal parasites, larvae internal or under skin; flukes
Class Monogenea	180	Exclusively parasitic; modified posterior holdfast with hooks; mainly ectoparasites on gills, fins, skin
Class Cestoda	70	Exclusively parasitic; long, usually segmented worms with suckers and/or hooks on holdfast; adults intestinal, larvae on viscera or in flesh; no digestive system; tapeworms
Phylum Nemathelminthes		
Class Nematoda	56	Slender worms, pointed or blunt at each end; complete digestive system; found in internal organs and viscera; roundworms
Phylum Acanthocephala	23	Exclusively parasitic; cylindrical with hooked probscis; no digestive system; adults intestinal, larvae on viscera; thorny-headed worms
Phylum Annelida	19	Suckers at each end; segmented ectoparasites; leeches
Phylum Mollusca	7	Clam-like; on gills; glochidia
Phylum Arthropoda		Exoskeleton with jointed limbs

Class Crustacea		
Subclass Branchiura	6	Flattened with dorsal shield; unsegmented, bilobed abdomen; 4 pairs of appendages; ectoparasites; fish louse
Subclass Entomostraca	29	Often highly modified; ectoparasites; copepods
Class Arachnoidea	3	Ectoparasites; mites
Total	703	

Table 2. Suppliers for sampling and laboratory equipment.

FIELD SAMPLING EQUIPMENT

Wildlife Supply Company (WILDCO) 301 Cass Street Saginaw, Michigan, USA 48602 Website: http://www.wildco.com	Tel: 517-799-8100 Fax: 517-799-8115 Email: goto@wildco.com
Kahlsico International Corporation P.O. Box 947 El Cajon, California, USA 92022-0947	Tel: 619-444-2158 Tel: 619-444-5944 Fax: 619-444-0207
Canadian distributor for Kahlsico: Geneq Inc. 8047 Jarry E. Montreal, Quebec H1J 1H6	Tel: 514-354-2511 Fax: 514-354-6948
223 Signet Drive Toronto, Ontario M9L 1V1	Tel: 416-747-9889 Fax: 416-747-7570

LABORATORY EQUIPMENT AND CHEMICALS/REAGENTS/STAINS

Laboratory equipment may be purchased at most scientific supply houses. Some examples are listed below.

Fisher Scientific 112 Colonnade Road Nepean, Ontario K2E 7L6	Tel: 800-234-7437 Fax: 800-463-2996 Website - http://www.fisher1.com
VWR Canlab All provinces (except Quebec) 2360 Argentia Road Mississauga, Ontario L5N 5Z7 or Quebec, Ottawa, Kingston 8567 Dalton Town of Mount Royal, Quebec H4T 1V5 Website - http://www.vwrsp.com	 Tel: 905-821-9410 Fax: 905-821-3460 Tel: 514-344-3525 Tel: 514-344-0133
Canadawide Scientific 2300 Walkley Road Unit 414 Ottawa, Ontario K1G 6B1	Tel: 800-2676-2362 Fax: 800-814-5162

Dissection instruments can be purchased at:

Fine Tools Scientific 202-277 Mountain Highway North Vancouver, British Columbia V7J 3P2 Website - http://www.finescience.com	Tel: 800-665-5355 Fax: 800-665-4544 Email: fstcan@axionet.com

Reagents, chemicals and stains can be purchased at:

Sigma-Aldrich Canada Ltd. 2149 Winston Park Drive Oakville, Ontario L6H 6J8 Website - http://www.sigald.sial.com/canada	Tel: 800-565-1400 Fax: 800-265-3858 Email: canada@sial.com

MS 222 can be purchased at:

Syndel Laboratories Ltd. 9211 Shaughnessy St. Vancouver, British Columbia V6P 6R5	Tel: 604-321-7131

Table 3. Recipes for standard fixatives, preservatives, and stains commonly used in parasitology. After Ash and Orihel (1991).

Material	Recipe
70% ethanol	740 mL 95% ethanol + 260 mL water
5% glycerol in 70 % ethanol	50 mL glycerol + 740 mL 95% ethanol + 210 mL water
10% buffered formalin	Dissolve 6.10 g dibasic sodium phophate (Na_2HPO_4) and 0.15 g monobasic sodium phosphate (NaH_2PO_4) in 800 mL 37.5% formaldehyde (=100% formalin) + 7200 mL water (Available commercially)
AFA (alcohol-formalin-acetic acid)	100 mL 100% formalin + 500 mL 95% ethanol + 50 mL glacial acetic acid + 450 mL water
Schneider's acetocarmine stain	45 mL glacial acetic acid + 55 mL water (slowly!). Add 5 g carmine powder, boil 15 min. Cool and filter. For use, add a few drops to small dish of 70% ethanol to make solution moderate to dark pink. (Available commercially)
Glycerol-alcohol mixtures for clearing large nematodes, to be used sequentially	1. 50 mL glycerol + 700 mL 95% ethanol + 250 mL water 2. 100 mL glycerol + 700 mL 95% ethanol + 200 mL water 3. 200 mL glycerol + 500 mL 95% ethanol + 300 mL water 4. 500 mL glycerol + 300 mL 95% ethanol + 200 mL water 5. 700 mL glycerol + 100 mL 95% ethanol + 100 mL water 6. 1000 mL glycerol
Lactophenol clearing agent	20 mL glycerol + 10 mL lactic acid + 10 mL melted phenol crystals + 10 mL water. Mix well.
Berland's fluid	5 mL 100% formalin + 95 mL glacial acetic acid

Table 4. References to identification manuals and host-parasite lists for freshwater fish parasites of Canada.

Parasite taxa	Reference	Comments
Protozoa	Lom and Dykova (1992)	Useful, expensive
Myxozoa	Lom and Dykova (1992)	Useful, expensive
Cnidaria	Arai, M.N. (1989)	Useful, inexpensive
Monogenea	Beverley-Burton (1984)	Useful, inexpensive
Trematoda	Gibson (1996)	Useful, inexpensive
	Schell (1985)	Good to genus level
Cestoda	Khalil et al. (1994)	Expensive; difficult to use; no host-parasite lists
	Schmidt (1986)	Expensive; good to genus level (some species); parasite species lists; host lists incomplete; uncritical assessment of parasite species
Nematoda	Moravec (1994)	Excellent; expensive; for European parasite fauna, but many species in Canada
Acanthocephala	Arai, H.P. (1989)	Useful, inexpensive (out of print?)
Copepoda	Kabata (1988)	Useful, inexpensive
Branchiura	Kabata (1988)	Useful, inexpensive
All taxa	Hoffman (1967)	Useful; simplistic; poor drawings; out of print
Host-parasite lists	Margolis and Arthur (1979)	Excellent, inexpensive
Host-parasite lists	McDonald and Margolis (1995)	Excellent, inexpensive

Appendix I. Sample data sheet

Fish parasite data sheet

Locality: _____ **Date:** _____

Host: _____ **No:** _____

Fork length: _____ **Weight:** _____ **Sex:** _____

Maturity: _____ **Condition:** Fresh ( ) Held ( ) Refrigerated ( ) Frozen ( ) Fixed ( )

Gonad weight: _____ **Stomach weight:** (full)_____ (empty) _____

Date of examination: _____ **Examiner:** _____

Blood smear ( ):	Esophagus:
External surface: Smear ( ):	Body cavity:
Fins:	Stomach:
Eyes L: R:	Mesenteries:
Gills L Arc 1 2 3 4	Gills R Arc 1 2 3 4
Operculum L: R:	Intestine: Smear ( ):
Nasal cavities:	Spleen: Smear ( ):
Mouth:	Liver: Smear ( ):
Brain: Smear ( ):	Gall bladder: Smear ( ):
Heart:	Urinary bladder: Smear ( ):
Swim bladder:	Kidney: Smear ( ):
Gonads: Smear ( ):	Muscle L: R: