Errata to *Fish Collection Methods and Standards, Version 4.0, RIC 1997*

1. Replace all references to *Reconnaissance (1:20 000) Fish and Fish Habitat Inventory: Standards and Procedures, RIC draft (1997*) with "*Reconnaissance (1:20 000) Fish and Fish Habitat Inventory: Standards and Procedures, RIC (1998*)."
2. The *Fish Collection Form and User Notes* in *Appendix A* - have been deleted. Replace all references to the Fish Collection Form and the associated User notes with "See *Reconnaissance (1:20 000) Fish and Fish Habitat Inventory: Data Forms and User Notes, RIC (1998)."* Appendix A now contains only *The Fish Species Codes for BC*.
3. Replace "All voucher specimens must be submitted in 50% isopropyl alcohol in the prescribed jars" with "All voucher specimens must be submitted in 40% isopropyl alcohol in the prescribed jars" in the first paragraph of section 7. *Fish Preservation Techniques.*
4. Replace the existing section 7.1 (under *Fish Preservation Techniques)* with the attached updated *version.*
5. Add "Standard procedures for specimen fixation and preservation for histology purposes can be obtained from Sally Goldes, BC Fisheries." at the end of the second paragraph of section *7.3 Collection and Preservation of Parasites and Other Health Related Specimens.*
6. Replace Sally Goldes' mailing address in section *7.3 Collection and Preservation of Parasites and Other Health Related Specimens* with the following:

   Sally Goldes
   Fish Health Unit
   BC Ministry of Fisheries
   2080-A Labieux Road
   Nanaimo, BC V9T 6J9

   Phone: (250) 751-3120
   Fax: (250) 751-3103
7. Add in Appendix A - *The Fish Species Codes for BC,* the following as a separate table, placed above the species code table:

GENERAL CODE	DESCRIPTION
NFC	No fish caught
SP	Unidentified species

8. Add in Appendix A - *The Fish Species Codes for BC,* the following as a separate table and notes, placed above the species code table and below the Table described in #7.

   "The following codes have been added to the Fish Species Code list to cover a) specific situations where identification to the species level is not possible, and b) fish that have been identified and verified as hybrids.

CODE	DESCRIPTION
DV/BT	Fish are either DV or BT, but suspect they are DV
BT/DV	Fish are either DV or BT, but suspect they are BT
RB/CT	Fish are either RB or CT, but suspect they are RB
CT/RB	Fish are either RB or CT, but suspect they are CT
TR	Fish are unidentifiable trout – only to be used for fry (< 70 mm in length)
DVxBT	Verified DV BT crosses. For other verified hybrids, contact the Ministry for appropriate codes

7. FISH PRESERVATION TECHNIQUES

Careful and correct preservation procedures in both the field and laboratory are important for ensuring the quality of the collected specimens or tissues. Fixatives of the correct concentration, appropriate containers, clean and sharp dissecting tools, waterproof data form/labels, and complete observations will all affect the quality and value of the sampling. Preservation techniques vary depending on how the samples will be used. All voucher specimens must be submitted in 40% isopropyl alcohol in the prescribed jars. The following sections outline some of the most common techniques, and describe the Ministry's requirement regarding the submission of samples.

7.1 Voucher Specimens

Voucher specimens are representative samples of species identified in the field, collected, and preserved to verify the field identification. **Only one representative sample of each red/blue listed/blue-listed species should be collected** (*see* Table 2). For species that are neither rare nor endangered, two to three specimens can be collected. (*Also see: Reconnaissance (1:20 000) Fish and Fish Habitat Inventory: Standards and Procedures, RIC draft (1998*). These specimens should represent the size variability encountered at the sampling site. Any mortality that occurs during fish capture for sampling can be submitted as voucher specimens.

7.1.1 Preservation

7.1.1.1 Anaesthetizing to kill

All fish must be euthanized before fixation by leaving them in high doses of the anaesthetizing solution. This is an ethical treatment of a live animal and serves a scientific purpose: anaesthetized fish relax and can be preserved in a more natural state. Once the opercular movements cease the fish can be considered dead.

7.1.1.2 Fixatives

- Fish must be fixed immediately after death to prevent tissue decomposition. The section describes some fixatives commonly used for fish voucher specimens.
- **Formalin** is commonly used to preserve collected specimens and it is available in liquid or powder forms *(Full strength liquid formalin is actually 37% formaldehyde dissolved in water)*. It is recommended that a solution of 10% formalin be used for the preservation of fish specimens. Formalin is slightly acidic and will de-calcify and soften bony structures. The addition of a buffering agent helps to retard this process. To make a 10% solution of **buffered formalin**, combine, by volume, 1 part full strength formalin with 9 parts distilled water and add approximately 3 ml of borax (buffering agent) per litre of solution (McAllister, 1965). Fish preserved in formalin will change in weight and length over time.
- **Paraformaldehyde**, a polymer of formaldehyde, can also be used to make a 10 % buffered formalin solution. The powder has the advantage of being relatively lightweight and easily transportable. A mixture of 16 g of paraformaldehyde and 4 g of anhydrous sodium

carbonate, with a small amount of Alconox (wetting agent) is prepared. 20 g of the powder mixture dissolved in 400 ml of distilled water produces a 10% buffered formalin solution (McAllister, 1965).

- **Alcohols**, such as ethanol and iso-propanol, are also used to fix and preserve fish specimens, especially if skeletal structures such as otoliths are to be examined. Alcohol is an excellent preservative but is not recommended for fixation of soft tissues.

- An alternative for preserving specimens is to quickly **freeze** them in dry ice or liquid nitrogen. Though freezing is not a recommended technique of choice for specimen preservation, it is one of the best methods to preserve the colours and tissues of the specimen. Samples must remain frozen until they arrive at the laboratory and can be permanently preserved. Fish frozen without initial preservation tend to fall apart when thawed. Partly thawing the specimen in 10% formalin solution is an option. However, frozen tissue can be used for genetic sampling. Logistically, freezing specimens may be hard to accomplish and maintain, especially on long field surveys.

- **Disposal of Formalin**

  Formalin must be oxidized to formic acid before disposal in the sanitary sewer as an aqueous waste. Protective gloves, clothes, and eye protection are mandatory when working with formalin. Slowly add while stirring, diluted formalin (1 ml of formalin to 10 mls of water) to an excess of household bleach (25 mls of household bleach for each ml of formalin). Stir for 20 minutes and then wash the solution into the drain with at least 50 times its volume of water. (This procedure is from Armour, Browne and Weir in “Hazardous Chemical Information and Disposal Guide," Dept. of Chemistry, University of Alberta.)

7.1.1.3 Labelling specimen

Two labels are needed for the specimen: a waterproof Specimen Label attached to the jaw or inserted into the mouth or opercular area of each specimen, and a waterproof Data Label on the outside of each jar (Figure 11). All labels must be written in pencil.

The **specimen label** contains the **fish identification number, species name** of the fish and the **collection method code**. The **data label** includes the **gazetted name, alias, watershed code, watershed/waterbody identifier** (if applicable), **reach number, site number**, **number of specimen submitted (No.)**, **collection date**, **crew’s name**(s), and a **contact phone number. F**or details on data label fields consult the Fish Collection Form User notes in the *Reconnaissance (1:20 000) Fish and Fish Habitat Inventory: Data Forms and User Notes, RIC (1998*). A brief description of the habitat and catch may also be included. The data label for a sample must be referenced to the field notes or data forms. The notes or forms will include the information recorded on the label but will also contain detailed information on habitat, size of sampling site, weather, sampling methods, etc.

Fish Voucher Specimen Data Label	
Gazetted name:	Alias:
Watershed code:	
Watershed/waterbody identifier:	
Reach no.: -	Site no: Date: - -
Species:	No.:
Crew:	Phone #:
Comments:	

Specimen label	Fish ID no.:
	Species name:
	Collection Mtd.:

Figure 11. Example of data and specimen label to be included in the jar with preserved specimens

Once the fixative has been added, the jar is sealed with Parafilm® (American National Can™), secured with plastic screw-type lids and placed on its side. This prevents curling of the specimen's body and abduction of the fins. For proper fixation, the animal should be immersed in the fixative for several days. Optimum fixation times for fish less than six inches in length is one to two weeks. For fish over six inches in length preservation time ranges from two to four weeks. The longest possible fixation time should be allowed as inadequately preserved specimens deteriorate rapidly.

7.1.1.4 Fixation Procedures

Specimens should be fixed soon after collection to limit deterioration of the tissues. All specimen must be **killed (**see **7.1.1)** before fixation. To fix the specimen, place it in a wide-mouthed glass jar, and fill with the fixative solution. Polypropylene lids with polyfoam liners must be used. (One suggested distributor is Ryco Packing (206) 872-0858 in Kent, Washington.) Specimens should be inserted into jars head first to make them easier to remove from the jars in the laboratory. Different species captured in the same set can be fixed and stored together. Though more than one animal can be fixed in the same jar, care must be taken not to pack too many fish in one jar.

The fish must be preserved in as natural a state as possible. Where possible, the specimen should float freely in the jar to avoid curling or bending. Before immersing large specimens, fixative should be injected directly into the body cavity to facilitate penetration and preservation of the internal organs. If syringes are not available, an incision can be made to the right of the ventral line to allow penetration of the fixative into the body cavity. The stomach should also be incised for internal fixation in order to prevent rotting due to

digestive juices. Care should be taken when making the incision to avoid damaging the internal organs.

7.1.1.5 Rinsing and Preserving Procedures

All voucher specimens must be submitted to the Ministry or to the Fish Identification agencies in 40% isopropyl alcohol. After fixation in formalin, the specimens must be thoroughly rinsed before preserving them in alcohol. Decant the formalin and place the jar under a slow, steady stream of running water for 1-2 days. If running water is unavailable, fill the jar with water. After several hours, decant and add fresh water. Repeat this procedure for several days until no formalin odour can be detected. If during rinsing the specimen shows signs of deterioration, transfer it directly to 40% isopropyl alcohol.

Formalin must be denatured before it is disposed, as described in section **7.1.1.3**.