Fish Histology

From Cells to Organs

Doaa M. Mokhtar

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Dedication

I dedicate this textbook and atlas to my love and my husband, Dr. Ahmed Ibrahim, PhD in Veterinary Surgery, Assiut University, Egypt, for his encouragement, time, and active support that made this work possible.

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LIST OF ABBREVIATIONS

AB	Alcian blue
ATP	adenosine triphosphate
AV	atrioventricular
BAT	biliary-arteriolar tract
BVT	biliary-venous tract
CNS	central nervous system
EGCs	eosinophilic granular cells
ELISA	enzyme-linked immunosorbent assay
GNRH	gonadotropin-releasing hormone
HE	hematoxylin and eosin
IgM	immunoglobulin M
MMCs	melanomacrophage centers
MMs	melanomacrophages
MRCs	mitochondria-rich cells
PAS	periodic acid–Schiff
PAS-AB	periodic acid–Schiff-alcian blue
PNS	peripheral nervous system
POFs	postovulatory follicles
PSC	pancreatic stellate cells
PVC	pavement cells
RCs	rodlet cells
RGL	relative gut length
VIP	vasoactive intestinal polypeptide
MT	Mallory triple stain
PAS-AB-HX	Periodic acid-Schiff-alcian blue-hematoxylin
PAS-HX	Periodic acid-Schiff-hematoxylin
RBCs	red blood cells
rER	rough endoplasmic reticulum
SEM	scanning electron microscopy
TB	Toluidine blue

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PREFACE

Histology is the discipline of biology that involves the microscopic examination of tissue sections in order to study their structure and correlate it with function. Histology can detect signs of disease not easily recognized on gross examination and can therefore be of interest in fish health supervision.

This book will provide the readers with the most contemporary and useful text possible. The book describes the most important recent developments in sciences of fish histology. I am also recognizing that the readers are faced with the tasks of learning an ever-increasing number of facts in an ever-decreasing period of time. Because of this, every attempt has been made to shorten the text wherever possible and to organize information in a way that will facilitate learning.

Fish constitute nearly 60% of all vertebrate species and are economically of major importance. The reporting of normal histology of fish tissues and organs serves as a foundation upon which to gather and build our ichthyopathology knowledge base.

The aim has been to present a general reference guide providing an extensive set of histological images of fishes. Although several studies treat histological aspects in relation to pathology, no recent synthesis on the normal histology of fish is available. Therefore, I believe that this textbook will be a main contribution to this field. The book is designed to provide students with a foundation in understanding and interpreting histologic and cytologic preparations and normal tissue components.

A text and colored atlas of histology of fish is designed for use by students and researchers, biologists, ichthyologists, fish farmers, veterinarians working in fisheries and, of course, by comparative histologists who want to learn more about the fish world. As a further aid to learning, numerous photomicrographs and electron micrographs amplify the text in addition to particular emphasis on diagrams and tables to summarize morphologic and functional features of cells, tissues, and organs.

All photomicrographs are original. Light microscopy has been illustrated with color photomicrographs. Tissue and organ samples chosen to illustrate this work have been selected from reared food fish, as well as from species in the aquarium and in the wild.

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First of all, thanks forever to ALLAH who is always with me.

For their help, advice, and/or support throughout months of dissections, cutting, staining, photographing, interpreting, writing, editing, and correcting of the present textbook and atlas, I would like to sincerely thank the following persons:

I express my sincere thanks and deep gratitude to Prof. Dr. A. H. S. Hassan, Prof. of Histology, Faculty of Veterinary Medicine, Assiut University, Egypt, for his continuous encouragement, valuable help, constructive criticism, advices during work, and scientific and untiring help that made this work possible.

I am indebted to Dr. Hanan H. Abd-ElHafeez, PhD in Histology, Faculty of Veterinary Medicine, Assiut University, Egypt, for giving me permission to use some micrograph and her valuable help, advices, and active support throughout the work.

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INTRODUCTION

It is recognized that a study of fish histology can provide a unifying background to physiology and pathology. In this textbook and atlas, the structure of tissues and organs of teleosts is described seeming to be helpful for both veterinary medical students and those interested in farm sciences. The information contained in this book emphasizes the relationships and concepts by which cell and tissue structures of fish are inextricably linked with their function. The book also describes the most recent development in the sciences of fish histology.

Fish histology from cells to organs cover the normal histology of six fish species. It provides original high quality photomicrographs, tables, updated terminology, and expanded information.

Fish histology from cells to organs begins with brief introduction into the histological techniques for fish sampling followed by an accurate up-to-date description of fish tissues, and finally I devote a chapter to each organ and organ systems in fish body. In addition, I place particular diagrams to illustrate the structure of organs and enhance the usefulness of the text.

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CHAPTER 1

INTRODUCTION TO HISTOTECHNIQUES AND FISH GROSS ANATOMY

CONTENTS

ABSTRACT

Six fish species studied in this book include: grass carp or white amur (*Ctenopharyngodon idella*), Nile catfish *(Clarias gariepinus)*, Nile tilapia (*Oreochromis niloticus*), redtail shark (*Epalzeorhynchos bicolor*), guppy (*Poecilia reticulata*), and molly fish (*Poecilia sphenops*). Immediately after death or euthanasia, the tissue or organ is cut into small pieces and fixed in Bouin's fluid for light microscopical studies. The fixed materials were further processed and sections were obtained at 3 µm and stained with Harris hematoxylin and eosin (HE). Hematoxylin stains the cell nucleus and other acidic structures in blue. In contrast, eosin stains the cytoplasmic proteins and a variety of extracellular structures from pink to red. Trichrome procedures help to differentiate collagen from muscle cells. In addition, Verhoff's and Weigert's elastica stains are ideal for elastic fibers. For histochemical staining, osmic acid reacts with fat to give a grey-black color; the periodic acid–Schiff (PAS) reaction and alcian blue (AB) identify varieties of glycosaminoglycans that are present in some tissues and cells. Grimelius silver impregnation displays some aspects of neuroendocrine cells. Iron HX and bromophenol blue are used to detect presence of proteins and Best's carmine for detection of glycogen. For semithin section and electron microscopic studies, tissues were fixed in a mixture of 3% paraformaldehyde–glutaraldehyde fixative. Then the samples were processed and semithin sections are cut at 1 µm thickness and stained with toluidine blue for light microscopy. Ultrathin sections were cut at 70 nm and were stained with uranyl acetate and lead citrate and examined by JEOL 100CX II transmission electron microscope. For scanning electron microscopy, the tissues are washed in 0.1 M cacodylate buffer and transferred to a 1% solution of tannic acid. The samples were processed and mounted on aluminum stubs and sputter-coated with gold/palladium. The specimens were examined with a JEOL JSM-5400 LV Scanning Electron Microscope. Pictures of gross anatomy of longitudinal and surface sections of whole fish were provided.

Six fish species studied in this book include; grass carp or white amur (*Ctenopharyngodon idella*), Nile catfish *(Clarias gariepinus*), Nile tilapia (*Oreochromis niloticus*), redtail shark (*Epalzeorhynchos bicolor*), guppy (*Poecilia reticulata*), and molly fish *(Poecilia sphenops*).

Grass carp or white amur (*Ctenopharyngodon idella*) (Fig. 1.1A) is a large cyprinid fish. They are native in large Asian rivers, such as Amur

River Basin in Russia and the West River in China. It is a fast growing herbivorous fish; it usually feeds on grass or other aquatic vegetations and can be grown together with other fish species.

Nile catfish (*Clarias gariepinus*) (Fig. 1.1B) is one of the most abundant and widely distributed fish in the Nile River. Catfish has a wide geographical spread, a high growth rate, resistant to handling and stress, and well appreciated in a wide number of African countries. It can be recognized by its long dorsal and anal fins, which gives it a rather eel-like appearance. The catfish is carnivorous in type, where tilapias are its most preferred food items especially the young ones followed by insects, crustaceans, and mollusks, respectively.

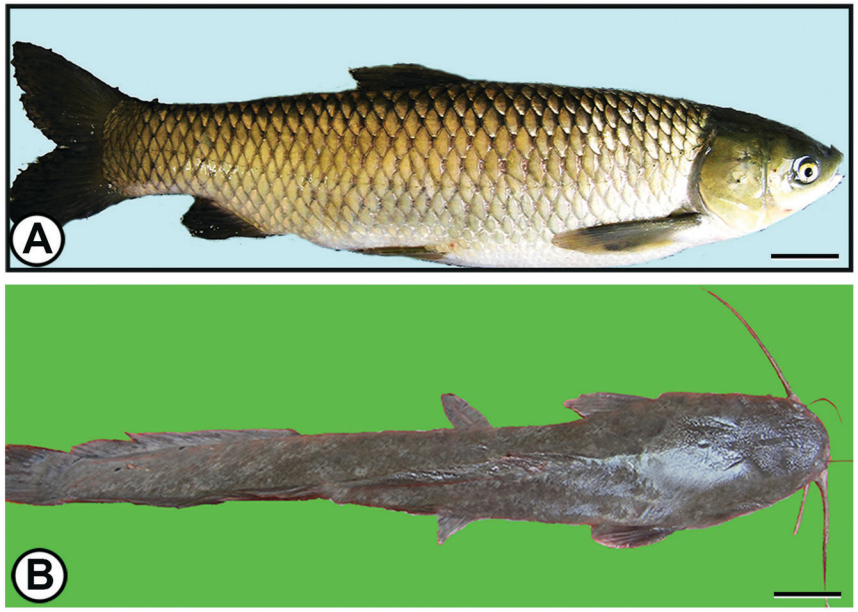

FIGURE 1.1 **(A)** The grass carp (*Ctenopharyngodon idella*). **(B)** Nile catfish (*Clarias gariepinus*).
(bars = 2cm)

Tilapia is a member of the family *Cichlidae*. Nile tilapia (*Oreochromis niloticus*) (Fig. 1.2A) is characterized by strong vertical black bands. This species is naturally distributed in Palestine, the Nile River as well as most parts of African Rivers and lakes. The *O. niloticus* is gonochoristic, with each individual possessing a single sexual phenotype. Nile tilapia is

characterized by extended spawning seasons, maturity at small size and a fast growth rate. It has been termed the aquatic chicken for its extraordinary production capabilities.

The guppy (*Poecilia reticulata*) (Fig. 1.2B), also known as million fish and rainbow fish is one of the most popular freshwater aquarium fish species. It is a member of the *Poeciliidae*, whose natural range is in South America, and is now found all over the world. The body of guppy is transparent and is covered with colorless scales and has ornamental dorsal and caudal fins. Guppies are used as a model organism in the field of ecology, evolution, and behavioral studies.

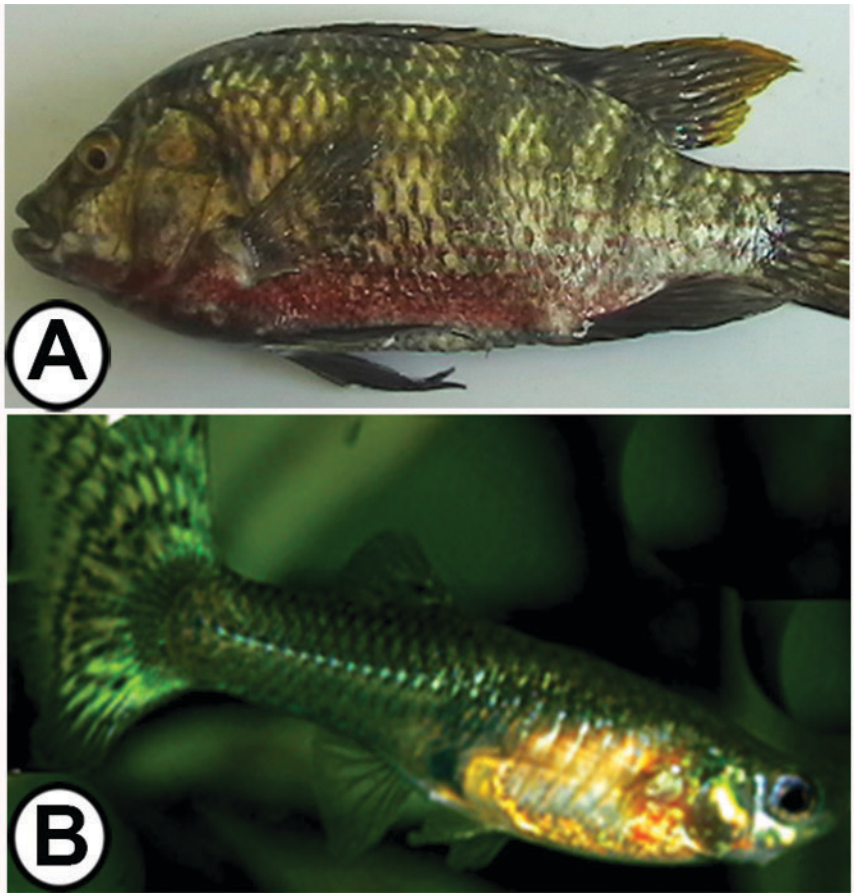

FIGURE 1.2 (A) Nile tilapia (*Oreochromis niloticus*). **(B)** The guppy (*Poecilia reticulata*).

Poecilia sphenops (Fig. 1.3A) is a species of fish, of the genus *Poecilia*, known under the common name Molly. They inhabit fresh water streams and coastal brackish and marine waters of Mexico. The Molly can produce fertile hybrids with many *Poecilia* species, most importantly the sailfin molly. Mollies rank as one of the most popular feeder fish due to high growth rate, birth size, reproduction, and brood number.

Redtail shark (*Epalzeorhynchos bicolor*) (Fig. 1.3B) is one of freshwater fish belongs to family *Cyprinidae* that originates from the streams and waterways of Thailand. It is characterized by black body and orange tail and its skin is covered with transparent scales.

FIGURE 1.3 (A) Molly fish *(Poecilia sphenops*). **(B)** Redtail shark (*Epalzeorhynchos bicolor*).
(bars = 2cm)

1.1 TISSUE PROCESSING FOR LIGHT MICROSCOPY

Immediately after death or euthanasia, the tissue or organ is cut into small pieces (1 × 1 × 0.5 cm) and immediately fixed in Bouin's fluid for 20 h (ideally preserves normal morphology and facilitates further processing).

The fixed materials were dehydrated in graded series of alcohols, cleared in methyl benzoate, and embedded in paraffin wax. Serial longitudinal and transverse sections were obtained at 3 μm and stained with Harris hematoxylin and eosin (HE). Hematoxylin stains the cell nucleus and other acidic structures (such as RNA-rich portions of the cytoplasm, lysosomes, endoplasmic *reticulum*, ribosomes, etc.) in blue. In contrast, eosin stains the cytoplasmic proteins and a variety of extracellular structures from pink to red. In addition to the widely, but not very specific used HE staining procedure, other stain combinations and techniques are available. For example, trichrome procedures (including three dyes), such as Mallory's and Crossmon's, help to differentiate collagen from muscle cells. In addition, Van Gieson Resorcin Fuchsin identifies collagen and muscle fibers, while Verhoff's and Weigert's elastica stains are ideal for elastic fibers.

1.2 HISTOCHEMICAL STAINING AND ENZYME HISTOCHEMISTRY

Osmic acid reacts with fat to give a grey-black color; the periodic acid–Schiff (PAS) reaction and alcian blue (AB) (pH 2.5) reveal different varieties of glycosaminoglycans of proteoglycans and glycoproteins that are present in some tissues and cells. Hematoxylin is the usual counterstain. The PAS-positive sites stain magenta/red; the AB-positive components stain blue.

Grimelius silver impregnation displays some aspects of neuroendocrine cells. Iron HX and bromophenol blue are used to detect presence of proteins. Representative sections were stained with Best's carmine for detection of glycogen. Long Ziehl–Neelsen for lipofuscins, improved Kupffer's gold chloride method for demonstration of stellate cells, and Maldonado's stain for demonstration of endocrine portion of the pancreas.

Acid phosphatase activity is identified with Gomori' lead nitrate. Lipase activity was demonstrated by Tween method and alkaline phosphatase activity by the Gomori calcium method.

1.3 SEMITHIN SECTIONS AND ELECTRON MICROSCOPIC STUDIES

Tissues were preserved by immersion in a mixture of 3% paraformaldehyde–glutaraldehyde fixative and left overnight. After fixation, the

samples were washed in 0.1 Mol l-1 phosphate buffer and osmicated by 1% osmium tetroxide in 0.1 Mol l-1 Na-cacodylate buffer at pH 7.3. After that, the samples were dehydrated in a graded series of ethanol followed by propylene oxide and embedded in araldite. The resin blocks are processed at electron microscopy unit in Assiut University, Egypt. Semithin sections are cut at 1 μm thickness with Reichert Ultracut S (Leica, Germany) and stained with toluidine blue for light microscopy. Ultrathin sections were done with Ultrotom VRV (LKB Bromma, Germany). The sections (70 nm) were stained with uranyl acetate and lead citrate and examined by JEOL 100CX II transmission electron microscope at the Electron Microscopy Unit of Assiut University.

For scanning electron microscopy, the tissues are washed in 0.1 M cacodylate buffer for 1 h and then transferred to a 1% solution of tannic acid for 2 h at room temperature. The pieces were washed again in buffer and post-fixed for 2 h in 1% osmium tetroxide. The post-fixed materials were washed and dehydrated in a series of increasingly concentrated solutions. They were then mounted on aluminum stubs and sputter-coated with gold/palladium for 3 min. The specimens were examined with a JEOL JSM-5400 LV Scanning Electron Microscope.

1.4 PICTURES OF GROSS ANATOMY

I have provided gross views of longitudinal and surface sections of whole fish. This allows a better understanding of the location of each organ.

KEYWORDS

- **guppy**
- **catfish**
- **tilapia**
- ***Poecilia***
- **hematoxylin**

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