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# Histological Observation of Tissues

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Honghao Zhao<sup>1</sup>, Jasmine Chong<sup>2</sup>, Rong Tang<sup>1</sup>, Li Li<sup>1</sup>, Jianguo Xia<sup>2</sup>, Dapeng Li<sup>1</sup>

<sup>1</sup>College of Fisheries, Hubei Provincial Engineering Laboratory for Pond Aquaculture, National Demonstration Center for Experimental Aquaculture Education, Huazhong Agricultural University, Wuhan 430070, China;

<sup>2</sup>Institute of Parasitology, and Department of Animal Science, McGill University, Saint-Anne-de-Bellevue, QC H9X 3V9, Canada

Metabolomics Protocols & Workflows  
Tech. support email: [bbmisraccb@gmail.com](mailto:bbmisraccb@gmail.com)



Dapeng Li

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**We use this protocol and it's working**

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## Abstract

The different kinds of stained histological sections are widely used to detect and observe physiological and pathological changes in various tissues.

## Safety warnings

- ⚠ Picric acid does not cause any direct allergies, but it would be converted into an allergenic chemical after contacting with tissue.

## Before start

Bouin's fixative was formulated by saturated solution of picric acid (75 ml), 40% aqueous formaldehyde (25 ml), and glacial acetic acid (5 ml), kept at room temperature.

## Blood Samples Collection

- 1 Both white muscle and gonad tissues were taken from 250 tails per experimental group (AF/GF).

## Storage of muscle samples used for H&E staining

- 2 White muscle samples were taken from each fish in GF and AF experimental groups, and temporarily stored in tissue fixative..

## Storage of gonad samples used for H&E staining

- 3 500 gonad samples were also collected from every experimental fish and kept in Bouin's fixative in 2 ml plastic tubes at 4 °C.

## Storage of samples used for Oil red O staining

- 4 The back and abdominal muscle samples were immediately harvested and frozen in liquid nitrogen, then were transferred and preserved at -80 °C until Oil red O staining.

## Preparation for Samples Staining

- 5 Serial transverse 10 µm-thick sections of muscles and gonads were stained routinely with hematoxylin & eosin (H&E). To determine the presence of fat in the muscles, frozen muscle tissues were stained with Oil red O solution, which would color any fat contained in the muscle.

## Sex Determination

- 6 The sex of each grass carp was determined by the contour of the gonad and further results of the gonad tissue slice (Jensen and Shelton, 1983; Ke, 1975).

## Sample Grouping

- 7 The sex of the grass carp was determined by the contour of the gonad and further results of the gonad tissue slice. Based on the results of the sex determination, metabolomic analysis of muscle samples were divided into four test groups (n = 10): female fish of the grass feeding group (FGF), male fish of the grass feeding group



(MGF), female fish of the artificial diet group (FAF), as well as male fish of the artificial diet group (MAF).

## The observation of Muscular Characteristics

- 8 A total of 200 - 400 fibers of per experimental group fish were studied using a Leica MZ 6 microscope for their cross sectional area. A size limit for identifying fibers was set at fiber diameters  $\geq 10 \mu\text{m}$  as the optical resolution below this limit did not allow for sufficient identification and accuracy in the analyses.