

FRASER RIVER ACTION PLAN



Protocols for
Reference and
Voucher Collections
of Aquatic
Invertebrates Stored
at the Royal British
Columbia Museum

Canada

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**Protocols for Reference and Voucher Collections
of Aquatic Invertebrates Stored at the
Royal British Columbia Museum**

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ABSTRACT

Protocols for Reference and Voucher Collections of Aquatic Invertebrates Stored at the Royal British Columbia Museum

Through the Fraser River Action Plan, Environment Canada provided initial funding to facilitate the deposition of aquatic invertebrate voucher specimens at the Royal British Columbia Museum.

The Museum will house and maintain the specimens and record them on a database. This report presents detailed protocols for handling, labelling and documenting aquatic invertebrate specimens from the time of collection to the time of arrival at the Royal British Columbia Museum.

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I. INTRODUCTION

During ecological, environmental and taxonomic studies, biological specimens are collected and identified. These identifications are used in subsequent analyses and reports to make certain conclusions. If these identifications are incorrect, false conclusions can be made. If results of future studies disagree with those of the initial study, the initial results can be verified only if representative specimens are available for re-examination. The information contained in these specimens is irreplaceable, because we cannot go back in time and re-sample. It is, therefore, imperative that in any study whose conclusions depend on the identification of biological specimens, provisions are made for the deposition of voucher specimens, preferably in a recognized museum with facilities for maintenance of biological collections. This is especially important in studies dealing with small aquatic invertebrates, which include many groups whose taxa are poorly known.

In recognition of the need to retain voucher collections, Environment Canada, through the Fraser River Action Plan, has provided funding for the deposition of voucher collections of aquatic invertebrates at the Royal British Columbia Museum (RBCM).

In British Columbia, the RBCM is the best location for the deposition of voucher collections because it has:

- 1) expertise to ensure collections and data are housed and maintained to museum standards;
- 2) an established system of storing, tracking, retrieving and lending specimens and associated data;
- 3) a database for electronic storage and retrieval of collection data for specimens in the RBCM collection, with links to the Canadian Heritage Information Network, a national database of museum and other collections;
- 4) taxonomic expertise;
- 5) availability of specimens and data to a wide arena of researchers and other interested parties; and
- 6) an established, peer-recognized reference collection.

This report describes procedures for handling, labelling and documenting aquatic invertebrate specimens from the time of collection to the time of arrival at the RBCM.

II. PROTOCOL FOR INVERTEBRATE COLLECTIONS

A. FIELD PROCEDURES

1. Anaesthetizing

Soft-bodied invertebrates should be routinely anaesthetized before fixing in formalin to prevent contraction and distortion of the specimens, making them difficult to identify. Many methods and chemicals may be used. Four methods routinely used at the RBCM are:

- a) Animals are relaxed by immersion in a 0.15% solution of propylene phenoxylol. For field collecting, a stock solution of 1.5% propylene phenoxylol is prepared by mixing 15 ml with one litre of warm tap water. One part of this solution is then mixed with nine parts of habitat water to produce a 0.15% solution in which the animals are placed.
- b) Menthol crystals sprinkled on the surface of the water can be used for some organisms, such as holothurians.
- c) Magnesium chloride is used at a concentration of 75 gm/litre of tap water, mixed with equal parts of habitat water. This generally requires a larger volume of relaxant at a higher concentration than propylene phenoxylol, so is not as convenient.
- d) Marine crustaceans can be anaesthetized by placing them in a container of seawater and allowing the water to warm to room temperature. This method does not work as well for freshwater crustaceans, which can often survive in warm water.

Generally for each major invertebrate taxon there is a method of anaesthetizing that works best. Collectors are advised to consult Knudsen (1966) or other references on biological techniques.

2. Fixation

The standard fixative is neutralized 10% formalin (approximately 4% formaldehyde). If large amounts of water or organic material are contained in the sample, 20% formalin may be required for proper fixation. Formalin should be neutralized with borax (sodium borate) at a concentration of approximately 15 ml per litre of 10% formalin. Check pH with litmus paper or pH meter and adjust to a pH of 8 or higher. Due to the caustic nature of formalin great care should be taken in transporting and using it. All containers used to store preservatives must have WHMIS (Workplace Hazardous Materials Information System) labels to identify contents and hazards. On ships or in vehicles care should be taken to

prevent breakage or leakage (see Transport of Hazardous Goods Regulations for procedures and maximum amounts). When no fume hood is available (as in the field) it is preferable to store, transfer and mix this chemical outside in the fresh air. Avoid breathing the fumes.

Sponges should be fixed in 70% ethanol, because formalin will dissolve silicious spicules important for identification. Aquatic insects may also be fixed in 70% ethanol if preferred.

The length of time that specimens take to fix depends on the size of the specimen: larger animals take longer to fix than small ones. As a general rule, leaving specimens in formalin for a week is sufficient to fix them. Smaller specimens may take less time and very large animals should be left longer.

3. Field Data

Data recorded should include at least the following information: detailed location, latitude and longitude (in degrees and minutes to two decimal places) or UTM, date, time, collector, depth of collection, substrate and method of collection. Other useful data that should be recorded, if possible, are water temperature, pH, salinity, conductivity and current speed. Appendix 1 contains an example of a RBCM voucher collection data sheet.

A unique field number should be devised to link specimens and field notes. The data for the site are recorded in a field note book under that field number. At the RBCM each curator uses a field number comprised of the first letter of his or her last name followed by the year, and the next consecutive collection number. For example, L990-5 denotes Lambert 1990 - 5th collection site. Each field number would have a set of station data and some notes on what was collected at that site. A collection is defined as a sample from one location at a specific time. In other words, specimens collected from the same site but on different days are considered different collections.

Field labels should be written on waterproof paper (such as Permafibre) in India ink or pencil. Do not use ballpoint or other kinds of ink which can run and disappear with time. Labels should be placed directly in the fluid with the specimen. ~~Do not~~ attach the label to the outside of the jar. Each label should contain a field number, date and location as a minimum. Ideally, put down complete location data. There are many examples of valuable specimens being rendered useless by lack of data in the jar and no paper documentation to go with them. If a mistake is made on the label, having several pieces of information allows you to cross check and pick out erroneous data. Appendix 2 contains samples of RBCM labels.

If time permits sort specimens to the lowest possible taxonomic level and place in jars of appropriate size. Specimens should be packed loosely so that the fixative can circulate freely around the specimens. If field work involves travelling by truck on bumpy roads, it

is advisable to use plastic containers to avoid possible breakage of glass. However, there is a trade-off: glass jars often seal better and the contents can be viewed more easily.

B. LABORATORY PROCEDURES

1. Sorting

Specimens in formalin should be transferred into 60% isopropanol as soon as possible upon return from the field. Aquatic insects should be transferred to 70% ethanol; if insects were originally fixed in 70% ethanol it should be changed at this time. Pour off the formalin under a fume hood and into a container for recycling. When changing any solution, use a sieve to prevent accidental loss of any specimens.

If not already sorted, all the specimens from a single collection locality should be divided into lots and placed in glass jars. A lot consists of one or more specimens of one species from a single locality. The jar should be large enough so that the specimens are not too crowded to allow the preservative to circulate. The minimum size jar is 4 oz. (100 ml). Tiny specimens should be placed in a screw-cap glass vial and then placed in a larger jar filled with the same preservative. The data label should go in the vial with the specimen.

It is important that every jar has a label that is either a duplicate of the field label or a final specimen label. With invertebrates it is not always practical to have everything identified to species, in some cases identification to genus or family is sufficient for the study in question.

Voucher specimens deposited at the RBCM should be sorted into separate containers according to **the taxonomic level identified in the report**. One vial or jar should be submitted for each taxon reported in the study. If identified to species **each vial or jar should contain only one species from one locality**. It is helpful to have more than one specimen of each species. All containers must be accurately labelled.

Field notes and a copy of any reports based on the voucher specimens should be submitted with the specimens.

Microscope slides may be submitted as vouchers providing they are permanent mounts and the specimen can be identified from the slide.

The identifier and date of identification for each lot must be recorded on the specimen label.

2. Containers

Larger alcohol-preserved specimens should be placed in wide-mouth, clear glass jars, large enough that the sample/specimen occupies no more than 50% of the volume (minimum size of 4 oz [100 ml]). The lid should be made of polypropylene and have a round polypropylene gasket placed under the lid to reduce evaporation. Metal or bakelite lids or lids with paper or cardboard liners must not be used.

Small specimens, such as copepods or cladocerans, should be stored in vials (minimum size 2 drams). Use only screw-cap vials with polyethylene cone inserts to prevent evaporation of the preservative. For long term storage, vials should be put in a larger jar containing the same preservative and concentration to prevent evaporation of preservative from the vial.

C. TRANSPORTATION

Prior to specimen shipment to the RBCM arrangements should be made with the Registrar, Biological Collections (604-387-1216) for their receipt. Please note that the federal Transport of Dangerous Goods Act regulates the shipping of formalin, ethanol and isopropanol. The Registrar can recommend shipping companies able to handle dangerous goods. Specimens or lots of specimens should be shipped in adequately padded wooden, plastic or metal shipping containers. These containers will be returned to the shipper if requested. Packaging of the specimens must be sufficient to prevent damage to the specimens and leakage of preservative during normal handling.

D. PROTOCOL SUMMARY

1. Anaesthetize soft-bodied specimens.
2. Fix in 10% neutralized formalin (70% ethanol for sponges), in the field.
3. Record complete collection data in a field book. Put a field label into the jar with at least the field number, location and date.
4. When completely fixed, transfer specimens to 60% isopropanol (most invertebrates) or 70% ethanol (aquatic insects and sponges).
5. Voucher collections sent to the RBCM should have **one species from one location at one time per jar or vial**. One jar or vial of each species is sufficient for voucher purposes. It is preferable, especially in the case of very small organisms, to submit more than one specimen of each species.
6. Specimens must be identified to at least the taxonomic level used in the report and be in good condition to allow for reidentification, if necessary. An appropriate label must be placed **inside** the container or on the slide, if slide mounts are used.
7. All data and any reports associated with the specimens should be sent to the RBCM with the specimens.
8. Contact the Biological Collections Registrar (604-387-1216) prior to the shipping of specimens. Contact the Chief of Biological Collections (604-387-3544) with any questions about supplies required for the preparation of aquatic invertebrates stored at the Royal British Columbia Museum.

References

- Knudsen J.W. 1966. Biological Techniques: Collecting, Preserving, and Illustrating Plants and Animals. Harper and Row, New York, 525 pp.

APPENDIX 1

Example of a RBCM voucher collection data sheet.

ROYAL BRITISH COLUMBIA MUSEUM
COLLECTION DATA

Field Collection No. Date.....

Location.....

.....

Latitude..... Longitude.....

Tide Height..... Time.....

Distance offshore..... Current.....

Capture depth..... Water depth.....

Method of collection.....

Vegetation.....

Substrate.....

Weather..... Water temperature..... ° C.

Salinity..... 0/00 Conductivity..... μ S/cm

Fixative..... Preservative.....

Collected by.....

COMMENTS:

ROYAL BRITISH COLUMBIA MUSEUM
COLLECTION DATA

Field Collection No. Date.....

Location.....

.....

Latitude..... Longitude.....

Tide Height..... Time.....

Distance offshore..... Current.....

Capture depth..... Water depth.....

Method of collection.....

Vegetation.....

Substrate.....

Weather..... Water temperature..... ° C.

Salinity..... 0/00 Conductivity..... μ S/cm

Fixative..... Preservative.....

Collected by.....

COMMENTS:.....

APPENDIX 2

Two Examples of sample bottle labels.

ROYAL BC MUSEUM

FIELDNO: DATE:
SPECIES:
LOCALITY:

COLLECTOR:
DEPTH: DATE:
DET:

LABELS
FOR LARGE
CONTAINERS

ROYAL BC MUSEUM

FIELDNO: DATE:
SPECIES:
LOCALITY:

COLLECTOR:
DEPTH: DATE:
DET:

ROYAL BC MUSEUM

FIELDNO: DATE:
SPECIES:
LOCALITY:

COLLECTOR:
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ROYAL BC MUSEUM

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DEPTH: DATE:
DET:

Labels for Small Containers

The back side of page presents the back of the label.

