



## **Parasitology Research, in press (accepted 24 January 2012)**

### **A quick and simple method, usable in the field, for collecting parasites in suitable condition for both morphological and molecular studies**

**Jean-Lou Justine<sup>1\*</sup>, Marine J. Briand<sup>2</sup>, Rodney A. Bray<sup>3</sup>**

**1** UMR 7138 Systématique, Adaptation, Évolution, Muséum National d'Histoire Naturelle, Case postale 51, 55 Rue Buffon, 75231 Paris cedex 05, France

**2** Laboratoire LIVE, Équipe Écologie Marine, Université de la Nouvelle-Calédonie, Campus de Nouville, 98851 Nouméa cedex, New Caledonia

**3** Department of Zoology, Natural History Museum, Cromwell Road, London SW7 5BD, UK

\* corresponding author; E-mail: justine@mnhn.fr

## **Abstract**

Many methods have been proposed for collecting and fixing parasites, but most were written before the molecular age, and were intended to be practised by experienced parasitologists in well-equipped laboratories. We describe here a very simple method, illustrated by photographs, for collecting helminths from the digestive tract of vertebrates. It only requires a few plastic vials, some ethanol and a means to heat water. Basically, the method consists of: (a) the extraction of all organs from the abdominal cavity; (b) opening the digestive system longitudinally; (c) agitate gut and contents in a saline solution (i.e. ca. 9% NaCl or ¼ sea water in tap water); (d) decant in saline as many times as needed to clean contents; (e) immediately fix parasites in near-boiling saline; (f) discard saline and keep specimens in 95% ethanol. Additional information is given for collecting parasites from fish gills with a similar process. The method will collect most helminths (digeneans, larval cestodes, nematodes, acanthocephalans) from the digestive tract, and monogeneans and isopod and copepod crustaceans from fish gills. The specimens will be suitable for both morphological study and DNA sequencing. The method is simple, fast, inexpensive, and can be used by untrained personnel, even in the field without electricity and without a binocular microscope. It can also be used by trained parasitologists who need to expedite treatment of abundant samples.

## **Keywords**

Method, morphology, DNA, helminth, fish

## Introduction

Parasites are one of the most neglected components in evaluations of biodiversity (Bouchet 2006; Poulin 2004; Whittington and Chisholm 2003); however, the simple observation that each free-living animal harbours at least one parasite species immediately suggests that the number of parasite species is at least equal to the number of non-parasitic species (Windsor 1995, 1998) and probably greater than it (Poulin and Morand 2000; Rohde 2001). Parasites are certainly a major component of biodiversity (Cribb and Bray 1998; Dobson et al. 2008; Poulin and Morand 2004) and ecosystems (Kuris et al. 2008; Lafferty et al. 2006) and thus deserve appropriate interest (Brooks and Hoberg 2000; Hoberg et al. 2009; Vannier-Santos and Lenzi 2011). However, parasitology has been often ill-considered (Vickerman 2009) and is still underrepresented in textbooks (Nichols and Gomez 2011). With the progressive decrease in the numbers of parasitologists trained in systematics (Brooks and Hoberg 2001; Cribb 2004; Poulin and Leung 2010) it is likely that further collection of parasites will be done by researchers or technicians who have not been thoroughly trained in parasitological methods. In addition, the development of molecular systematics demands that specimens should be collected in conditions suitable for both morphological and molecular methods; this is not the case for most methods published in classic papers or handbooks.

Most parasitological books, including the oldest ones (e.g. Baylis 1915; Braun and Lühe 1910; Dujardin 1845; Van Beneden 1878, 1887) include a chapter about the processing of parasites. They describe many methods, but agree at least on one point, the need to use only freshly killed hosts to obtain living parasites.

A variety of general methods for the collection and preservation of helminths have been published since the 1950s; methods sometimes varied very much from teams to teams, with controversies arising about the pro and cons of flattening and the use of various chemicals (Berland 1961a, b, 1984; Bray 1984; Burt 1984; Chubb 1962; Cooper 1988; Durette-Desset 1984; Fagerholm 1979; Garvin et al. 1997; Gibson 1979, 1984; Gläser 1965; Gusev 1983; Hanson Pritchard and Kruse 1984; Hargis 1953; Hendrix 1994; Huber 1998; Kennedy 1979; Lichtenfels 1984; Lim 1991; McCue and Thorson 1963; Moravec 1994, 2001; Newman and Cannon 1995; Pritchard and Kruse 1982; Rogers 1966; Unnithan 1957; Williams et al. 1991; Yamaguti 1965a, b, 1968). Various papers have addressed the influence of methods on the measurements of specimens (Bakke 1988; Criscione and Font 2001; Fagerholm 1979; Fagerholm and Lövdahl 1984; Justine 2005, 2007) or the processing of monogenean sclerites (García-Vásquez et al. 2011; Kritsky et al. 1978; Maillard et al. 1982; Malmberg 1957; McDougal and Mizelle 1969; Milne and Avenant-Oldewage 2006; Mo and Appleby 1990; Shinn et al. 1993;

Richards and Chubb 1995; Rohde 1987). The interest in describing new methods is still alive and many recently published works address specific parasites or methods (Albuquerque et al. 2009; Bruno et al. 2006; Cribb et al. 2004; Deveney and Whittington 2001; Galli et al. 2006; Galli et al. 2007; Parker et al. 2010; Snyder and Clopton 2005; Wong et al. 2006).

The emergence of molecular methods has prompted a renewed interest in methods which can process material for both morphological and molecular methods (Harris et al. 1999; Košková et al. 2011; Naem et al. 2010; Strona et al. 2009; Toe et al. 1997; Yoder et al. 2006).

We propose here a method for collecting parasites which can be used by any person who has at least some basic knowledge of vertebrate anatomy, but has no specific training in parasitology. This method modifies Cribb & Bray's "gut wash" method (2010), recently published but in use in various laboratories for years before its publication, and intensively used by one of us (JLJ) on more than 2,000 fish, with molecular (Bray et al. 2009; Černotíková et al. 2011; Miller et al. 2009, 2010a, b; Olson et al. 2010; Perkins et al. 2009) and morphological results (lists in Justine 2010; Justine et al. 2010a, b) published on several parasite groups. Our method requires only access to hot water and alcohol (ethanol) and a few plastic vials and does not require the use of a binocular microscope; it therefore can be practised in the field in harsh conditions, even without electricity. It can be used, also, by trained parasitologists who need to process abundant samples in a limited time. The method is illustrated by photographs. Examples are based on fish, but the method can be used for all vertebrates.

## **Description of the method**

### **Freshness of material**

Parasitological examination should be practised only on freshly killed hosts. "Freshly" usually means hours, but can be extended to 24h if the hosts are kept in a refrigerator. Frozen-thawed material must not be used. Parasites should be alive.

### **Material and chemicals needed**

#### **Material**

- Forceps.
- Scissors.
- Several wide mouthed bottles (size related to the size of the fish).
- Plastic Petri dishes or plastic plates (size related to the size of the fish).
- Small or medium-sized plastic vials for storage (as many as fishes  $\times$  2 if both intestine and gills are studied).
- Facilities for boiling water (any methods from wood fire to solar apparatuses).

### Chemicals

- Ethanol 95%, 20 cl per fish (small species) to more than 1 litre per fish (big species).
- Seawater (for gills of marine fish) and for preparation of saline for gastrointestinal tract (see below). If not available: NaCl.
- Tap water, or any reasonably clean water.

### Method for gastrointestinal parasites (Figure 1)

Before processing, prepare saline solution. When near to sea, the best is to mix one part of seawater and three parts of tap water (Cribb and Bray 2010). Filtering is unnecessary. Away from the sea, use 0.9% solution of NaCl in water (9 grams in 1 litre).

#### Step 1: obtain gastrointestinal tract.

- Open the abdominal cavity, from anus, and anteriorly.
- Cut the digestive system at the level of the anus and oesophagus; if necessary, cut additional attachments.
- Extract all organs and put them on a flat container of appropriate size (a Petri dish for small animals, or a plastic tray).
- Discard liver, spleen and pancreas: keep only the tubular digestive system.
- Untangle the intestine (usually better done with fingers than with metal instruments).

#### Step 2: open gastrointestinal tract.

- Open the whole digestive system longitudinally (from anus to oesophagus, or vice versa, including stomach) with scissors.
- If pyloric caeca are present, try to open at least some of them
- Open gall bladder.
- Discard large undigested food items, especially those found in stomach.
- Drop entire digestive system into a vial of compatible dimensions (from 20 cm<sup>3</sup> for a small host to several litres to a large one).
- Use saline to rinse the dish on which the digestive system has been open and add rinsing liquid to vial.
- Fill about one third of vial with saline.

**Step 3:** shake.

- Close vial.
- Vigorously shake vial for about one minute. You should obtain a coloured and unattractive mix.

**Step 4:** decant and clean.

- Fill vial up to top with saline.
- Allow the contents to settle for about 1-2 minutes.
- Gently incline vial and discard upper three quarters of the mix.
- Check for transparency: if the content is transparent as water, proceed to main step 5.
- If the content is not transparent, fill vial again with saline, wait for settling, and carefully discard three quarters of the liquid. Redo this step as many times as necessary.

**Step 5:** fix with hot water.

- Gently incline vial and discard almost all liquid, taking care that no solid matter (visible with the naked eye) is discarded.
- Pour near-boiling water in vial, fill.
- Wait at least one minute.
- For small vials and small hosts, continue process in same vial; for large hosts and vials, transfer contents of large vial to a smaller vial which will be used for storage.

**Step 6:** add ethanol.

- Gently incline vial and discard almost all liquid, still taking care that no solids are discarded.
- Add ethanol up to top of vial.
- The ethanol-solids ratio should be higher than 5:1.

**Step 7:** label vial.

- Label vial immediately; labels should be hand-written in pencil on tracing paper (waterproof polyester paper is good, but more expensive) and placed within the vial.
- Computer labels are sometimes damaged by ethanol; use only pencil.
- Avoid external labelling of vials with markers; most are erased by alcohol, which can possibly leak. Labels should be *within* the vial. For information on labelling see (Huber 1998).
- The vial should contain a transparent solution, the gut tissue and the label; the gut should not be discarded because some helminths (large digeneans, certain nematodes) remain attached even after the shaking.
- Close vial. You're done.

**Method for fish gills (Figure 2)**

The method is basically the same but does not require multiple decantations. For freshwater fish, use tap water; for sea fish, use seawater.

**Step 1:** obtain gills.

- Excise gills by cutting each gill at upper and lower extremity.
- Put all gills in a vial.

**Step 2:** fix with hot water.

- Pour near-boiling hot water on gills.
- Wait one-two minutes. It is normal if the gills change colour and if the extremities of their filaments curl.

**Step 3:** add ethanol.

- Gently incline vial and discard most liquid, still watching that no solids are discarded.
- Add ethanol up to top of vial.
- The ethanol-solids ratio should be higher than 5:1.

**Step 4:** label.

- Same remarks as above (step 7 of gastrointestinal tract)

- The vial should contain the gills, a transparent solution and the label. It is paramount to keep the gills because most monogeneans and copepods will be still attached.
- Close vial. You're done.

## **Conservation and transport**

Optimal preservation of DNA will be attained by keeping the vials in a refrigerator. Vials can simply be kept as they were prepared for years. If the parasitological specialist who performs the triage is elsewhere (often in another continent) the problem arises of how to send vials containing ethanol, which is forbidden by some postal services. It is possible to discard almost all the ethanol to keep only the specimens wet (this also saves weight) without deterioration of the material if the vials are refilled with ethanol as soon as they arrive at their destination.

## **Discussion**

### **1. The result of a compromise of morphology and molecules**

A method suitable for both morphological and molecular studies is obviously the result of a compromise. Formalin fixation (either hot water followed by formalin, or immersion of living specimens in hot formalin) is often considered the best method for obtaining perfect slides of unflattened digeneans (Cribb and Bray 2010), monogeneans (Kritsky and Bakenhaster 2011; Monteiro et al. 2010) and to collect nematodes (Moravec and Justine 2005). However, formalin is not suitable for molecular studies (see below). Freezing at  $-80^{\circ}$  is generally considered one of the best methods to preserve DNA (Nagy 2010), but frozen specimens are usually very bad for morphology (with the exception of monogeneans if only sclerites, not the soft organs, are considered). Also, each parasite group (monogeneans, digeneans, nematodes, acanthocephalans, and also crustaceans) will be best preserved for morphology with some subtle variations; our method is probably not the best for morphology, but will provide specimens which will be acceptable.

### **2. The need for a solution with correct osmotic pressure**

Parasites should be collected and washed in a solution of osmotic pressure close to their original milieu. The use of saline is essential for the collection of gastrointestinal parasites (either for freshwater or seawater

fish); saline solution mimics the osmotic pressure found in the lumen of the intestine. As an example, digeneans are damaged after a few minutes in hypo-osmotic solutions (i.e. tap water) and especially lose tegumental spines, which are important for systematics. The easiest method for the preparation of 0.9% NaCl saline is a mixing of  $\frac{1}{4}$  seawater and  $\frac{3}{4}$  tap water (Cribb and Bray 2010).

External parasites (especially gill parasites) live either in fresh water or seawater, and should be collected in the corresponding milieu.

### **3. The advantages of washing**

One of the advantages of the initial washing and the subsequent successive decantation processes is that the parasites are cleaned of the intestinal mucus: such specimens are particularly well suitable for classical morphological examination, and are also much better for further scanning electron microscope (SEM) examination (Moravec and Justine 2010a, b).

### **4. Hot fixation vs freezing**

Freezing of specimens has been proposed for the collection of monogeneans (e.g. Mizelle 1936, 1938; McDougal and Mizelle 1969), because freezing-thawing does not alter the sclerotised parts of monogeneans, on which much morphological work is based for this group of animals (e.g. Justine and Grugeaud 2010; Pariselle et al. 1991; Rohde 1987). Freezing is certainly an excellent method for DNA processing (Nagy 2010); however, we do not recommend freezing for helminths other than monogeneans because morphological studies (especially of digeneans) require that the body keeps its original shape and proportions; for Bunkley-Williams and Williams (1994), “freezing destroys almost all parasites”.

The advantage of hot water is that it very efficiently relaxes most helminths. Hot water fixation works very well on flatworms (monogeneans, trematodes) (Cribb and Bray 2010; Kritsky and Bakenhaster 2011; Monteiro et al. 2010) and nematodes (Moravec and Justine 2010a, b), and provides acceptable results on some acanthocephalans (unpublished). Parasitic crustaceans on gills, as other arthropods, do not need to be relaxed and fixed in hot water but are still usable if they have been heated. Hot water fixation does not damage DNA (see e.g. Miller et al. 2009, 2010a, b).

The use of our method without the hot water step will result on contracted helminths which will be completely unsuitable for morphological analysis, and should absolutely be avoided.

Other hot fixatives have been used, such as FAA (formalin-acetic-alcohol) (e.g. Kritsky and Fennessy 1999), hot formalin (e.g. Bakke 1988; Rohde and Watson 1985) or hot 70% ethanol (e.g. Berland 1961a) but

manipulation of these hot, 'steaming' chemicals is more dangerous than that of hot water because of toxic fumes (formalin, FAA) and possible explosion or flaming (ethanol); hot water is certainly less dangerous, and can be used easily in the field. For the same reason of toxicity, we exclude the use of mercury compounds (i.e. Manter 1926).

## 5. Ethanol vs formalin and DMSO

Ethanol and formalin were often compared in classical methods, usually with some advantage found for formalin for morphology (Schmidt 1986; Cribb and Bray 2010). However, formalin damages DNA and formalin-fixed material can be used for molecular methods only at the expense of additional and costly effort (e.g. Herniou et al. 1998; Jeon et al. 2011; Klopfleisch et al. 2011; Nadler 1999; Simsek et al. 2011) or is simply useless (Chakraborty et al. 2006). Ethanol has been tested against various other chemicals for the preservation of DNA (e.g. Hajibabaei et al. 2006; King and Porter 2004; Nagy 2010; Post et al. 1993; Quicke et al. 1999; Srinivasan et al. 2002) and is routinely used for DNA studies in helminths (e.g. Shinn et al. 2010).

DMSO (dimethyl sulphonyde) or DESS (a solution of DMSO, disodium EDTA and saturated NaCl) is considered a good preservative of DNA (Yoder et al. 2006); it is suitable for light microscope morphological studies of nematodes (Naem et al. 2010; Yoder et al. 2006) or monogeneans (Strona et al. 2009) but apparently disrupts morphology in certain arthropods (Frampton et al. 2008). One of the advantages of DESS is that it is not flammable and considered non-toxic and thus allows specimens to be sent by post. However, DESS has not been tested on digeneans. It is more expensive and less easy to obtain than ethanol (Frampton et al. 2008).

We do not recommend acetone, although a good preservative for DNA (Fukatsu 1999), because of its toxicity and unknown effect on morphology of helminths. In addition, acetone dissolves many plastics.

In our method, we recommend ethanol for the following reasons. Ethanol:

- (1) Is suitable for molecular methods;
- (2) Produces material well suitable for morphological methods;
- (3) Is easily found in most countries;
- (4) Is of low toxicity and can be manipulated by untrained personnel;
- (5) Has a relatively low cost.

The quality of ethanol is paramount for the efficiency of the method, and particularly the quality of further molecular work. Absolute (100%) ethanol is not necessary, because it is very expensive; also, absolute ethanol might contain benzene (Nagy 2010). 95% ethanol solution is the best compromise. However, the manipulator should pay attention to the components of the remaining 5%: it should be water, and no other

solvent. Alcohol sold for flaming purposes has the advantage of a lower cost, but its proportion of ethanol is unknown, and it is usually mixed with methanol (wood alcohol, methyl alcohol) and many other components (to make it unsuitable for human taste) which vary from countries to countries (Lachenmeier et al. 2007); this should be avoided.

Ethanol solutions at 70% or 80% are not acceptable, because ethanol is diluted when added to the remaining of saline (main step 6 of the intestine method, main step 3 of gill method). If only 70% or 80% ethanol (with no other chemical added) is available, it can be used if the material is left overnight in this solution in the labelled vial, then next morning, most of the liquid is discarded and replaced with fresh ethanol solution.

The ethanol-material ratio in the final vial should be higher than 5:1 (Nagy 2010).

## **6. Flaws of this method**

The authors certainly do not pretend that the method will retrieve all parasites. Parasites from special locations, such as trematodes from the scales of fish and others (Cribb and Bray 2010), will not be collected; however, gastrointestinal trematodes represent 95% of fish trematodes (Cribb and Bray 2010). The list of flaws of methods used for parasitological research can be very long: see e.g. (Justine et al. 2010a, b). The shaking part will probably damage most adult cestodes, which will be broken into many proglottids (Cribb and Bray 2010) and thus should not be used for the spiral intestine of sharks or rays; however, the method works very well on most larval cestodes (tetraphyllids), even the tiniest; larval trypanorhynch in the gut (*Nybelinia*) will be correctly preserved. Larval trypanorhynch cestodes which are in cysts in the abdominal cavity will be collected, if the cysts are kept with the intestinal mass, but probably in suboptimal conditions for morphological study because the tentacles will not be everted (Campbell and Beveridge 1994). Proper collection of trypanorhynchs requires the cysts to be individually open; the cysts can then be added to the gastrointestinal mixture before adding hot water.

However, the method will retrieve, in conditions acceptable for both good morphological and molecular works most flatworms (digeneans, larval cestodes) and nematodes of the gut lumen; these constitute the majority of internal macroparasites found in a vertebrate. Nematodes will be usable for SEM observations (Moravec and Justine 2010a, b). The additional use of the method on fish gills will provide monogeneans in acceptable condition. Gill parasitic crustaceans (mainly copepods and isopods) will also be collected; although heating is generally not necessary nor advised for these groups, the method will provide specimens of acceptable quality; however, not all ectoparasitic crustaceans will be collected by our method, because some easily separate from the fish (Grutter 1995).

## 7. Separating collection and triage in time and place

Our method described above is mainly based on the “gut wash” method of Cribb and Bray (2010) and resembles the method proposed for monogeneans by Thatcher (2006); however, it differs in that (a) the “wash” obtained after decantation is not immediately examined by the trained parasitologist, but is fixed in hot water and preserved in ethanol for further processing; and (b) it extends the method to all groups of gastrointestinal and gill parasites. Cribb & Bray’s method has been intensively used by the senior author over several years, on more than 2,000 fish (Justine 2010; Justine et al. 2010a, b) for the collections of all gastrointestinal parasites (including trematodes, and also all others) and has been found much more time effective than the direct examination of guts; material collected by this method has been forwarded to various specialists of various helminth groups, who were satisfied by the quality of the material, both for morphological (lists of papers in Justine 2010) and molecular studies (Bray et al. 2009; Černotíková et al. 2011; Miller et al. 2009, 2010a, b; Olson et al. 2010; Perkins et al. 2009).

Our method can be practised by a person who is not trained for parasitology, but it requires future processing of the samples by trained parasitologists. Most parasitologists will agree that the triage and collection of living parasites, which are coloured and moving, is easier and more effective and pleasant than that of whitish immobile ethanol-fixed specimens; however, our method separates the two steps of sample collecting, the collection itself and the triage, leaving the second part to trained parasitologists and thus reducing the risk of errors by untrained manipulators. The method can also be easily explained to untrained technicians, and it is hoped that the present step-by-step explanation and photographs will make it easily understandable by a reader untrained in parasitology. Finally, it can be practiced in remote exotic places without modern comfort (which are usually the places in the world where biodiversity is at its greatest), while further processing can be done in well-equipped laboratories.

Another possible use of our method is to treat abundant material in a limited time. Pritchard and Kruse (1982), wrote “the collector must be ready to work hard and long”. However, there are limits to human stamina, even for the most trained and motivated parasitologists, when they receive at the same time more freshly killed hosts specimens than that they can process while they are still fresh: in such cases, our method can be an alternative to the classical collection of living parasites.

## Acknowledgments

David Gibson (NHM, London) provided literature; Sarah Samadi and Regis Debruyne (MNHN, Paris) discussed DNA conservation. Students (Cyndie Dupoux, Adeline Grugeaud, Isabelle Mary, Sophie Olivier, Charlotte Schoelinck, Aude Sigura) helped in testing various methods; Eva Řehulkova (Masaryk University, Czech Republic) helped in the field.

## References

- Albuquerque MC, Ederli NB, Monteiro CM, Rodrigues MLA (2009) Técnica de montagem permanente e morfometria de *Ancylostoma caninum* (Ercolani, 1859), *Oxyspirura mansoni* (Cobbold, 1879) Ransom, 1904 e *Oesophagostomum columbianum* Curtice, 1890. Rev Bras Med Vet 31:80-84
- Bakke TA (1988) Morphology of adult *Phyllostomum umblae* (Fabricius) (Platyhelminthes, Gorgoderidae): the effect of preparation, killing and fixation procedures. Zool Scr 17:1-13
- Baylis HA (1915) Instructions for collectors: No. 12.- Worms. British Museum (Natural History), London
- Berland B (1961a) Nematodes from some Norwegian marine fishes. Sarsia 2:1-50
- Berland B (1961b) Use of glacial acetic acid for killing parasitic Nematodes for collection purposes. Nature 191 (4795):1320-1321
- Berland B (1984) Basic techniques involved in helminth preservation. Syst Parasitol 6 (4):242-245. doi:10.1007/bf00012195
- Bouchet P (2006) The magnitude of marine biodiversity. In: Duarte CM (ed) The Exploration of Marine Biodiversity. Scientific and Technological Challenges. Fundacion BBVA, Bilbao, pp 31-64
- Braun M, Lühe M (1910) A handbook of practical parasitology. John Bale, Sons and Danielsson, London
- Bray R, Waeschenbach A, Cribb T, Weedall G, Dyal P, Littlewood D (2009) The phylogeny of the Lepocreadioidea (Platyhelminthes, Digenea) inferred from nuclear and mitochondrial genes: Implications for their systematics and evolution. Acta Parasitol 54 (4):310-329. doi:10.2478/s11686-009-0045-z
- Bray RA (1984) The curation of helminths at the British Museum (Natural History). Syst Parasitol 6:251-253
- Brooks DR, Hoberg EP (2000) Triage for the biosphere: the need and rationale for taxonomic inventories and phylogenetic studies of parasites. Comp Parasitol 67:1-5
- Brooks DR, Hoberg EP (2001) Parasite systematics in the 21st century: opportunities and obstacles. Trends Parasitol 17 (6):273-275
- Bruno DW, Nowak B, Elliott DG (2006) Guide to the identification of fish protozoan and metazoan parasites in stained tissue sections. Dis Aquat Org 70:1-36
- Bunkley-Williams L, Williams EH (1994) Parasites of Puerto Rican freshwater sport fishes. Puerto Rico Department of Natural and Environmental Resources, San Juan, and Department of Marine Sciences, University of Puerto Rico, Mayaguez,
- Burt MDB (1984) Problems with tense tapeworms. Syst Parasitol 6:249
- Campbell RA, Beveridge I (1994) Order Trypanorhyncha Diesing, 1863. In: Khalil LF, Jones A, Bray RA (eds) Keys to the Cestode parasites of Vertebrates. CAB International, Wellingford, pp 51-148

- Černotíková E, Horák A, Moravec F (2011) Phylogenetic relationships of some spirurine nematodes (Nematoda: Chromadorea: Rhabditida: Spirurina) parasitic in fishes inferred from SSU rRNA gene sequences. *Folia Parasitol* 58:135-148
- Chakraborty A, Sakai M, Iwatsuki Y (2006) Museum fish specimens and molecular taxonomy: A comparative study on DNA extraction protocols and preservation techniques. *J Appl Ichthyol* 22 (2):160-166. doi:10.1111/j.1439-0426.2006.00718.x
- Chubb JC (1962) Acetic acid as a diluent and dehydrant in the preparation of whole, stained helminths. *Biotech Histochem* 37:179-182
- Cooper DW (1988) The preparation of serial sections of platyhelminth parasites, with details of the materials and facilities required. *Syst Parasitol* 12:211-229
- Cribb B, Armstrong W, Whittington I (2004) Simultaneous fixation using glutaraldehyde and osmium tetroxide or potassium ferricyanide-reduced osmium for the preservation of monogenean flatworms: An assessment for *Merizocotyle icopae*. *Microsc Res Tech* 63 (2):102-110. doi:10.1002/jemt.20015
- Cribb TH (2004) Living on the edge: parasite taxonomy in Australia. *Int J Parasitol* 34:117-123
- Cribb TH, Bray RA (1998) Trematodes of fishes: a test case for predictions of parasite biodiversity on the Great Barrier Reef. In: Greenwood JG, Hall NJ (eds) Australian Coral Reef Society 75th Anniversary Conference. School of Marine Science, The University of Queensland, Brisbane, Heron Island October 1997, pp 43-56
- Cribb TH, Bray RA (2010) Gut wash, body soak, blender, and heat-fixation: approaches to the effective collection, fixation and preservation of trematodes of fishes. *Syst Parasitol* 76:1-7
- Criscione CD, Font WF (2001) Artfactual and natural variation of *Oochoristica javaensis*: Statistical evaluation of in situ fixation. *Comp Parasitol* 68 (2):156-163
- Deveney M, Whittington ID (2001) A technique for preserving pigmentation in some capsalid monogeneans for taxonomic purposes. *Syst Parasitol* 48 (1):31-35. doi:10.1023/a:1026558405617
- Dobson A, Lafferty KD, Kuris AM, Hechinger RF, Jetz W (2008) Homage to Linnaeus: How many parasites? How many hosts? *Proc Natl Acad Sci U S A* 105:11482-11489. doi:10.1073/pnas.0803232105
- Dujardin F (1845) Histoire naturelle des helminthes ou vers intestinaux. Librairie encyclopédique de Roret, Paris
- Durette-Desset M-C (1984) Techniques de récolte, de fixation et de conservation des Nématodes parasites de Vertébrés. *Syst Parasitol* 6:248
- Fagerholm H-P, Lövdahl M (1984) Induced morphometric variation in the preparation of nematode parasites for the LM and SEM. *Syst Parasitol* 6 (4):245-247. doi:10.1007/bf00012196
- Fagerholm HP (1979) Nematode length and preservatives, with a method for determining the length of live specimens. *J Parasitol* 65 (2):334-335
- Frampton M, Droege S, Conrad T, Prager S, Richards MH (2008) Evaluation of specimen preservatives for DNA analyses of bees. *J Hymenoptera Res* 17:195-200

- Fukatsu T (1999) Acetone preservation: a practical technique for molecular analysis. *Mol Ecol* 8 (11):1935-1945. doi:10.1046/j.1365-294x.1999.00795.x
- Galli P, Strona G, Villa AM, Benzoni F, Stefani F, Doglia SM, Kritsky DC (2006) Three-dimensional imaging of monogenoidean sclerites by laser scanning confocal fluorescence microscopy. *J Parasitol* 92:395-399
- Galli P, Strona G, Villa AM, Benzoni F, Stefani F, Doglia SM, Kritsky DC (2007) Two-dimensional versus three-dimensional morphometry of monogenoidean sclerites. *Int J Parasitol* 37 (3-4):449-456
- García-Vásquez A, Shinn A, Bron J (2011) Development of a light microscopy stain for the sclerites of *Gyrodactylus* von Nordmann, 1832 (Monogenea) and related genera. *Parasitol Res*: in press. doi:10.1007/s00436-011-2675-y
- Garvin MC, Bates JM, Kinsella JM (1997) Field techniques for collecting and preserving helminth parasites from birds, with new geographic and host records of parasitic nematodes from Bolivia. *Ornithol Monogr* (48):261-266
- Gibson DI (1979) Materials and methods in helminth alpha-taxonomy. *Parasitology* 79:r36
- Gibson DI (1984) Technology as applied to museum collections: the collection, fixation and conservation of helminths. *Syst Parasitol* 6:241
- Gläser H-J (1965) Zur Kenntnis der Gattung *Dactylogyrus* Diesing 1850 (Monogenoidea). *Z. ParasitenKde* 25(5):459-484
- Gusev AV (1983) Methods for collection and preparation of monogeneans parasitizing fish. Nauka, Leningrad
- Gutter AS (1995) Comparison of methods for sampling ectoparasites from coral reef fishes *Mar Freshw Res* 46:897-903
- Hajibabaei M, Smith MA, Janzen DH, Rodriguez JJ, Whitfield JB, Hebert PDN (2006) A minimalist barcode can identify a specimen whose DNA is degraded. *Mol Ecol Notes* 6 (4):959-964. doi:10.1111/j.1471-8286.2006.01470.x
- Hanson Pritchard M, Kruse G (1984) Making the best of things: reclaiming specimens. *Syst Parasitol* 6 (4):253-255. doi:10.1007/bf00012201
- Hargis WJ, Jr. (1953) Chloretone as a Trematode relaxer, and its use in mass-collecting techniques. *J Parasitol* 39 (2):224-225
- Harris PD, Cable J, Tinsley RC, Lazarus CM (1999) Combined ribosomal DNA and morphological analysis of individual gyrodactylid monogeneans. *J Parasitol* 85 (2):188-191
- Hendrix SS (1994) Marine flora and fauna of the eastern United States. Platyhelminthes: Monogenea. NOAA Technical Report NMFS 121. US Department of Commerce, Seattle
- Herniou EA, Pearce AC, Littlewood DTJ (1998) Vintage helminths yield valuable molecules. *Parasitol Today* 14 (7):289-292

- Hoberg EP, Pilitt PA, Galbreath KE (2009) Why museums matter: a tale of pinworms (Oxyuroidea: Heteroxynematidae) among pikas (*Ochotona princeps* and *O. collaris*) in the American West. *J Parasitol* 95:490-501
- Huber JT (1998) The importance of voucher specimens, with practical guidelines for preserving specimens of the major invertebrate phyla for identification. *J Nat Hist* 32 (3):367-385
- Jeon H-K, Kim K-H, Eom KS (2011) Molecular identification of *Taenia* specimens after long-term preservation in formalin. *Parasitol Int* 60 (2):203-205
- Justine J-L (2005) Species of *Pseudorhabdosynochus* Yamaguti, 1958 (Monogenea: Diplectanidae) from *Epinephelus fasciatus* and *E. merra* (Perciformes: Serranidae) off New Caledonia and other parts of the Indo-Pacific Ocean, with a comparison of measurements of specimens prepared using different methods, and a description of *P. caledonicus* n. sp. *Syst Parasitol* 62 (1):1-37. doi:10.1007/s11230-005-5480-0
- Justine J-L (2007) *Huffmanella* spp. (Nematoda, Trichosomoididae) parasites in coral reef fishes off New Caledonia, with descriptions of *H. balista* n. sp. and *H. longa* n. sp. *Zootaxa* 1628:23-41
- Justine J-L (2010) Parasites of coral reef fish: how much do we know? With a bibliography of fish parasites in New Caledonia. *Belg J Zool* 140 (Suppl.):155-190
- Justine J-L, Beveridge I, Boxshall GA, Bray RA, Moravec F, Trilles J-P, Whittington ID (2010a) An annotated list of parasites (Isopoda, Copepoda, Monogenea, Digenea, Cestoda and Nematoda) collected in groupers (Serranidae, Epinephelinae) in New Caledonia emphasizes parasite biodiversity in coral reef fish. *Folia Parasitol* 57:237-262
- Justine J-L, Beveridge I, Boxshall GA, Bray RA, Moravec F, Whittington ID (2010b) An annotated list of fish parasites (Copepoda, Monogenea, Digenea, Cestoda and Nematoda) collected from Emperors and Emperor Bream (Lethrinidae) in New Caledonia further highlights parasite biodiversity estimates on coral reef fish. *Zootaxa* 2691:1-40
- Justine J-L, Grugeaud A (2010) Does the number of sclerotised structures used for the systematics of monogeneans change with age? A study of the monocotyloid *Dendromonocotyle pipinna*. *Parasitol Res* 107:1509-1514
- Kennedy MJ (1979) Basic methods of specimen preparation in parasitology. International Development Research Centre, Ottawa
- King JR, Porter SD (2004) Recommendations on the use of alcohols for preservation of ant specimens (Hymenoptera, Formicidae). *Insectes Soc* 51 (2):197-202. doi:10.1007/s00040-003-0709-x
- Klopfleisch R, Weiss ATA, Gruber AD (2011) Excavation of a buried treasure – DNA, mRNA, miRNA and protein analysis in formalin fixed, paraffin embedded tissues. *Histol Histopathol* 26:797-810
- Košková E, Matějusková I, Civiánová K, Koubková B (2011) Ethanol-fixed material used for both classical and molecular identification purposes: *Eudiplozoon nipponicum* (Monogenea: Diplozoidae) as a case parasite species. *Parasitol Res* 107 (4):909-914. doi:10.1007/s00436-010-1949-0

- Kritsky D, Bakenhaster M (2011) Monogenoidean parasites of the gill lamellae of the sheepshead *Archosargus probatocephalus* (Walbaum) (Perciformes: Sparidae) from the Indian River Lagoon, Florida, with descriptions of four new species of *Euryhaliotrema* Kritsky & Boeger, 2002 (Dactylogyridae). *Syst Parasitol* 78 (1):57-68. doi:10.1007/s11230-010-9282-7
- Kritsky DC, Fennessy CJ (1999) *Calicobenedenia polyprioni* n. gen., n. sp. (Monogenoidea: Capsalidae) from the external surfaces of wreckfish, *Polyprion americanus* (Teleostei: Polyprionidae), in the North Atlantic. *J Parasitol* 85:192-195
- Kritsky DC, Leiby PD, Kayton RJ (1978) A rapid stain technique for the haptor bars of *Gyrodactylus* species (Monogenea). *J Parasitol* 64:172-174
- Kuris AM, Hechinger RF, Shaw JC, Whitney KL, Aguirre-Macedo L, Boch CA, Dobson AP, Dunham EJ, Fredensborg BL, Huspeni TC, Lorda J, Mababa L, Mancini FT, Mora AB, Pickering M, Talhouk NL, Torchin ME, Lafferty KD (2008) Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature* 454 (7203):515-518. doi:10.1038/nature06970
- Lachenmeier DW, Rehm J, Gmel G (2007) Surrogate alcohol: What do we know and where do we go? *Alcohol Clin Exp Res* 31 (10):1613-1624. doi:10.1111/j.1530-0277.2007.00474.x
- Lafferty KD, Dobson AP, Kuris AM (2006) Parasites dominate food web links. *Proc Natl Acad Sci U S A* 103 (30):11211-11216. doi:10.1073/pnas.0604755103
- Lichtenfels J (1984) Methods for conserving, storing, and studying helminths in the U.S. National Parasite Collection. *Syst Parasitol* 6 (4):250-251. doi:10.1007/bf00012199
- Lim LHS (1991) Preparation of Museum specimens - Monogenea. *Fish Health Sect Newsletter* 2:10-11
- Maillard C, Gonzalez J, Noisy D (1982) A scanning electron microscope study of the male copulatory sclerite of the monogenean *Diplectanum aequans*. *Parasitology* 84:63-64
- Malmberg G (1957) Om förekomsten av *Gyrodactylus* på svenska fiskar (In Swedish.). *Skrifter Utgivna av Södra Sveriges Fiskeriförening, Årsskrift* 1956:19-76
- Manter HW (1926) Some North American fish Trematodes. *Illinois Biological Monographs*, 10. University of Illinois, Urbana
- McCue JF, Thorson RE (1963) A rapid method for collecting large numbers of intestinal helminths. *J Parasitol* 49 (6):997
- McDougal HD, Mizelle JD (1969) Studies on Monogenetic Trematodes. XLIV. A new method for collection of Monogenea. *Trans Am Microsc Soc* 88 (3):445-447
- Miller TL, Adlard RD, Bray RA, Justine J-L, Cribb TH (2010a) Cryptic species of *Euryakaina* n. g. (Digenea: Cryptogonimidae) from sympatric lutjanids in the Indo-West Pacific. *Syst Parasitol* 77:185-204
- Miller TL, Bray RA, Goiran C, Justine J-L, Cribb TH (2009) *Adlardia novaecaledoniae* n.g., n. sp. (Digenea: Cryptogonimidae) from the fork-tailed threadfin bream *Nemipterus furcosus* (Val.) (Perciformes: Nemipteridae) off New Caledonia. *Syst Parasitol* 73:151-160

- Miller TL, Bray RA, Justine J-L, Cribb TH (2010b) *Varialvus* gen. nov. (Digenea, Cryptogonimidae), from species of Lutjanidae (Perciformes) off the Great Barrier Reef, New Caledonia and the Maldives. *Acta Parasitol* 55 (4):327-339. doi:10.2478/s11686-010-0045-z
- Milne SJ, Avenant-Oldewage A (2006) The fluorescent detection of *Paradiplozoon* sp. (Monogenea: Diplozoidae) attachment clamps' sclerites and integumental proteins: research communication. *Onderstepoort J Vet Res* 73:149-152
- Mizelle JD (1936) New species of Trematodes from the gills of Illinois fishes. *Am Midl Nat* 17 (5):785-806
- Mizelle JD (1938) Comparative studies on Trematodes (Gyrodactyloidea) from the gills of North-American fresh-water fishes. *Illinois Biological Monographs*, 17(1). The University of Illinois Press, Urbana
- Mo TA, Appleby C (1990) A special technique for studying haptor sclerites of monogeneans. *Syst Parasitol* 17:103-108
- Monteiro C, Kritsky DC, Brasil-Sato M (2010) Neotropical Monogenoidea. 55. Dactylogyrids parasitising the pintado-amarelo *Pimelodus maculatus* Lacépède (Actinopterygii: Pimelodidae) from the Rio São Francisco, Brazil. *Syst Parasitol* 76 (3):179-190. doi:10.1007/s11230-010-9250-2
- Moravec F (1994) Parasitic nematodes of freshwater fishes of Europe. *Academia, Praha*
- Moravec F (2001) Trichinelloid nematodes parasitic in cold-blooded vertebrates. *Academia, Praha*
- Moravec F, Justine J-L (2005) Two species of *Philometra* (Nematoda, Philometridae) from serranid fishes off New Caledonia. *Acta Parasitol* 50 (4):323-331
- Moravec F, Justine J-L (2010a) Some trichinelloid nematodes from marine fishes off New Caledonia, including description of *Pseudocapillaria novaecaledoniensis* sp. nov. (Capillariidae). *Acta Parasitol* 55:71-80
- Moravec F, Justine J-L (2010b) Two new genera and species of cystidicolids (Nematoda, Cystidicolidae) from marine fishes off New Caledonia. *Parasitol Int* 59:198-205. doi:doi:10.1016/j.parint.2010.01.005
- Nadler S (1999) Nucleotide sequences from vintage helminths: fine wine or vinegar? *Parasitol Today* 15 (3):122
- Naem S, Pagan C, Nadler SA (2010) Structural restoration of nematodes and acanthocephalans fixed in high percentage alcohol using DESS solution and rehydration. *J Parasitol* 96:809-811
- Nagy Z (2010) A hands-on overview of tissue preservation methods for molecular genetic analyses. *Org Divers Evol* 10 (1):91-105. doi:10.1007/s13127-010-0012-4
- Newman LJ, Cannon LRG (1995) The importance of the fixation of color, pattern and form in tropical Pseudocerotidae (Platyhelminthes, Polycladida). *Hydrobiologia* 305 (1-3):141-143
- Nichols E, Gomez A (2011) Conservation education needs more parasites. *Biol Conserv* 144 (2):937-941. doi:10.1016/j.biocon.2010.10.025
- Olson PD, Cairra JN, Jensen K, Overstreet RM, Palm HW, Beveridge I (2010) Evolution of the trypanorhynch tapeworms: Parasite phylogeny supports independent lineages of sharks and rays. *Int J Parasitol* 40:223-242

- Pariselle A, Lambert A, Euzet L (1991) A new type of haptor in mesoparasitic monogeneans of the genus *Enterogyrus* Paperna, 1963, with a description of *Enterogyrus foratus* n. sp. and *E. coronatus* n. sp., stomach parasites of cichlids in West Africa. *Syst Parasitol* 20 (3):211-220. doi:10.1007/bf00009785
- Parker JH, Curran SS, Overstreet RM, Tkach VV (2010) Examination of *Homalometron elongatum* Manter, 1947 and description of a new congener from *Eucinostomus currani* Zahuranec, 1980 in the Pacific Ocean off Costa Rica. *Comp Parasitol* 77 (2):154-163
- Perkins EM, Donnellan SC, Bertozzi T, Chisholm LA, Whittington ID (2009) Looks can deceive: Molecular phylogeny of a family of flatworm ectoparasites (Monogenea: Capsalidae) does not reflect current morphological classification. *Mol Phylogenet Evol* 52:705-714. doi:doi:10.1016/j.ympev.2009.05.008
- Post RJ, Flook PK, Millest AL (1993) Methods for the preservation of insects for DNA studies. *Biochem Syst Ecol* 21 (1):85-92
- Poulin R (2004) Parasite species richness in New Zealand fishes: a grossly underestimated component of biodiversity? *Divers Distrib* 10:31-37
- Poulin R, Leung TLF (2010) Taxonomic resolution in parasite community studies: are things getting worse? *Parasitology* 137:1967-1973. doi:doi:10.1017/S0031182010000910
- Poulin R, Morand S (2000) The diversity of parasites. *Q Rev Biol* 75 (3):277-293
- Poulin R, Morand S (2004) *Parasite Biodiversity*. Smithsonian Books, Washington
- Pritchard MH, Kruse GOW (1982) *The collection and preservation of animal parasites*. University of Nebraska Press, Lincoln and London
- Quicke DLJ, Lopez-Vaamonde C, Belshaw R (1999) Preservation of hymenopteran specimens for subsequent molecular and morphological study. *Zool Scr* 28 (1-2):261-267. doi:10.1046/j.1463-6409.1999.00004.x
- Richards GR, Chubb JC (1995) Trichrome staining of *Gyrodactylus* sclerites and soft tissues following fixation in ammonium picrate-glycerin, including an improved rendition of the haptoral bars of *G. tumbulli*. *J Helminthol* 69:149-154
- Rogers WA (1966) Three new species of *Pseudomurraytrema* (Trematoda: Monogenea) from gills of catostomid fishes. *J Parasitol* 52 (3):462-465
- Rohde K (1987) Different populations of *Scomber australasicus* in New Zealand and south-eastern Australia, demonstrated by a simple method using monogenean sclerites. *J Fish Biol* 30 (6):651-657. doi:10.1111/j.1095-8649.1987.tb05794.x
- Rohde K (2001) Marine parasite diversity and environmental gradients. In: Levin S (ed) *Encyclopedia of Biodiversity*, vol 1. Academic Press, New York, pp 73-88
- Rohde K, Watson N (1985) Morphology, microhabitats and geographical variation of *Kuhnia* spp. (Monogenea: Polyopisthocotylea). *Int J Parasitol* 15 (5):569-586
- Schmidt GD (1986) *CRC Handbook of tapeworm identification. vol Platyhelminthes Cestoda Systématique*. CRC Press, Boca Raton, Florida

- Shinn AP, Collins C, Garcia-Vasquez A, Snow M, Matejusova I, Paladini G, Longshaw M, Lindenstrøm T, Stone DM, Turnbull JF, Picon-Camacho SM, Rivera CV, Duguid RA, Mo TA, Hansen H, Olstad K, Cable J, Harris PD, Kerr R, Graham D, Monaghan SJ, Yoon GH, Buchmann K, Taylor NGH, Bakke TA, Raynard R, Irving S, Bron JE (2010) Multi-centre testing and validation of current protocols for the identification of *Gyrodactylus salaris* (Monogenea). *Int J Parasitol* 40 (12):1455-1467
- Shinn AP, Gibson DI, Sommerville C (1993) An SEM study of the haptor sclerites of the genus *Gyrodactylus* Nordmann, 1832 (Monogenea) following extraction by digestion and sonication techniques. *Syst Parasitol* 25 (2):135-144. doi:10.1007/bf00009983
- Simsek S, Kaplan M, Ozercan I (2011) A comprehensive molecular survey of *Echinococcus granulosus* in formalin-fixed paraffin-embedded tissues in human isolates in Turkey. *Parasitol Int* 109 (2):411-416. doi:10.1007/s00436-011-2269-8
- Snyder SD, Clopton RE (2005) New methods for the collection and preservation of spirorchiid trematodes and polystomatid monogeneans from turtles. *Comp Parasitol* 72:102-107
- Srinivasan M, Sedmak D, Jewell S (2002) Effect of fixatives and tissue processing on the content and integrity of nucleic acids. *Am J Pathol* 161 (6):1961-1971
- Strona G, Stefani F, Galli P (2009) Field preservation of monogenean parasites for molecular and morphological analyses. *Parasitol Int* 58 (1):51-54
- Thatcher VE (2006) Amazon Fish Parasites. ABLA Series, Vol. 1. Aquatic Biodiversity of Latin America (ABLA Series). Pensoft, Sofia, Bulgaria
- Toe L, Back C, Adjami AG, Tang JM, Unnasch TR (1997) *Onchocerca volvulus*: comparison of field collection methods for the preservation of parasite and vector samples for PCR analysis. *Bull W H O* 75 (5):443-447
- Unnithan RV (1957) On the functional morphology of a new fauna of Monogenea on fishes from Trivandrum and environs. Part I, Axinidae Fam. nov. *Bull Cent Res Inst Univ Kerala Ser. C*, 5:27-122
- Van Beneden P-J (1878) Les commensaux et les parasites dans le règne animal. Librairie Germer Baillière et Cie, Paris
- Van Beneden P-J (1887) Animal parasites and messmates. Appleton, New York
- Vannier-Santos MA, Lenzi HL (2011) Parasites or cohabitants: cruel omnipresent usurpers or creative "éminences grises"? *J Parasitol Res* 2011, id 214174, 19 pages. doi:10.1155/2011/214174
- Vickerman K (2009) "Not a very nice subject." Changing views of parasites and parasitology in the twentieth century. *Parasitology* 136 (12):1395-1402. doi:10.1017/s0031182009990825
- Whittington ID, Chisholm LA (2003) Biodiversity of marine parasites in Australia: More than just a list of largely invisible creatures. *Rec S Aust Mus Monogr Ser* 7:51-60
- Williams EHJ, Bunkley-Williams L, Dowgiallo MJ, Dyer WG (1991) Influence of collection methods on the occurrence of alimentary canal helminth parasites in fish. *J Parasitol* 77:1019-1022

- Windsor DA (1995) Equal rights for parasites. *Conserv Biol* 9 (1):1-2. doi:10.1046/j.1523-1739.1995.09010001.x
- Windsor DA (1998) Most of species on Earth are parasites. *Int J Parasitol* 28:1939-1941
- Wong WL, Tan WB, Lim LHS (2006) Sodium dodecyl sulphate as a rapid clearing agent for studying the hard parts of monogeneans and nematodes. *J Helminthol* 80:87-90. doi:doi:10.1079/JOH2005320
- Yamaguti S (1965a) New digenetic trematodes from Hawaiian fishes, I. *Pac Sci* 19:458-481
- Yamaguti S (1965b) Preparation of stained whole mounts of flatworms. *Trans Am Microsc Soc* 84:602-603
- Yamaguti S (1968) *Monogenetic Trematodes of Hawaiian Fishes*. University of Hawaii Press, Honolulu
- Yoder M, De Ley IT, King IW, Mundo-Ocampo M, Mann J, Blaxter M, Poiras L, De Ley P (2006) DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. *Nematology* 8:367-376

## Figure Legends

### **Figure 1: Collecting helminths from the gastrointestinal tract.**

- A. Open fish from anus.
- B. Open abdominal cavity and extract all organs. Discard liver.
- C. Moisten organs with saline.
- D. Open stomach longitudinally.
- E. Open entire intestine longitudinally.
- F. Drop entire digestive system in a container and close it.
- G. Vigorously shake container. Allow decantation for 1-2 minutes.
- H. Discard upper part. Repeat until remaining liquid is transparent.
- I. Add near-boiling water. Discard most of it.
- J. Fill vial with ethanol.
- K. Add internal label in vial.

### **Figure 2. Collecting gills with helminths and other parasites.**

- A, B. Cut each gill at both extremities.
- C. Collect gills in sea water (for sea fish) or tap water (for freshwater fish).
- D. Put gills in container and add near-boiling water.
- E. Discard most water.
- F. Fill vial with ethanol. Add internal label in vial.



