

## TISSUE COLLECTING, PREPARATION AND PRESERVATION PROTOCOLS FOR THE KU BIODIVERSITY INSTITUTE DIVISION OF ICHTHYOLOGY

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### Pre-collection

In order to ensure that we are not duplicating effort and wasting valuable freezer space, please obtain a list of tissues already in the tissue collection from the relevant collecting area or taxonomic group before collecting. The collection can be searched online by going to <http://ichthyology.biodiversity.ku.edu/> and clicking on the tissue database link in the right hand menu. Be aware however, that some species duplication is a necessary part of the tissue collection and is scientifically valuable e.g. differing geographical areas, differing dates of collection etc. If you are unsure please consult either the collection manager or curator.

If at all possible, obtain tissue numbers in advance (tags printed from thermal transfer printer obtainable from Collection Manager together with strings and hole punch for voucher tags). This will make incorporation into the collection that much easier and ties between tissues and vouchers that much more visible.

Detailed field notes for all vouchers collected should be taken. Information that should be taken includes field number, date, collector's names, detailed collecting locality including country, state/province, distance to nearest town/place and latitude and longitude in decimal degrees.

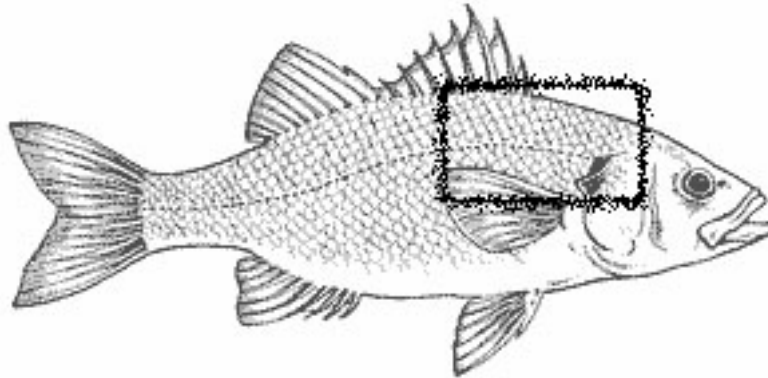
Physical voucher specimens should be collected along with tissues to allow for species identification after the fact. If physical voucher specimens are not possible then good quality digital photo's of vouchers should be taken (from multiple angles if necessary but at least a lateral left side view) and lodged with the collection. In some instances (particularly for small specimens where a whole specimen is needed for tissue) "pseudo-vouchers" may also be used where additional specimens of the same species collected at the same site may be used as vouchers. In these cases positive identification of species should be ensured.

If possible, photo's (either digital or film) should be taken of ALL vouchers before dissection so that life-like colors of specimens can be preserved. These images will be linked within the database to the tissue and voucher. Photo's should be taken of the left hand side of the fish with the head facing to the left on a blank background (uniform color) with some indication of tissue number as well as scale evident.



### Collection of tissues

Tissues should be taken from **two specimens of each species** to serve as duplicates per species. These specimens should preferably be the two largest specimens of the species collected. Specimens should be **kept cold** on ice or frozen in a freezer if there is going to be any delay between capture and tissue taking. **Gloves** are to be worn during the entire process to ensure sterile conditions and prevent contamination of samples.



The most common area for removal of tissues is the **right shoulder** just behind and above the gill arch. It is here that the flesh is the thickest and easiest to extract. This leaves the left side of the fish intact for photography and subsequent identification verification. The fish should be **descaled** in the area from which tissues are to be taken. The **skin should then be wiped clean** with ethanol. This also has the added bonus effect of making the skin slightly firmer and thus easier to slice. Using a fresh or freshly cleaned (with 99% ethanol) scalpel blade cut **two to three pieces of tissue** (enough to fill half of a 2.0ml cryotube each) from flank muscle on the **left hand side of the fish**. These pieces act as duplicates per individual. Using the end of the scalpel blade or cleaned forceps, place tissue in cryotube and immediately fill with 99% ethanol. Seal the tube well. Ensure that scalpel is either replaced or wiped clean together with forceps (if used) with 99% ethanol between each individual. Write tissue number on side of tube and record tissue number, species name, field number and number of tubes (duplicates)

in field log. If no tissue number has been given, record field number, species name and tube number (1-3 for each duplicate) on the side of the tube. Place in cryobox.

If at all possible, do not use a whole individual as a tissue sample as this prohibits the retaining of a voucher for the specimen. In some cases, due to the size of some species it is virtually impossible to section the specimen. In these cases a whole specimen may be used as a tissue sample but a duplicate individual should be retained as a voucher for that tissue. Photo vouchers can also be taken in these cases.

### **Fixation and Preservation**

Follow the guidelines as prescribed in the Fish Collection and Preparation Protocols document for all voucher specimens.

### **Vouchers**

Vouchers are to be tagged with tissue numbers (through gill arch if possible and tied securely), injected (body cavity and large muscle volumes) with and placed in 10% formalin and lodged either with KU or another institution. The voucher numbers from other institutions should be made available to KU as soon as they have been cataloged.

### **Supplies**

Scalpels (KU Union – Excel Hobby scalpel and interchangeable blades in packs of 100)

Surgical gloves – Fisher Brand Nitrile (Fisher Catalog No. 19-050-221A-E (XS-XL))

Ethanol – KU Chemistry Stores or Wetlab

Formalin – KU Chemistry Stores or Wetlab

Squeeze bottles – Fisherbrand (Fisher Catalog No. 03-409-23B)

Cryotubes – Nalgene (Fisher Catalog No. 03-337-7W)

Cryoboxes – Nalgene (Fisher Catalog No. 15-350-107B)

Tags – obtainable from Collection manager, preprinted off thermal transfer printer

Cotton thread – WalMart - Aunt Lydia's Crochet 100% mercerized Cotton – 1000 yards

Waterproof notebook – KU Union 'Rite-in-the-Rain' All weather Transit Book

Liquipac drums