2 Functional Anatomy

Abstract

The microscopic examination of stained sections and the ability to interpret the relationship between fine structure and function is essential. The recognition and interpretation of physiological and pathological processes requires a thorough understanding of normal tissue structure and microanatomy, and importantly the variations within species are crucial for correct interpretation. This chapter covers the physiological changes, sexual maturation and aging processes that are 'normal', and inherently different from those resulting from injury, infection or disease.

Keywords

Histology • Anatomy • Normal structure • Salmon • Trout

Microanatomy or histology, the science of tissues, is the microscopic examination of thin, stained sections that allows the interpretation of the relationship between fine structure and function. Individual cells and tissues may undergo changes during physiological responses, sexual maturation and aging, that are 'normal' and largely different from those resulting from injury, infection or disease processes. Therefore, knowledge of the normal structure and variations within species is of crucial significance for correct interpretation and understanding of pathological changes.

Within the body, the coelomic cavities (pericardial and abdominal), various organs and components, are surrounded or held in position by layers of a serous membrane, the peritoneum, a mesothelium and connective tissue containing blood and secondary (lymph) circulation which also lines the septum transversum that separates both cavities. In this way the outermost layer covering the different regions of the alimentary canal known as the 'serosa', is effectively the 'visceral peritoneum', and covers other viscera lying within the 'peritoneal cavity or space', hence they are not in the body cavity but enveloped within a double layer of peritoneum, and analogous to pushing a finger within a balloon. This also determines that some organs are 'retroperitoneal', for example, the kidney.

Throughout this chapter we will refer to the functional unit of an organ as the parenchyma, while the space in between these parts, cells or functional units, is referred as the interstitium. The latter may contain various cellular and extracellular elements, supporting structures or secretions. For example, in the kidney the nephrons and the blood vascular system are embedded in the meshwork of the interstitium, with non-renal elements such as haematopoietic, secretory and supportive cells; whereas the liver consists of hepatocytes, with a meshwork of connective tissue, namely the framework of the organ.

In this chapter an overview of the anatomy of salmonids is presented by 'systems' (e.g. respiratory system, excretory system) and organised by a functional approach.

The staining of light micrographs are only identified when haematoxylin and eosin (H&E) is not used, and the magnification for the images are given either as a scale bar or if without a scalebar are identified as low power ×4–10; medium power ×20–40; high power ×60–100 (Fig. 2.1).

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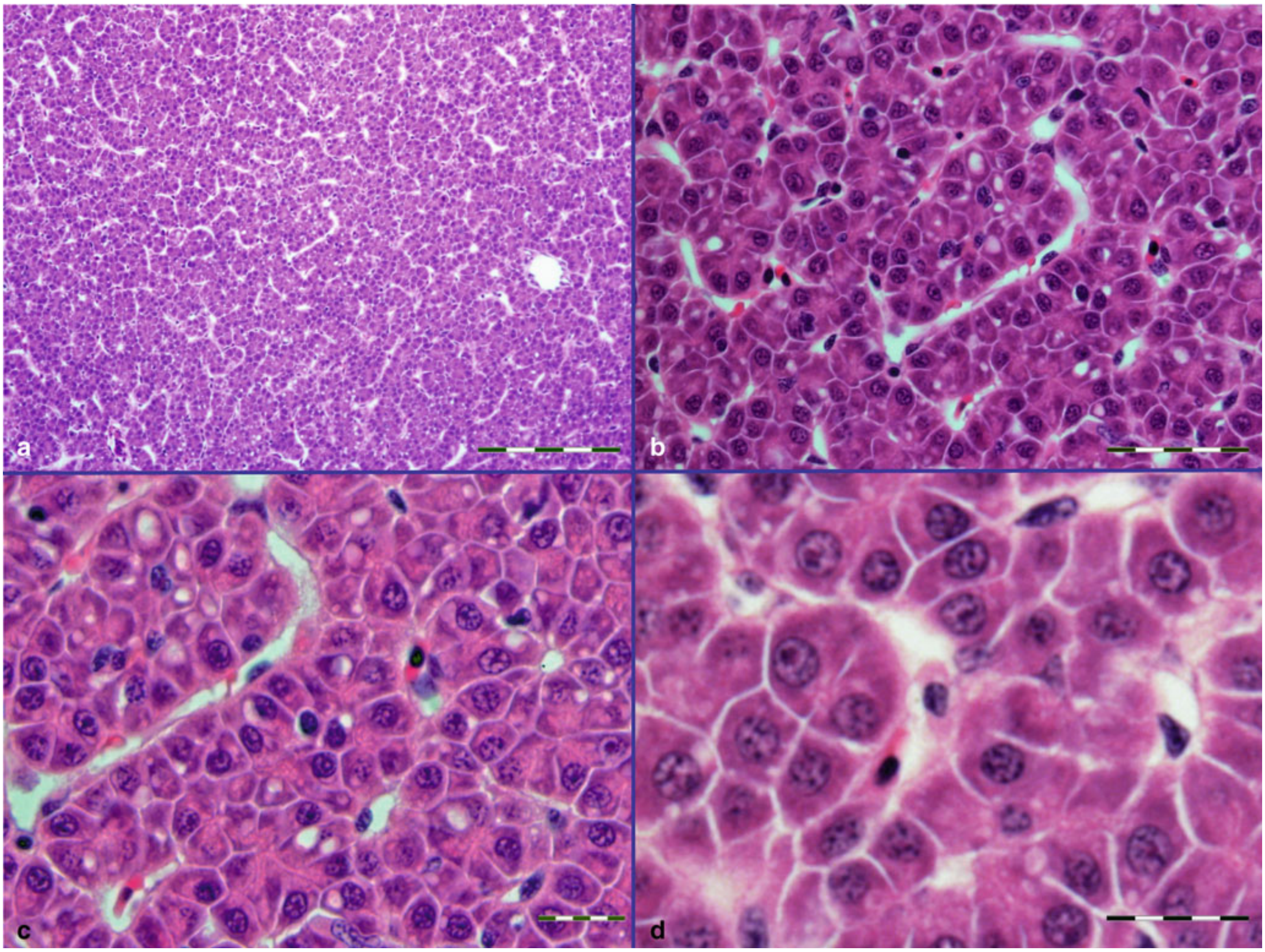

Fig. 2.1 Sections to illustrate different magnifications with representative bar scales. (**a**) Bar scale = 100 μm, ×20. (**b**) Bar scale = 50 μm, ×40. (**c**) Bar scale = 20 μm, ×60. (**d**) Bar scale = 20 μm, ×100

2.1 Respiratory System (including the operculum and pseudobranch)

Salmonids have four gill arches bilaterally placed on each side of the head, each supporting a holobranch with its two hemibranchs, the double vertical rows of gill filaments (Fig. 2.2). A series of cartilaginous or bony projections, the gill rakers, protrude forwards from the pharyngeal margin of the gill arch. These are relatively sparse in most salmonids but show a wide variation of morphologies in different species, and may form a fine grid that helps filter planktonic organisms from the water, at the same time preventing food particles from entering the gill chamber. Each hemibranch comprises a row of posterior-laterally oriented filaments with its respiratory epithelium covered lamella on each side (Fig. 2.3). Anatomically the filaments looks like a 'feather', supporting on each side a continuous symmetrically-spaced individual lamella. The filaments are supported along their proximal half by an interbranchial septum of connective and muscle tissues, but the septum is reduced to about a third of the filament length or even absent in more advanced fish. Each lamella consists of a supportive scaffold of pillar cells among which blood supply enters and leaves the lamella, which are covered by a thin double-layer epithelium separated by a space in which migrating inflammatory cells may be seen. The inner layer of the epithelium sits on a basement membrane that traverses the opposing face of the lamella in grooves located within the pillar cells, and in this way provides additional tensile support. However, the bulk of the respiratory epithelium obvious through light microscopy, is the outer squamous layer that provides a large and intimate interface with the water for exchange of gases, acid–base regulation, osmoregulation and excretion of nitrogenous waste products. Chloride and mucous cells, normally found near the base of the lamellae, may also be found distally under pathological conditions, especially the mucous cells (Fig. 2.4). Chloride cells are highly rich in

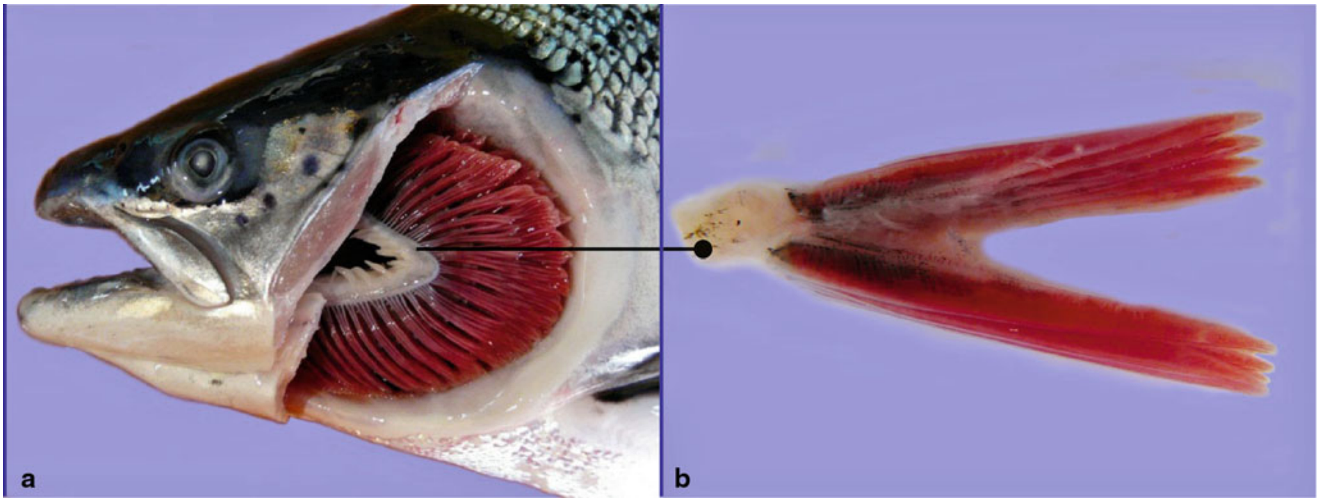

Fig. 2.2 (**a**) Gills of an adult wild Atlantic salmon, left operculum is removed. The complete gill arch with its gill rakers can be seen. (**b**) Transversal section of one holobranch showing the hemibranchs with the vertical rows of gill filaments

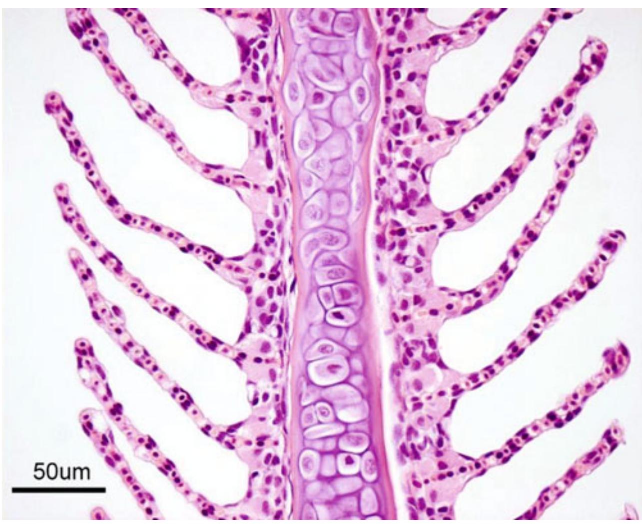

Fig. 2.3 Gill filament, central cartilage and lamellae from adult Atlantic salmon. Chloride cells are located near the base of the lamellae

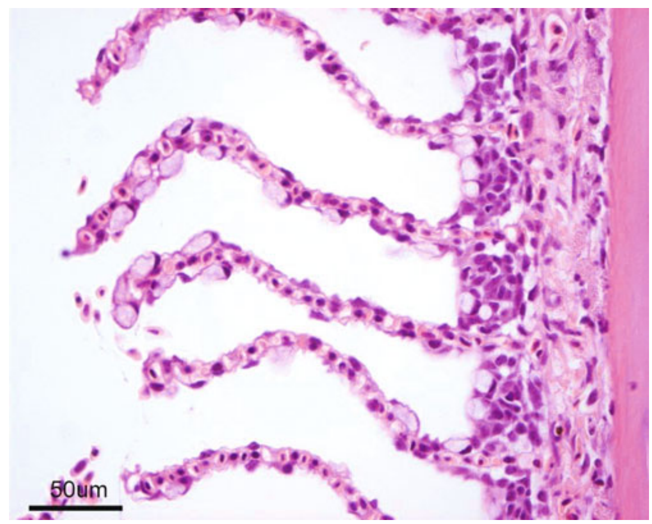

Fig. 2.4 Goblet (mucous) cells on lamellae of farmed adult Atlantic salmon

mitochondria and responsible for the secretion of sodium chloride from the blood, they are prominent in fish living in the marine environment and characteristic during smoltification, but their number may also increase in several pathological conditions. Other cells within the filament interstitium include lymphocytes, eosinophilic granular cells (EGCs) (see Fig. 4.15), macrophages, neuroepithelial cells and rodlet cells. Granular and neuroepithelial cells are obvious at the base of the lamella and more frequent in marine than in freshwater fish. Intraepithelial lymphocytic cell accumulations can be seen at the caudal edge of interbranchial septum in Atlantic salmon, and shown to be a lymphoid tissue and probably of significance for surveillance of gill infections (Fig. 2.5).

Blood flow in the lamella capillaries is opposite to the ventilatory water flow (‘counter current’) ensuring effective gas exchange between blood and water across the respiratory epithelium. Venous blood from the ventral aorta diverges to the afferent arterioles and capillaries of the lamellae where gas exchange takes place and becomes arterial blood. Arterial blood is drained through the efferent branchial artery and into the dorsal aorta. The water flow over the lamellae is continuous and achieved by means of the buccal-opercular pump.

Dorsally, on the inner surface of each operculum is the pseudobranch, a rudimentary first gill arch (Figs. 2.6 and 2.7). The pseudobranchial cells are in close proximity to a network of blood vessels and may play a role in the blood supply to the retina and in osmoregulation and sensing.

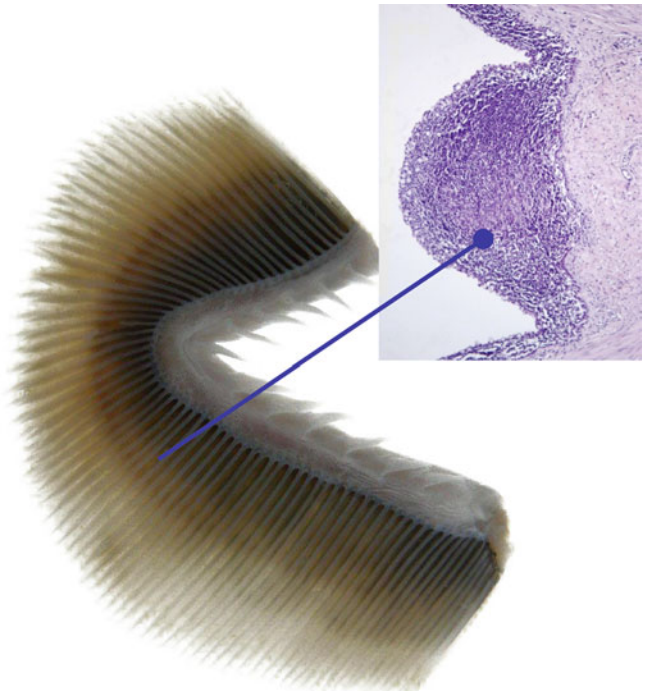

Fig. 2.5 Gills and location of interbranchial lymphoid tissue from Atlantic salmon. *Insert* medium power

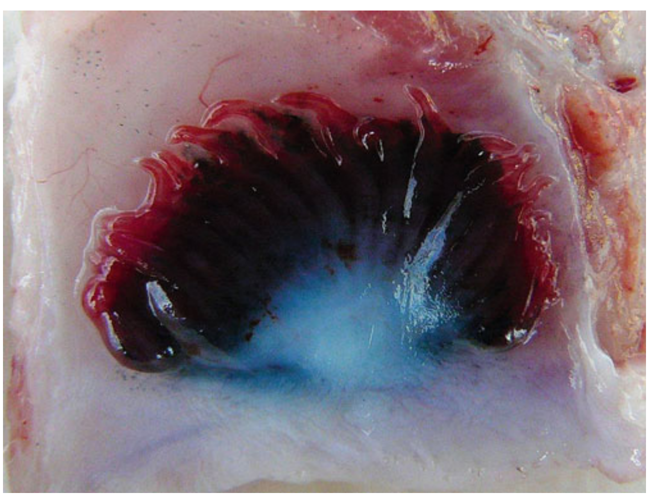

Fig. 2.6 Pseudobranch from an adult sea water farmed Atlantic salmon

The gills are frequently involved in several pathological conditions of diverse aetiology, but lesions should be differentiated from artefacts and post-mortem changes. Due to their delicate structure, exposed location, abundant blood supply and large surface, gills quickly undergo post-mortem changes that can make histopathological interpretation difficult (see Fig. 4.31).

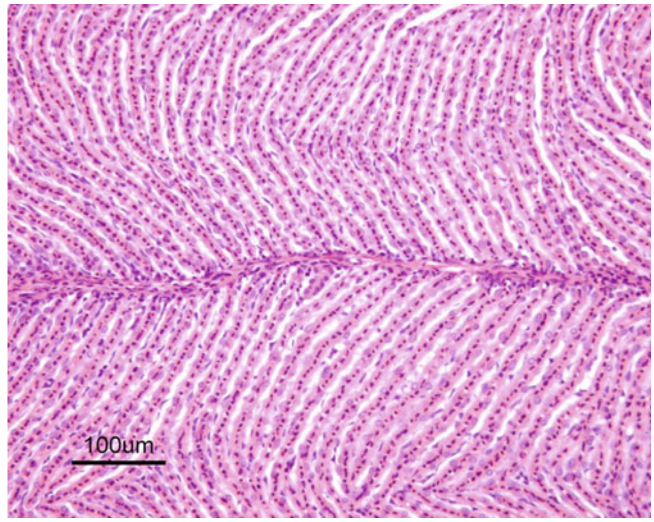

Fig. 2.7 Pseudobranch lamellae of adult Atlantic salmon

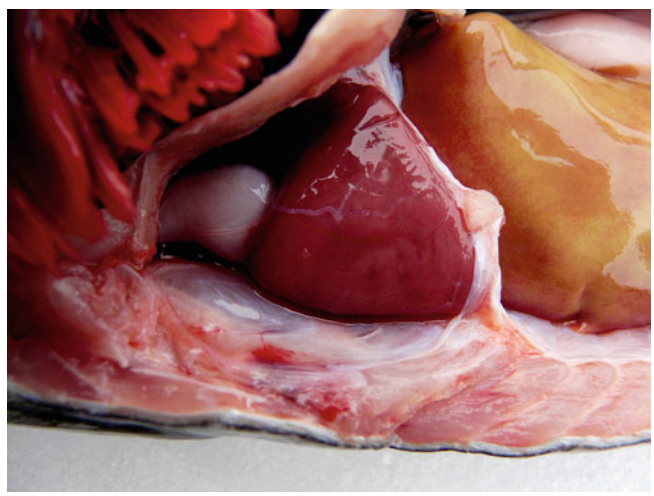

Fig. 2.8 Position of normal heart in the pericardial cavity of adult Atlantic salmon

2.2 Circulatory System (Cardiovascular and Secondary Circulation)

2.2.1 The Cardiovascular System

The cardiovascular system is a simple loop with the heart, gills and systemic circulation in series. The deoxygenated blood is pumped from the ventricle to the gills where it leaves as oxygenated blood to be delivered directly to body organs and tissues.

The heart is located in the pericardial cavity antro-ventrally to the peritoneal cavity, and is separated from the latter by the septum transversum (Fig. 2.8). It consists of four compartments: the sinus venosus, the atrium, the ventricle

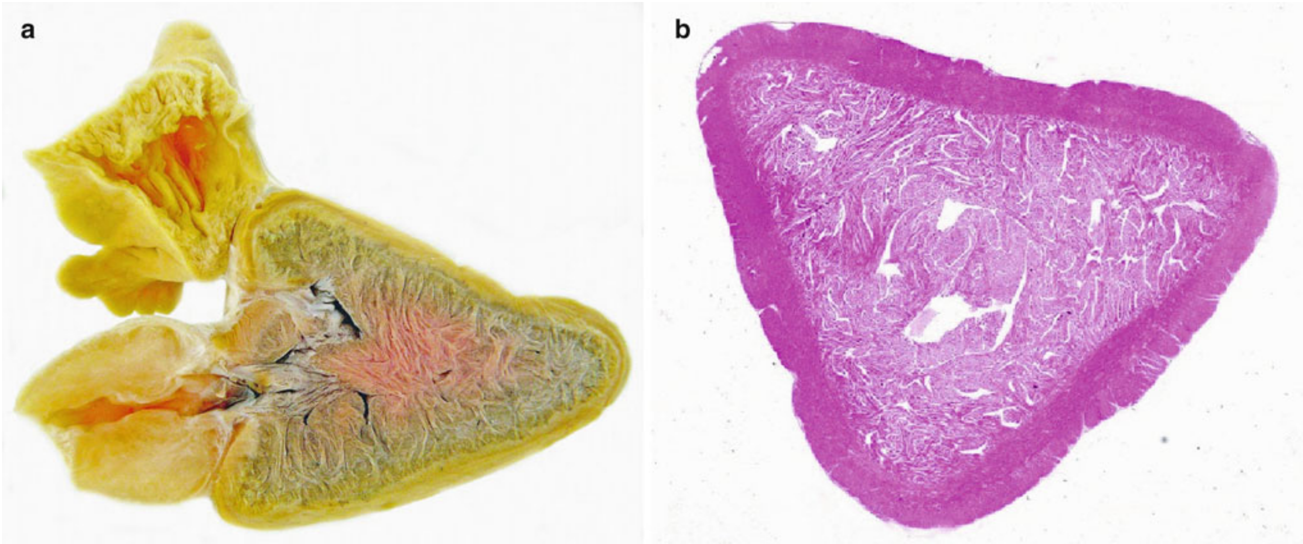

Fig. 2.9 (**a**) Sagittal section through the heart of wild Atlantic salmon showing atrium, ventricle with spongy and compact myocardium and bulbous arteriosus. Formalin fixed specimen. (**b**) Stained transverse section of ventricle from Atlantic salmon

Fig. 2.10 Coronary vessels on the surface of bulbus arteriosus and the ventricle in adult farmed Atlantic salmon

and the bulbus arteriosus, interposed by the conus arteriosus. The former was considered to have been lost through evolution but recent work has clearly shown its presence and fundamental role in the heart outflow tract, where it supports the conus valves previously named bulbo-ventricular valves. Deoxygenated blood from the cardinal and hepatic veins flows into the thin-walled sinus venosus and passes through the sino-atrial valve and then into the thin-walled spongy atrium. Blood is drawn from the atrium via the atrio ventricular valves into the thick-walled and muscular ventricle. The ventricle has a pyramidal shape and consists of two muscular layers: the outer compact myocardium with its own supply of oxygenated blood, and the inner spongy myocardium (Fig. 2.9). The blood supply to the outer myocardium is via the coronary artery (Fig. 2.10), a branch of the hypobranchial artery from the second gill arch that runs caudally on the ventral side of the bulbus arteriosus, before it bifurcates and spreads over the ventricle surface. The inner spongy myocardium has no blood supply of its own, but is supplied with oxygen and nutrients from the venous blood being pumped through the organ. An example of the microanatomy of an artery and vein is shown in Fig. 2.11. The last chamber is the highly compliant bulbus arteriosus with thick walls composed of fibro-elastic and connective tissue. This chamber functions as a depulsator and delivers a steady blood flow to the ventral aorta. The adventitia of the organ consists of blood vessels and large nerve bundles in a collagen matrix. All chambers of the heart are covered by a flat epithelium called the epicardium which fuses with the pericardium that covers the inner surface of the pericardial cavity. All inner surfaces are covered by the endocardium. The thickness of the outer, compact myocardium may vary with age, sex and the habitat of the fish (Fig. 2.12). The absolute and relative thickness of the compact myocardium increases with age and is thicker in males than in females, and thicker in fish living in running water than those living in lakes. Generally, wild fish have a thicker compact myocardium than farmed fish of the same size. The cardiac striated muscle (myocardium) is differentiated from skeletal striated muscle by the branching structure of the fibres and centrally located nuclei (Fig. 2.13).

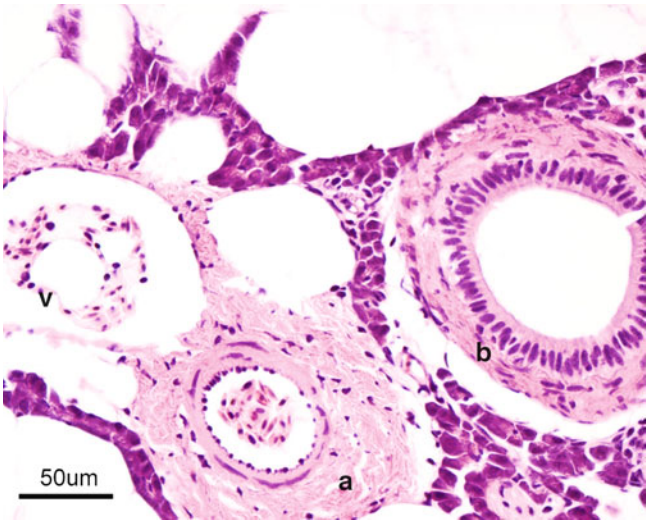

Fig. 2.11 Transverse section through thick walled artery (*a*) and thin walled vein (*v*) from Atlantic salmon, note presence of bile duct (*b*)

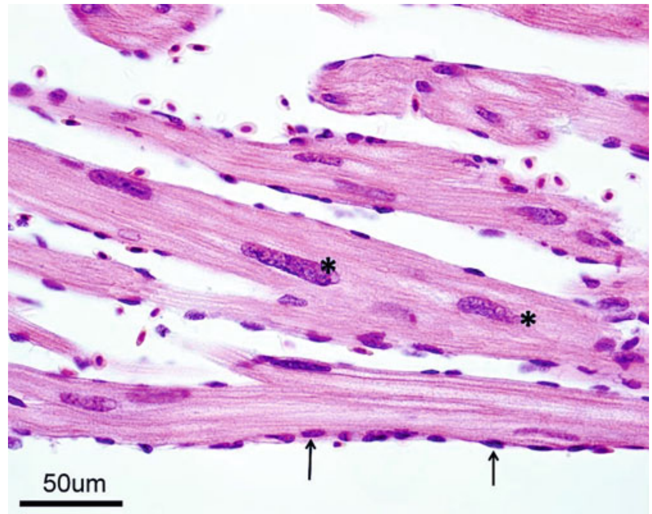

Fig. 2.13 Longitudinal section of spongy myocardium of Atlantic salmon; note central location of nuclei (*) and endocardial cell nuclei (*arrows*)

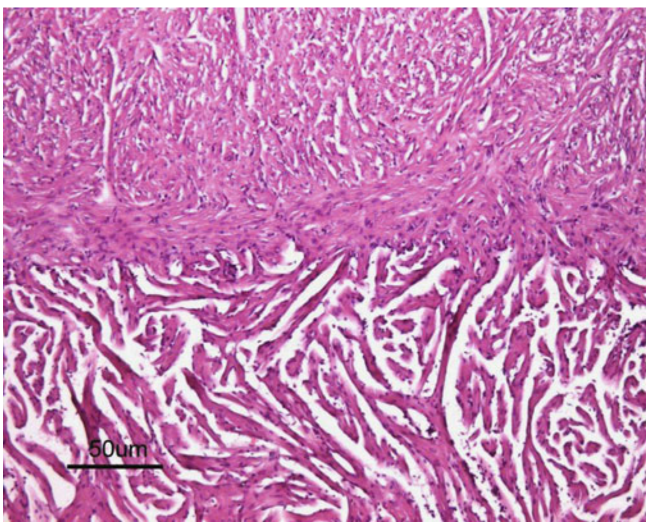

Fig. 2.12 Interphase between compact and spongy myocardium of ventricle of Atlantic salmon parr

Blood Cells

Blood is composed of humoral (plasma) and cellular (blood cell) components. In contrast to mammals, fish red blood cells (erythrocytes) are nucleated and ovoid in shape, 13–16 μm long and 7–10 μm broad. Erythrocyte numbers may vary, but are usually in the range of 1.05×10^6–3.0×10^6 mm^3. Giemsa staining shows that mature cells have a dense chromatin, purple-red centrally-located nucleus, and a clear homogenous, light red cytoplasm. The later reflects the absence of organelles and the quantity of haemoglobin present which in mature cells is very abundant. The peripheral blood is mainly composed of mature erythrocytes, although immature and developmental stages can be distinguished. Immature erythrocytes are known as reticulocytes, they are rounder with a relatively larger nucleus. Five categories can be recognized based on structure, distribution and quantity of basophilic substances within their cytoplasm. Normally, these represent about 1 % of the total count in healthy fish.

Lymphocytes in fish constitute 70–90 % of the total number of leucocytes. Lymphocytes are arbitrarily separated into categories of large (10–15 μm diameter) and small (7–10 μm diameter), which may represent different functional stages. The round or oval nucleus virtually occupies the whole cell leaving a narrow margin of basophilic cytoplasm. The cytoplasm may show pseudopodia-like projections on the surface. Both T and B lymphocytes forms are recognised in fish, playing a most significant role in the innate and the acquired immune responses.

Thrombocytes are responsible for blood clotting and are important in homeostasis and defence. Typically they are elongated but can be also be spindle-shaped and ovoid, with an indentation. They are of variable size (5–8 μm long) with a light basophilic rim of cytoplasm and a densely staining nucleus that occupies most of the cell. Between 1 and 6 % of the total white cells in rainbow trout are thrombocytes.

Neutrophils are morphologically similar to those in mammals and are commonly found at sites of inflammation. The eccentric nucleus is often kidney-shaped, although in mature cells two or five lobed nuclei may be recognized which are connected to each other by threads of nuclear material. These cells vary from 4 to 13 μm in diameter. There is evidence of phagocytic capacity in Atlantic salmon however much lower than the ‘professional’ macrophages, therefore unlikely that phagocytosis is their primary function. Their role seems to be more important in extracellular killing through enzymatic and other antimicrobial secretions.

Monocytes form about 0.1 % of the circulating leucocytes and are partially differentiated end cells which under appropriate conditions will develop into mature cells of the mononuclear phagocyte system. Monocytes are 9–25 μm in diameter with a lighter staining cytoplasm than small lymphocytes and contain small granules with a large nucleus.

Differentiation of phagocytes into macrophages usually takes place when they become extravascular, migrating from the vessels into the tissues and therefore usually not seen in circulation. Their phagocytic capability is well documented, but resting macrophages in tissues are difficult to distinguish from fibrocytes in H&E stained sections.

2.2.2 Secondary Circulation

Studies indicate that fish lack a true lymphatic system but a second vascular system derived from and connected to the primary circulation is present. Analogies between the lymphatic and the secondary system have been noted and observed in skin, fins, gills, oral mucosa and lining of the peritoneum, however it remains to be determined whether the secondary system is the antecedent of a lymphatic system or a coincidentally similar structure.

The spleen is usually dark red or almost black in colour and a discrete organ with sharply defined edges located near the greater curvature of the stomach. A thin serous capsule covers the surface. The spleen functions as a haematopoietic organ, a temporary blood bank and as a remover of circulating antigens and effete blood cells. Occasionally, two or more spleens may be recorded and the organ may also be located elsewhere in the abdominal cavity (Fig. 2.14). The spleen structure is provided by a capillary and a connective tissue meshwork among which the cells fill up the interspaces i.e. erythroblasts, mature and immature erythrocytes, lymphocytes, monocytes and macrophages. The parenchyma of the spleen is composed of white pulp, namely lymphoid tissue, surrounding small arteries which diffusely intermeshes with the haematopoietic red pulp, composed of a reticular cell network and supporting blood-filled sinusoids. There is no sharp demarcation between red and white pulp as the parts rich in erythrocytes and those rich in lymphocytes are intermingled (Fig. 2.15). The ellipsoids form the main elements of the spleen and are a thick-walled filter capillary network gradually forming from the artery which enters the organ. Each ellipsoid comprises a thick basement membrane-bound tube within which the vessels run and is separated from the membrane by a layer of sheathed components. Degradation products of senescent erythrocytes are stained yellow by H&E and known as haemosiderin, a common feature in the spleen parenchyma. Perl's staining is used to differentiate haemosiderin deposits (see Fig. 4.22). Variable numbers of melanomacrophages may be found scattered in the spleen tissue. Phagocytic cells capable of trapping large quantities of particulate matter from the circulating blood, once replete migrate to the ellipsoids to the melanomacrophages.

Fig. 2.14 Spleen in abdominal cavity of Atlantic salmon, note duplicate organ in this case

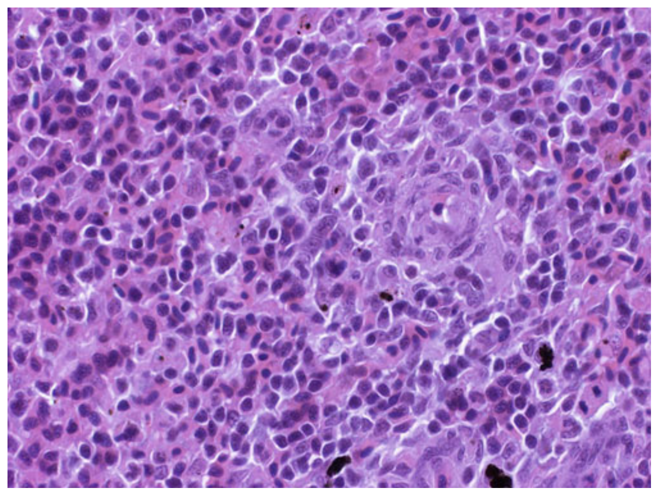

Fig. 2.15 Spleen of adult Atlantic salmon. Medium power

2.3 Integument System

The structure of the skin varies to some extent among species but is basically composed of two layers: an outer epidermis and an underlying dermis. The epidermis constitutes the barrier between the body and the aquatic environment and can be divided into two additional layers. The outer epithelium is composed of stratified squamous cells and a basal layer of undifferentiated cuboid germinal cells. The depth is greatest on the head and over the fins where scales are absent. The thickness of epidermis and number of mucous-secreting goblet cells in the epidermis varies between species and location on the body. Their number may increase during sexual maturation and spawning migration, and lymphocytes can also be present in the epidermis (Fig. 2.16). Mucous cells have characteristic basal, compact nuclei. The epidermis sits on a thin acellular layer that can be observed by light microscopy and is the fusion of the basal and the reticular lamina known as the basement membrane, although the components, i.e. the basal lamina, can only be observed by electron microscopy.

The dermis is mainly composed of collagenous connective tissue. Two layers can be distinguished histologically, the upper stratum spongiosum comprised of a loose network of collagen and reticulin fibres and also containing pigment cells (chromatophores), and the lower stratum compactum composed of a collagenous dense matrix providing structural strength to the skin.

Scales are translucent acellular plates of dermal origin that project into the epidermis. They are composed of a mineralized matrix anchored in dermal pockets between layers of collagen in the stratum spongiosum, and the epidermal basement membrane. Scales have variable size, for example, they are small in Arctic char but large in whitefish. In addition, grayling scales differ from those of other salmonid species by their large size and shape with characteristic indentations on the caudal edge (ctenoid type scale in contrast to cycloid type in other salmonids).

A thin, cellular layer covers the entire scale and is distinct from other epidermal tissues, the scleroblasts which are a rich source of calcium. During periods of starvation or sexual maturation calcium from the scales may be reabsorbed by osteoclasts leaving a scar in the outer margin of the scale (‘spawning scar’).

The hypodermis is the layer between deeper layer of the dermis and the underlying muscle and is composed of loose connective tissue and some fat cells. In the head region, the hypodermis is indistinguishable from the stratum compactum of the dermis.

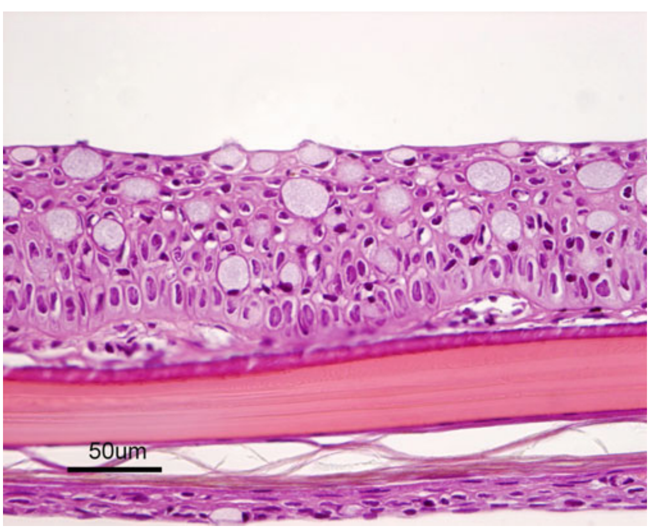

Fig. 2.16 Epidermis with goblet cells on top of a scale. Adult Atlantic salmon

2.4 Musculoskeletal System

2.4.1 The Skeletal System

The skeletal system includes the bones of the skeleton and the cartilage, ligaments, and other connective tissue that stabilize or connect the bones. The bulk of the body muscle is organized in four quadrants, with the striated muscle further organized in blocks or myotomes or myomere (Figs. 2.17, 2.18 and 2.19). The myosepta separates but also holds the myotomes together. The bulk of the skeletal muscle consists of anaerobic white fibres with a relatively poor vascularisation and few mitochondria and is used for bursts and strong swimming activity. The red aerobic muscle

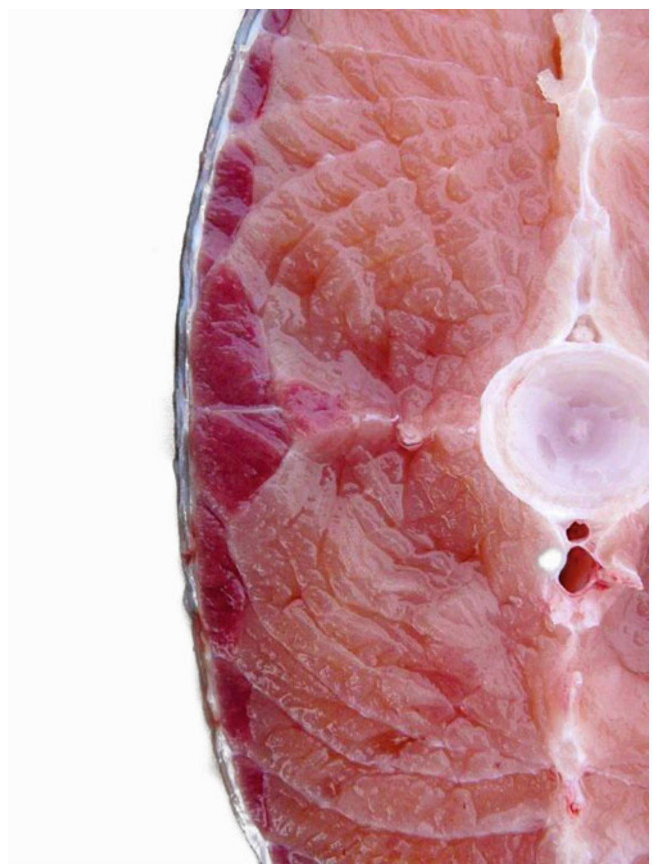

Fig. 2.17 Transverse section through body muscle of adult Atlantic salmon showing red and white muscle

is organized as a triangular band along the flank just beneath the lateral line and is highly vascularised, rich in myoglobin, glycogen and lipids with numerous mitochondria (Fig. 2.20). Red muscle is used for long-term sustained swimming and moderate speed swimming activity. Skeletal striated muscle differs from the heart striated muscle with a peripheral instead of a central nucleus.

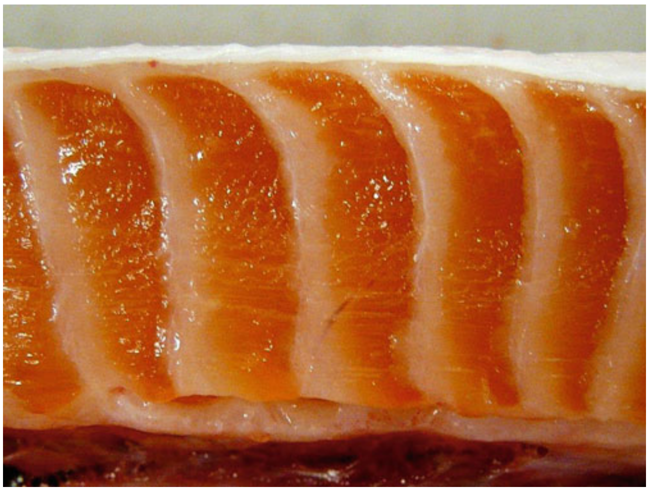

Fig. 2.18 Myotomes in the belly flap of an adult Atlantic salmon

2.4.2 Fins

Salmonids have a complete set of fins. Each fin is covered by stratified squamous epithelium continuous with the epidermis of the body. The dermis has a reduced stratum compactum and a thicker hypodermis compared to that of the main body. Median fins of include the dorsal, adipose, caudal and anal, and the paired sets are the pectoral and pelvic fins supported by bony girdles, which are embedded in the ventral body musculature as floating structures. Unpaired fins are generally supported on small bones within the musculature septa. The caudal fin is supported by a greatly modified, posterior most caudal vertebrae, flattened into an almost symmetrical plate against which the flexible fin rays of the caudal fin articulate. The adipose fin located between the dorsal and caudal fins, despite its name, has no adipose tissue or bony support. A clearer idea of its role has emerged with evidence of sensory function reported, suggesting it may act as a precaudal flow sensor, therefore its removal can be detrimental to swimming efficiency.

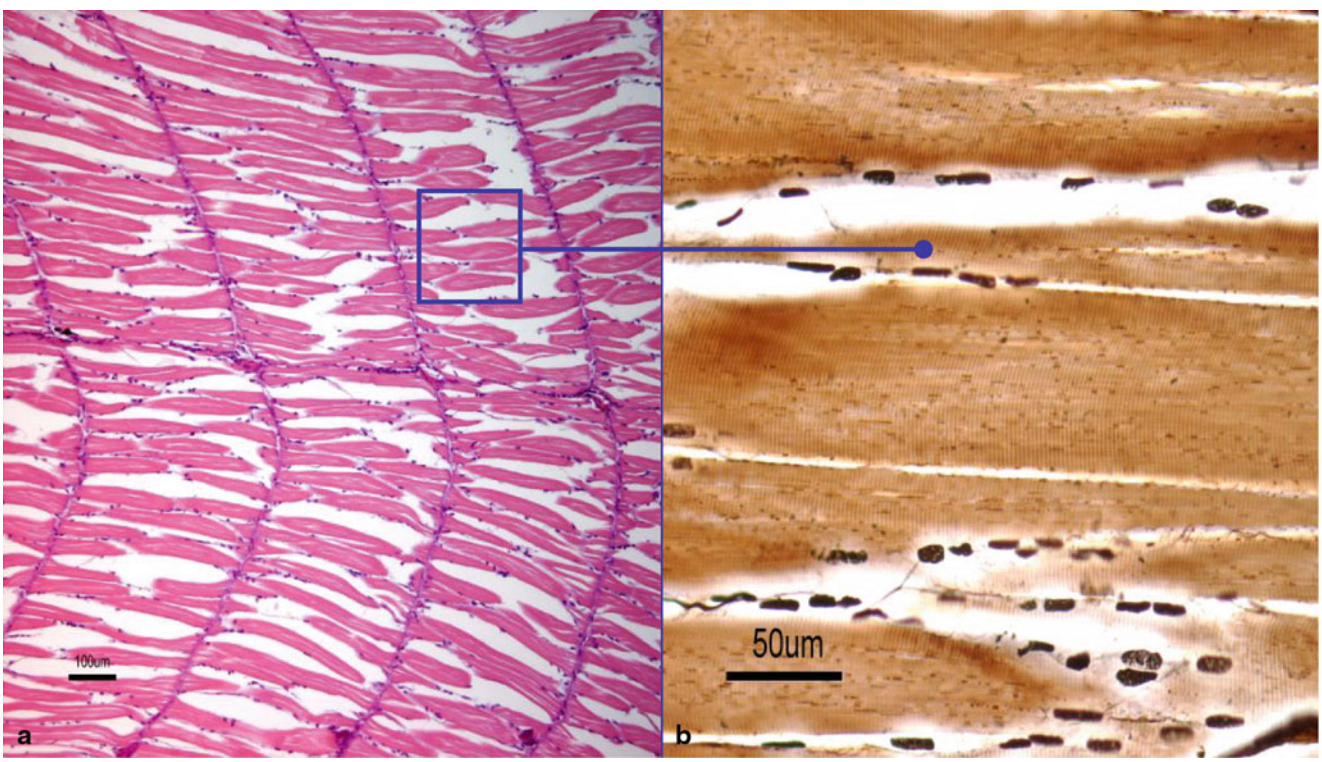

Fig. 2.19 (**a**) Longitudinal section through body muscle of Atlantic salmon fry showing arrangement of myotomes. (**b**) *Insert* is stained with Wilder silver stain

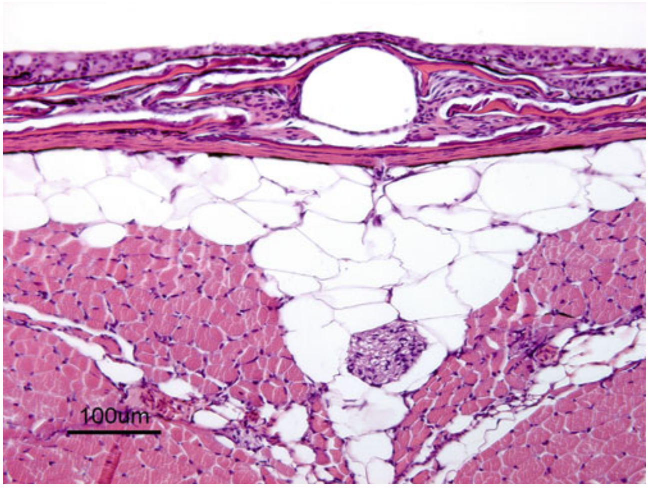

Fig. 2.20 Transverse section through Atlantic salmon parr showing epidermis, scales, lateral line, red and white muscle. Low power

Fig. 2.21 Horizontal section of rainbow trout showing head kidney. Note Y-shaped portion at cranial end

2.5 Excretory System

Salmon, like most fish, release their nitrogenous wastes as ammonia and the gills play an important role by excreting this compound through diffusion into the surrounding water. However the core of the excretory system remains in the kidney, with primary functions performed by filtering wastes from the blood to maintain the body fluid levels, collect and excrete the waste products and maintain pH.

2.5.1 Kidney

The kidney comprises tightly fused units giving the appearance of a single organ. The kidney is located retroperitoneally along most of the length of the body cavity, ventral to the vertebral column and dorsal to the swim bladder. The anterior section, also known as the cranial or head kidney is composed entirely of haematopoietic and lymphoid tissue (Fig. 2.21), while the posterior section has the excretory role and the functional unit are the nephrons (glomeruli and tubules) embedded in haematopoietic tissue (Fig. 2.22).

The typical nephron of salmonids living in fresh water is characterized by a relatively large glomerulus that fills up the Bowman's capsule (Fig. 2.23). From the latter, the renal tubule begins with the short neck segment characterized by low cuboidal epithelium with long cilia. This section is divided into a first segment with eosinophilic, cuboidal to columnar epithelium with a distinct brush border, and a second segment with a taller columnar epithelia and a centrally located oval nucleus. The latter has a prominent brush border but lack the extensive tubular system in the

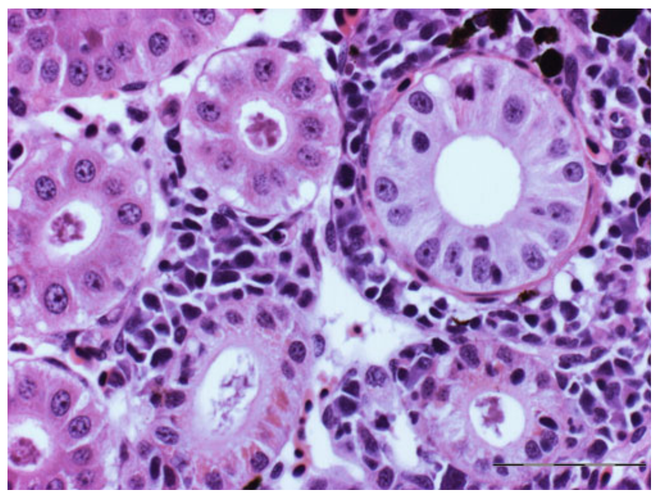

Fig. 2.22 Kidney tubules and interstitial tissue

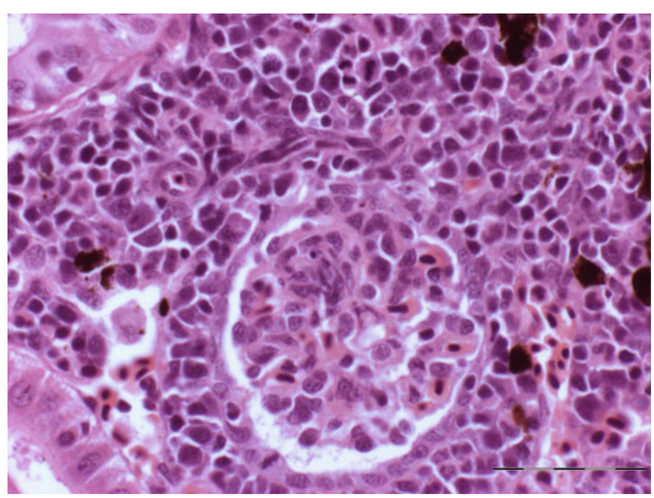

Fig. 2.23 Glomerulus in the interstitial renal tissue of adult Atlantic salmon

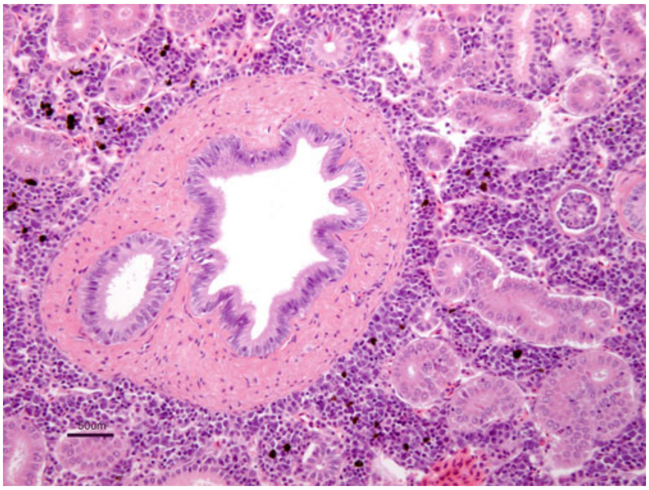

Fig. 2.24 Collecting duct and urether with wall of smooth muscle in farmed rainbow trout

Fig. 2.25 Ureters of an adult Atlantic salmon fusing to form a small urinary bladder

epithelium of the first segment. A variable intermediate segment may be distinguished, with a lower and more cuboidal epithelium. The brush border becomes intermittent and as it reaches the distal segment, they are absent. Each collecting duct system terminates in a mesonephric duct (Fig. 2.24). Histologically the proximal tubules have a wider lumen compared to that of the neck region and the distal tubules. Within the glomerulus, erythrocytes can be distinguished within the capillary lumen as well as the nuclei of mesangial cells, capillary endothelial cells and the podocytes of the visceral epithelium of the Bowman's capsule. Salmonids from the marine environment have fewer and smaller glomeruli and the distal part of the tubule is lacking. Collecting ducts pass urine into two ureters which fuse to form the urinary bladder (Fig. 2.25).

Functionally, the principle role of the posterior kidney is maintenance of a stable internal environment with respect to

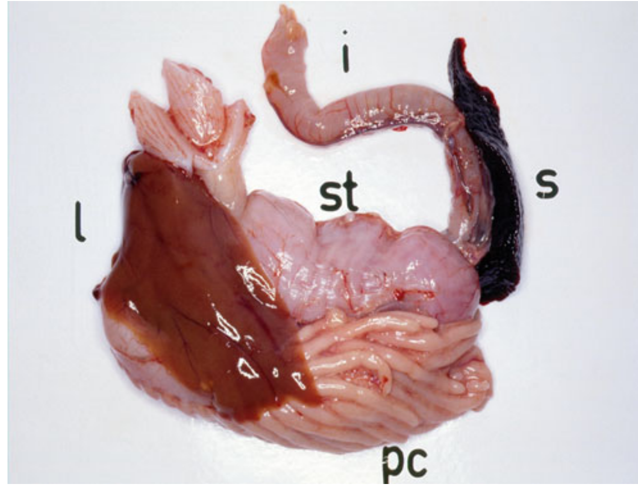

Fig. 2.26 Visceral organs of the digestive system from Atlantic salmon; liver (*l*), pyloric caeca (*pc*), spleen (*s*), stomach (*st*), intestine (*i*)

water and salts, therefore it needs to adapt to the external water conditions. Accordingly, in the freshwater the fish is hypertonic and the nephron must conserve salts and eliminate excess water which enters the body through the gills. Conversely, in the marine environment, the fish is hypotonic, the urine produced is scant and contains various di- and trivalent electrolytes as well as nitrogenous end-products. The nephron must conserve water through a reduction in urinary volume in order to prevent dehydration. This function is accomplished by a high glomerular filtration rate, reabsorption of salts in the proximal tubules and further concentration of the urine in the distal segment. Ammonia, urea and monovalent electrolytes are mainly excreted through the gills.

2.6 Digestive System

The digestive system is composed of the alimentary canal and digestive glands (gastric glands, pyloric caeca, liver, pancreas and intestinal glands, Fig. 2.26). The following regions are generally distinguished: oral cavity, pharynx, oesophagus, stomach and intestine. Functionally, the role of the digestive tract is the hydrolysis of food items.

The oral cavity contains the tongue and teeth. The tongue is relatively poorly developed in and is typically a rather rigid structure of connective tissue covered with epithelium and many unicellular glands (Fig. 2.27). The mucosal epithelium of the tongue consists of stratified epithelium and contains many taste buds and mucous cells. A lamina propria and a thin submucosa are present in the oral cavity wall, but the muscularis mucosae and submucosa are not recognized. The teeth are joined by connective tissue to the bone. The pulp of teeth is composed mainly of connective tissue and

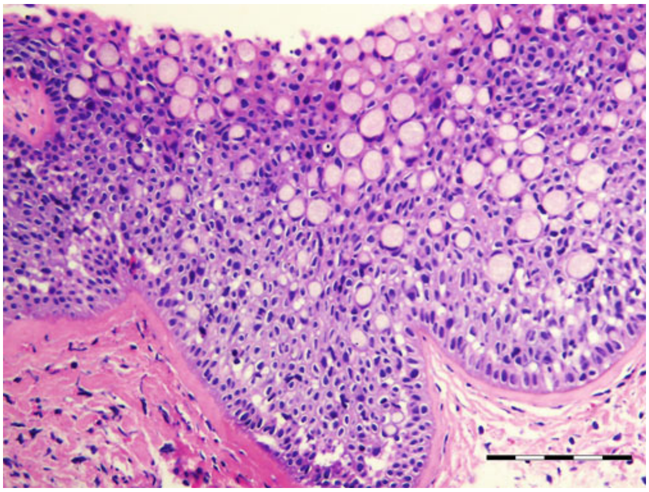

Fig. 2.27 Transverse sections of tongue from rainbow trout

occupies the centre of the tooth. Odontoblasts are arranged at the outermost region of the pulp and secrete dentin. The presence of teeth does not imply chewing activity but often have a role in grabbing and tearing food.

Although the histological structure of the alimentary canal varies along its length, it is basically composed of the following layers from inside out: mucosa, submucosa, muscularis and serosa (Fig. 2.28). The mucosa comprises a simple columnar epithelium with mucous cells; loose connective tissue makes up its lamina propria, richly supplied with blood capillaries. The submucosa supports the mucosa and joints it to the underpaying muscle layer and is composed of dense irregular connective tissue. The muscularis mucosa is a thin layer of longitudinal smooth muscle and the serosa is effectively a sheet of the visceral peritoneum.

The mucosa of the pharynx consists of shallow folds of stratified epithelium. Epithelial cells of the outermost layer are flat, but those at the base are columnar. Mucous cells are present and especially numerous at the bottom part of the

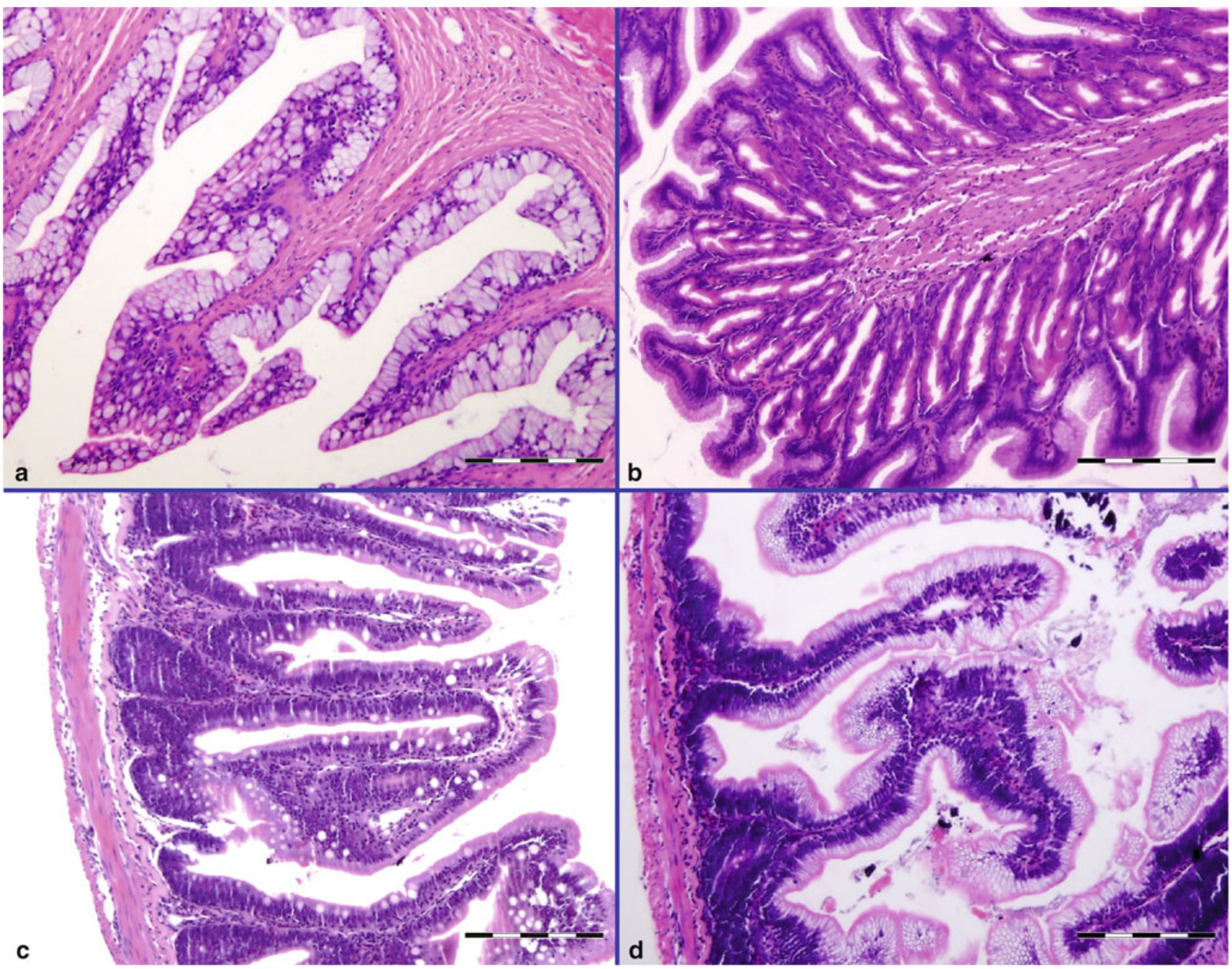

Fig. 2.28 Transverse sections of gut from rainbow trout. (**a**) Oesophagus. (**b**) Stomach. (**c**) anterior gut. (**d**) Anterior gut. Bar = 200 μm

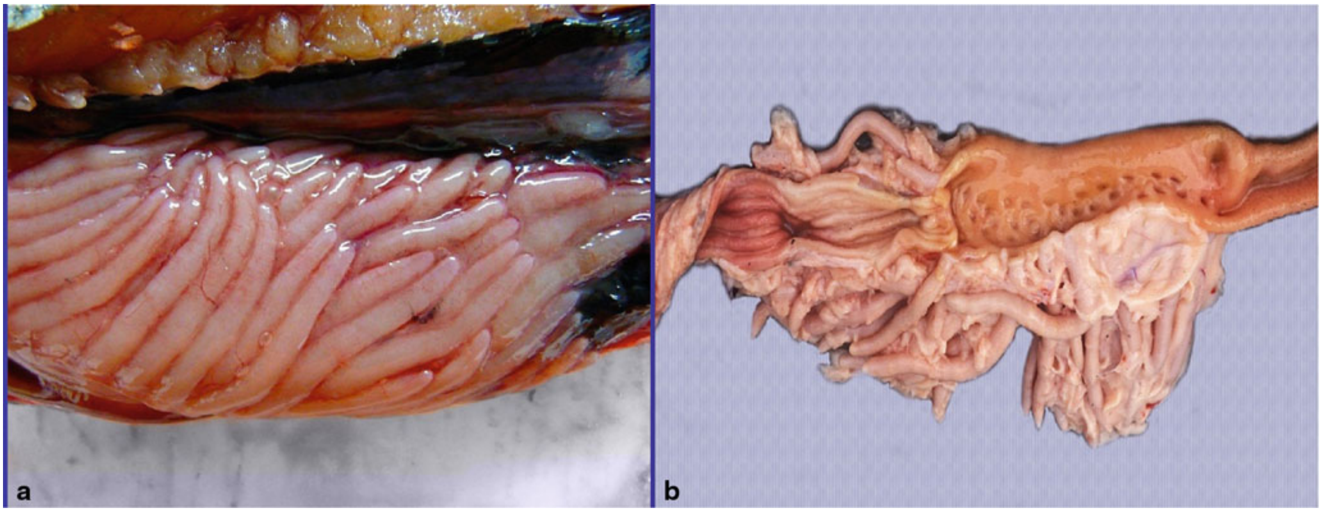

Fig. 2.29 (**a**) Pyloric caeca of adult Atlantic salmon. (**b**) Opened stomach and pyloric region showing gastric folds and the openings into the pyloric caeca

folds (crypts). The pharynx mucosa contains a lamina propria, but is devoid of a muscularis mucosa. The muscularis is composed of a thick outer layer of circular muscle and a thin layer of longitudinal muscle. Both layers of the muscularis are striated.

The oesophagus is a short, muscular thick-walled tube with longitudinally arranged folds of mucosa to facilitate swallowing and propulsion of food particles. At the entrance to the oesophagus the mucosa contains many mucous cells and taste buds. This region usually lacks a muscularis mucosa, distinguishing it from the rest of the digestive tract.

In the stomach and intestine the stratum compactum is located between the lamina propria and the muscularis mucosa which is composed of dense collagen fibres. Eosinophilic granular cells (EGC's) may be present and form a layer at the inner and outer sides of this stratum and are considered to be closely related to fish mast cells. This layer of EGC's is sometimes referred to as the stratum granulosum.

The stomach is U-shaped and composed of the cardiac portion (anterior), fundus and pyloric portion. Each region has a simple folded mucosal epithelium. At the cardiac portion the folds are shallow and become deeper at the fundus and pyloric portion. The epithelium varies between cuboidal and highly columnar, and the nuclei are generally located in the basal region of the cell. Gastric glands are located in the lamina propria and often into the crypts of the mucosal folds.

The fundic region of the stomach is a blind sac, pouching off from the main tube of the organ and characterized by numerous gastric glands although, in contrast, they may be absent in the pyloric region.

Pyloric caeca are blind-ended, finger-like projections that extend outwards from the pyloric valve region of the stomach and the anterior intestine (Fig. 2.29). Their structure and function resemble that of the intestine with a multi-folded intestinal type epithelium, and regions where fats are broken down into fatty acids and glycerine. They expand the nutrient absorption surface but also contribute to the salt and water balance, therefore playing a functional role in osmoregulation.

Fig. 2.30 Mesentery with blood vessels of the posterior intestine in adult Arctic char

The intestine extends from the end of the pyloric portion of the stomach to the vent and includes the duodenum, anterior intestine, posterior intestine and rectum. The main function of the intestine is uptake of lipids, proteins and ions. The mesentery, a double layer of peritoneum, represents the peritoneal fold that attaches the small intestine to the posterior body wall. Blood vessels and nerves for the intestine are located in the mesentery (Fig. 2.30). The bile and pancreatic ducts and pyloric caeca open into the duodenum. The anterior and posterior intestine can be distinguished from each other by the shape of their respective mucosal

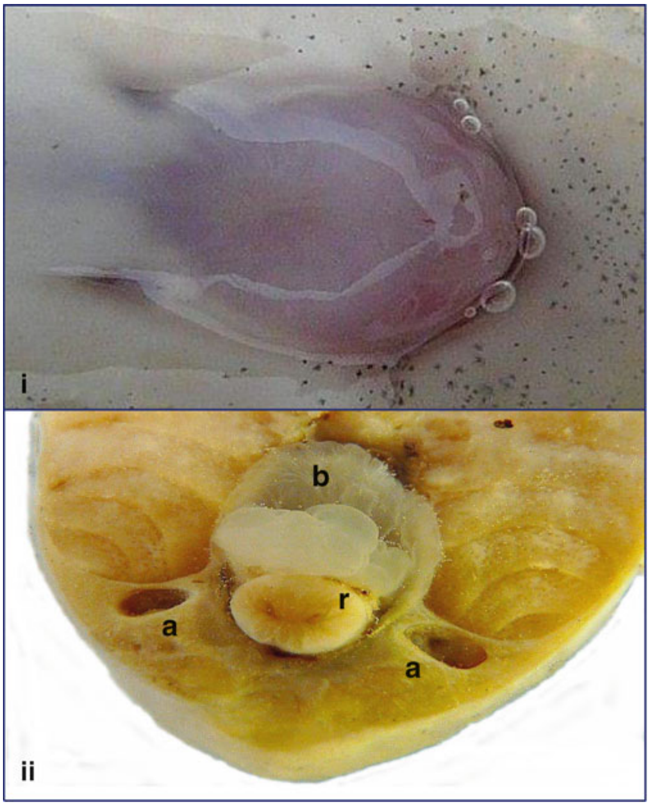

Fig. 2.31 (**i**) External view of vent area of Atlantic salmon. (**ii**) Transverse section through formalin fixed Atlantic salmon, vent area. End of bladder (*b*), rectum (*r*), abdominal pores (*a*)

folds. In rainbow trout for example, the anterior region has shallow folds and the posterior region deep folds with a thicker muscle layer with many mucous cells.

The vent is equipped with a muscular sphincter. The rectum connects with the vent located cranial to the anal fin and has a thicker muscular wall than the intestine and is capable of considerable distension. The term 'vent' specifically defines the external opening of the alimentary canal or the anus, although in a wider sense the term is used to refer to the region that includes the rear portion of the alimentary canal, the urogenital papilla, genital cavity and pore, and last portion of the urinary canal and bladder, surrounded by tissues of the posterior abdominal wall, abdominal pores and underlying adipose and muscle in the immediate area (Fig. 2.31). Abdominal pores are a paired communication between the abdominal cavity and the exterior at the rear of the abdominal cavity, and they lead to the exterior through the body wall one at each side within or behind the vent and urogenital region.

2.6.1 Liver and Gall Bladder

The liver is a large reddish-brown organ normally located in the left anterior part of the abdominal cavity, with its cranial

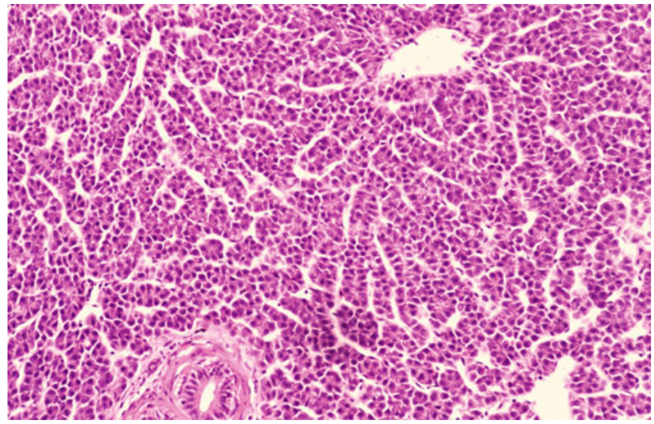

Fig. 2.32 Normal liver parenchyma from Atlantic salmon. Note a vein (*top*) and bile duct (*bottom*). Low power

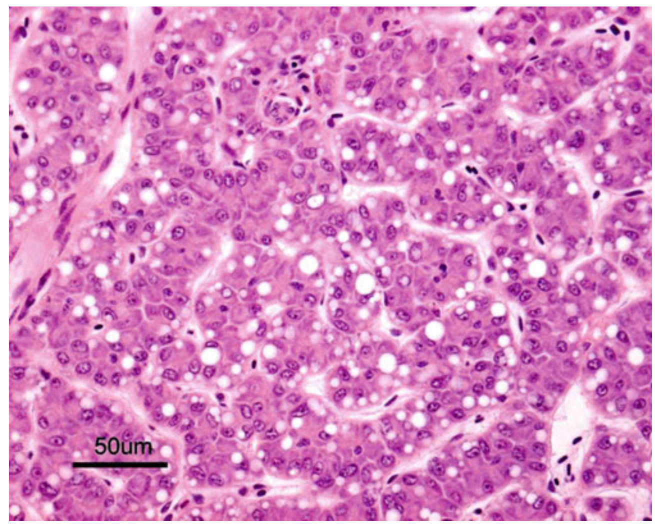

Fig. 2.33 Normal parenchyma of Arctic char liver

part close to the septum transversum. The parenchyma is composed of chords of cuboidal hepatocytes supported by lattice fibres and some connective tissue (Fig. 2.32). Each cell has a roundish polygonal cell body containing a clear spherical nucleus, usually with one nucleolus and contains variable amounts of lipid and glycogen, depending on normal variation or the nutritional status of the fish. The normal appearance of a liver from Arctic char is included for comparative purposes (Fig. 2.33). Blood is filtered through a network of sinusoids running between poorly defined, cord-like structures of hepatocytes. Phagocytosis and particulate antigens presentation represent an important immune function. Both the hepatic artery and portal vein enter the liver.

The hepatocytes secrete bile into the bile canaliculi where it is carried into the extracellular bile canaliculi to form the bile duct, which subsequently joins with the hepatic duct and opens into the duodenum. A branch of the hepatic duct called ductus cysticus, leads into the gall bladder where the

Fig. 2.34 Full gall bladder in adult farmed Atlantic salmon

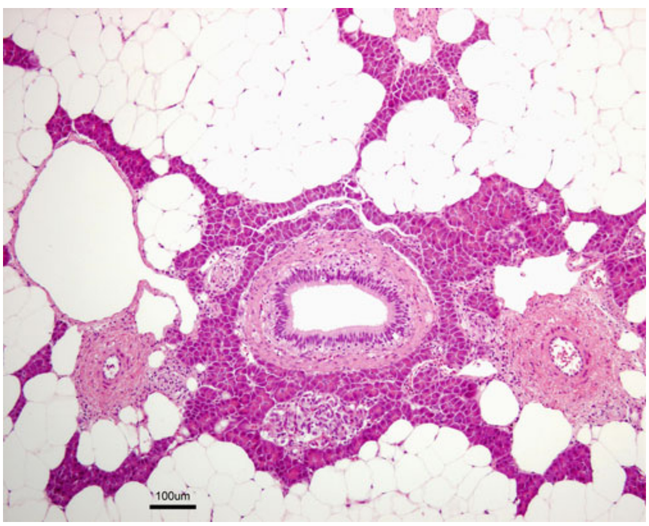

Fig. 2.35 Normal pancreas with endocrine, exocrine tissue and ducts

bile is stored (Fig. 2.34). The wall of the gallbladder is thin, contractile, and will contract when food, especially fatty food, passes through the duodenum.

2.6.2 Pancreas

The pancreas is a diffuse organ which is interspersed throughout the adipose tissue and surrounds the mesenteric fat mainly among the pyloric caeca. Functionally, the pancreas is both a digestive organ and an endocrine gland. The exocrine tissue is organized in distinct clusters ('nests') of 'acinar cells' of strongly basophilic cytoplasm and therefore stains purple with H&E (Fig. 2.35). The triangular or polygonic cells have basally located, well-defined nuclei and nucleoli. In actively feeding fish, bright eosinophilic secretory zymogen granules are present in the glandular cytoplasm. Dark staining is apparent when the quantity of zymogen granules is high and pale when the quantity of zymogen granules is low. A cell which has atrophied as a result of starvation or disease, contains few zymogen granules and consequently becomes basophilic and small in size. Digestive enzymes secreted from these cells are carried into the ascending intestine through a series of ducts and participate in the breakdown of proteins, fats and carbohydrates.

The endocrine portion of the pancreas is lightly capsulated and occur in clusters of glandular cells, the Islets of Langerhans', occurring among fat cells or surrounded by exocrine tissue. It is composed of cords of cells and generally recognized as having three functionally independent cell types: alpha (A), beta (B) and delta (D) cells that secrete hormones, including glucagon and insulin.

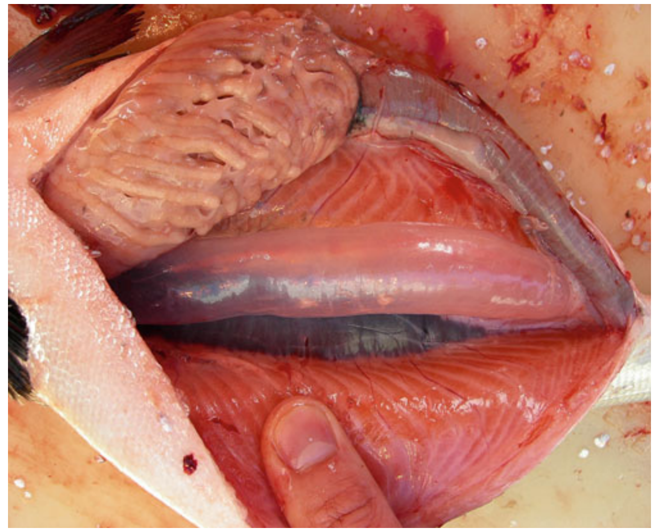

Fig. 2.36 Swim bladder from Arctic char

2.6.3 The Swim or Air Bladder

The swim or air bladder develops as an out pushing from the anterior part of the gastrointestinal tract and is a hydrostatic organ that can be filled or emptied to regulate buoyancy. In primitive fish it remains connected to the oesophagus by a tube, the ductus pneumaticus (physostomous fish), while in higher teleosts the connection is lost during development (physoclistous) and the swim bladder filling depends on a 'gas gland' (Figs. 2.36 and 2.37). Anatomically, the gas-filled swim bladder is a conspicuous organ located along the dorsal wall of the peritoneal cavity. Histologically three layers can be distinguished, the inner mucosa, the muscle layer and the outermost fibrous connective tissue.

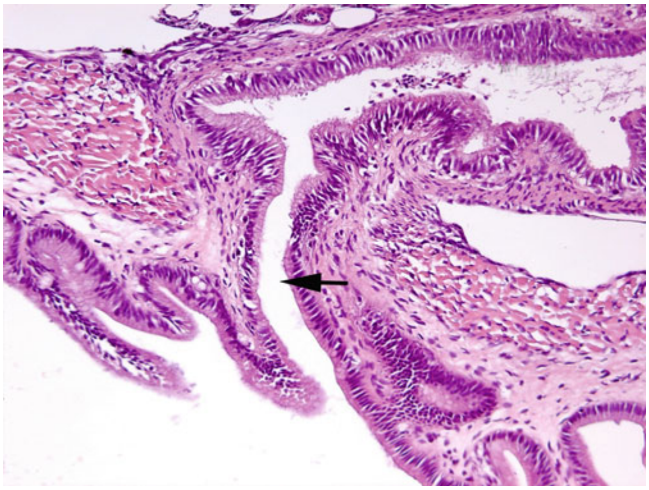

Fig. 2.37 Pneumatic duct (*arrow*) between oesophagus (*bottom*) and swimbladder (*top*). Low power

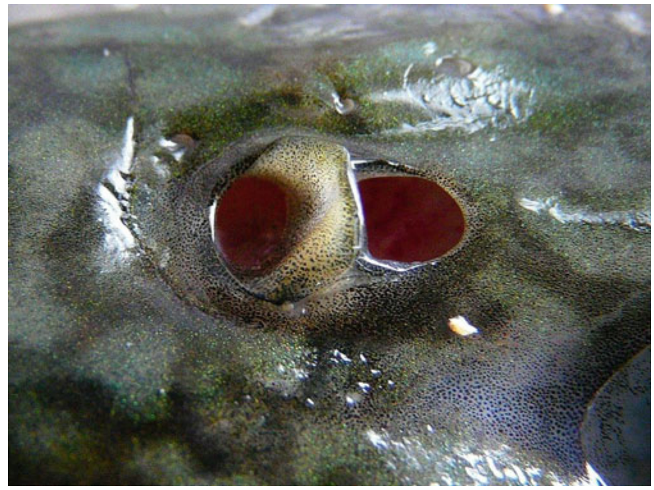

Fig. 2.38 Nostrils of an adult lake trout

2.7 Sensory System

Fish sensory systems include mechano, olfactory and taste receptors, equilibrium, hearing as well as vision organs. The olfactory sensory neurons give a sense of smell mediated by specialized sensory cells of the nasal cavity (Fig. 2.38). For the purpose of this chapter we will only refer to the mechano reception, exemplified by the lateral line and vision systems, as the most important regions for tissue pathology and infection.

2.7.1 The Mechanosensory Lateral Line

The mechanosensory lateral line system comprising a series of receptor organs, composed of neuromasts, located on the epithelium or within canals on the head and trunk, and innervated by several lateral line nerves which project to the hindbrain. This sensory system can detect movement and vibrations in the surrounding water, allowing the fish to respond to unidirectional or oscillatory movements, at relatively short distance. The trunk lateral canal is easy to observe as a fairly straight and clearly defined line, running along the middle top section of the flanks (Fig. 2.39). It comprises short segments of overlapping tubed scales (the lateral line scales) with a pore present at each end of the canal segments that links to adjacent overlapping scales to form a continuous viscous fluid-filled canal. Additional pores piercing the canal walls might be present and provide additional access to the external environment. A neuromast in located within each lateral line scale and additional superficial ones or ‘accessory neuromasts’, may also be located in the epithelium in proximity to the trunk canal.

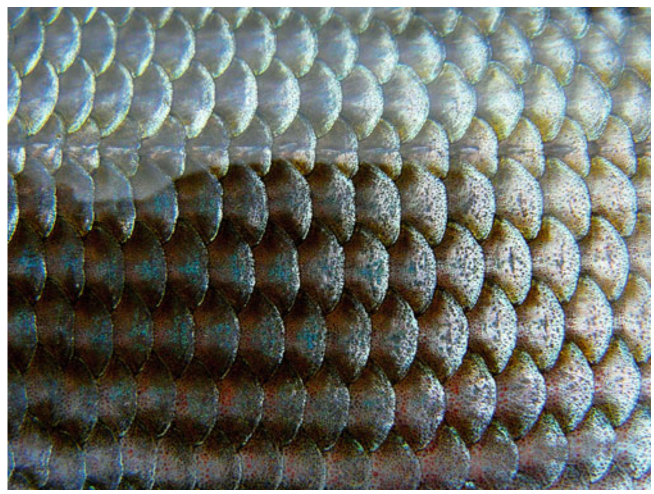

Fig. 2.39 Normal skin with scales of grayling, lateral line is visible

2.7.2 Visual

The fish eye is a delicate and highly specialised structure that is particularly vulnerable because of its exposure to the environment and absence of protective eyelids. The eye is similar to that of other vertebrates (Fig. 2.40) and its function is to collect and focus the light and convert it into a nervous impulse. The components of the eye are the cornea, iris, lens, sclera, choroid and retina (Fig. 2.41). The cornea is non-pigmented and consists of a stratified squamous epithelium on a thick basement membrane and has a refractive index similar to water and therefore, irrelevant as an optical surface. The lens is a spherical ball consisting of three layers: a first encapsulating sheath of non-transparent material which is secreted by the second layer, an underlying tissue of physiologically active cells which are nucleated and capable of division and secretion, and a third tissue immediately

Fig. 2.40 Eye of a grayling

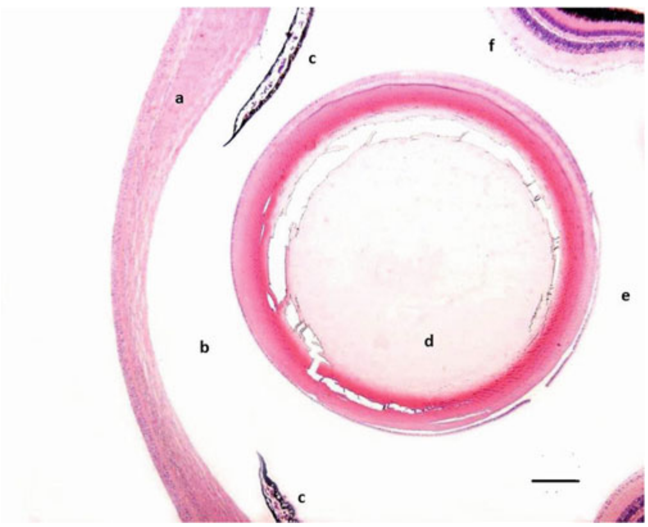

Fig. 2.41 Lateral view of the salmonid eye. (*a*) Cornea. (*b*) Anterior chamber. (*c*) Iris. (*d*) Lens (*e*) Vitrous body. (*f*) Retina

beneath consisting of non-nucleated long, slender, transparent cells in parallel rows that occupy the greatest volume. The lens protrudes through the iris providing a wide angle of view. In order to accommodate changing sight requirements, the retractor lentis muscle must be drawn inwards towards the retina. The iris is fixed as the sphincter and the dilator muscles are poorly developed. The innermost element of the eyeball is the photo-sensitive retina. Choroid vessels form a subscleral network of capillaries which provide nourishment for the retina. Eight layers are recognized: pigment epithelium elements, rods and cones, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer and nerve fibre layer (Fig. 2.42).

A counter current system is present in the choroid layer of the retina. This vascular structure (the choroid rete mirabile) is supplied by a branch of the ophthalmic artery and blood

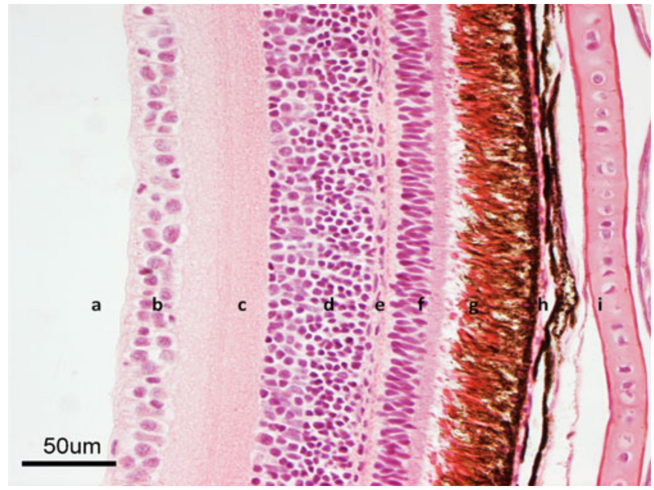

Fig. 2.42 Retina of coho salmon showing the various layers. (*a*) Vitreous body. (*b*) Ganglion cell axons. (*c*) Inner plexiform and ganglion cell layer. (*d*) Inner nuclear layer. (*e*) Outer plexiform layer. (*f*) Nuclei of rods and cones cells. (*g*). Rods and cones. (*h*) Pigmented epithelium. (*i*) Scleral cartilage

from of the choroidal vessels initially passes through the pseudobranch. The correlation between the oxygen pressure and development of the rete suggest that the choroid rete mirabile plays a role in establishing the high oxygen pressure of the retina.

2.8 Nervous System

The nervous system can be divided into the cerebrospinal system, i.e., the brain, spinal cord, ganglia, cranial and spinal nerves, and the autonomous or vegetative system, comprising ganglia and sympathetic and parasympathetic nerves that works in close integration and interdependency with the endocrine system. Overall the main function is integration and control of organs and the communication with the outside environment. Histologically, the nervous system cellular elements are the neurons and the neuroglia and consist of two basic types of cell: neurons and glial cells.

2.8.1 Brain

The brain has the same basic regions as that of other vertebrates, but the proportions between the anatomical units are different, particularly the mesencephalon with the optic lobes being very conspicuous (Fig. 2.43). The brain is traditionally divided into five different units: telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon. The protective layers around the brain are known as the meninges, and represent an important barrier against

Fig. 2.43 Exposed brain of adult Atlantic salmon

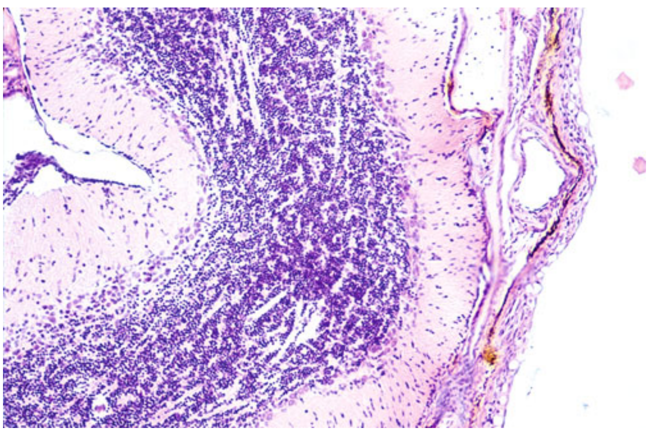

Fig. 2.44 Granular layer of cerebellum. Low power

pathogens between the blood and the cerebrospinal fluid (Fig. 2.44). They are small compared to its mammalian counterparts and are dominated by the olfactory lobes. In contrast to mammals, the histological conspicuous six-layered neocortex is absent.

The ventral part of the diencephalon, including the hypothalamus, is termed the infundibulum and is well developed in salmonids. Three important proteins are produced here and important components of the cerebrospinal fluid.

The telencephalon structures are located in the ventral midline of the diencephalon: chiasmaopticus, the pituitary gland (hypophysis) and a ventral choroid plexus called 'saccus vasculosus'. In the optic chiasma, the nerve fibres from the retina are crossing before entering the brain. The complex pituitary gland is a neuro-epithelial structure orchestrating several other endocrine organs and is involved in osmoregulation, gonadal development, growth and melanization. The saccus vasculosus is a sac-like structure with blood sinusoids quite similar to the choroid plexus of the eye, and communicates with the lumen of the third ventricle. On the dorsal side of the diencephalon is the pineal gland (epiphysis). This is a well vascularised non-image forming photoreceptor structure, located under a thin non-pigmented area of the skull that permits light to reach the structure ('pineal window'). This structure can detect alterations in ambient light (decreasing light in fall, increasing light in the spring) and is of importance for regulation of seasonal physiology such as smoltification. The mesencephalon is highly developed and conspicuous with its two optic lobes (tectumopticum) reflecting the importance of vision for these fish. Centrally, there is a large lumen (ventriculus mesencephali) filled with cerebrospinal fluid. The metencephalon has a well-developed, partly folded dorsal part called the cerebellum, responsible for movement coordination. The myelencephalon is the origin of the cranial nerves and the beginning of the spinal cord (medulla oblongata).

2.8.2 The Sympathetic and Parasympathetic Ganglions and Nerves

The sympathetic and parasympathetic ganglions and nerves of the autonomous nervous system are responsible of regulating several functions and mostly antagonist responses to that of the cerebrospinal system, as both innervate most internal organs including digestive tract, heart and gills.

2.9 Endocrine System

Glandular derivatives such as thyroid, thymus and ultimobranchial bodies are formed from the pharynx during embryological development. Thyroid hormones play a supportive role in sea water acclimation and follicles are distributed throughout the connective tissue of the pharyngeal area. They may also be observed around the eye, ventral aorta, hepatic veins and anterior kidney, and are similar histologically to mammalian thyroid tissue (Fig. 2.45). The thymus is located on the dorsolateral wall of the pharynx with its ventral surface covered with mucosal epithelium (Fig. 2.46).

At the junction of the head and posterior kidney are the corpuscles of Stannius, a sac-like body which has an endocrine role in calcium metabolism. The corpuscles of Stannius are not always visible macroscopically and may be embedded deep in the renal tissue (Figs. 2.47 and 2.48). Variable amounts of individual or clustered pigment-

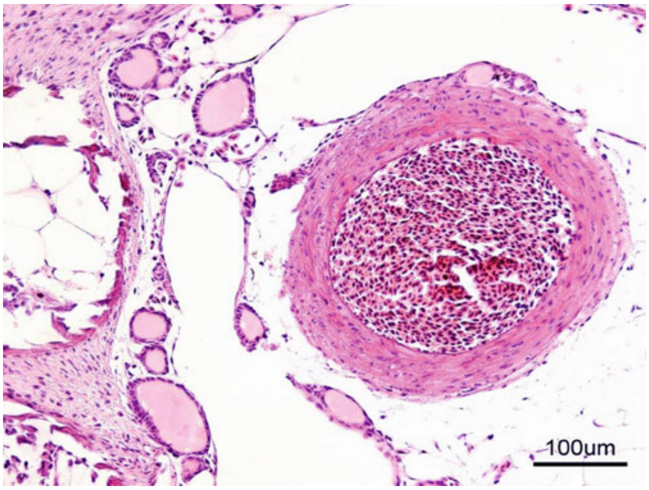

Fig. 2.45 Thyroid follicles located near the ventral aorta of an Atlantic salmon parr

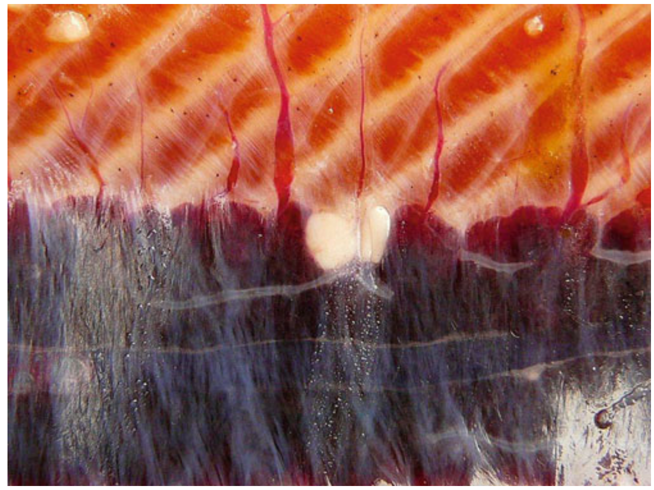

Fig. 2.47 Corpuscle of Stannius near the margin of the kidney in brook trout

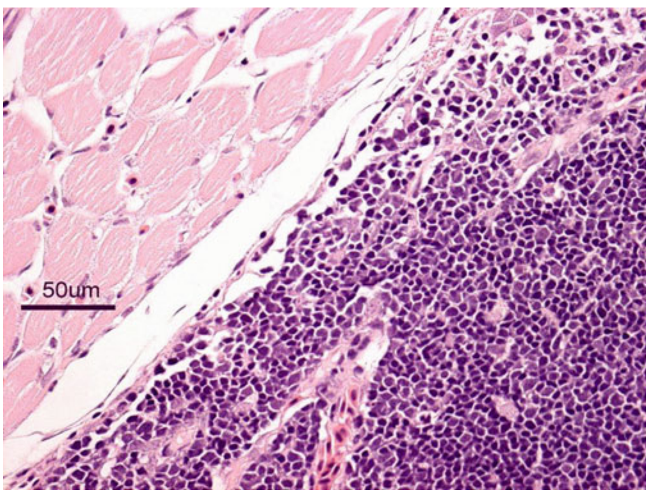

Fig. 2.46 Thymus of Atlantic salmon parr

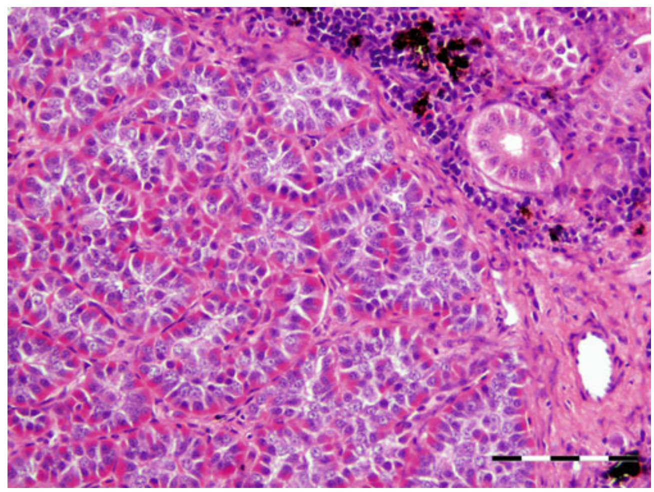

Fig. 2.48 Corpuscle of Stannius (*left*) within the kidney of Atlantic salmon. Bar = 100 µm

containing melanomacrophages are normally present in the interstitial renal tissue. Their number typically increases with age and with disease conditions. The kidney also contains endocrine elements such as chromaffine cells located in the wall of the posterior cardinal vein that release adrenaline and noradrenaline into the circulation, and interrenal tissue which in most teleosts is located around major veins (Fig. 2.49).

Ventral to the oesophagus in the septum transversum separating the heart from the abdominal cavity is the small endocrine ultimobranchial gland, derived from the pharynx. This secretes the hormone calcitonin which lowers serum calcium levels and acts with hypocalcin (secreted by the corpuscles of Stannius) to regulate calcium metabolism. The epiphysis projecting from the epithalamus produces melatonin.

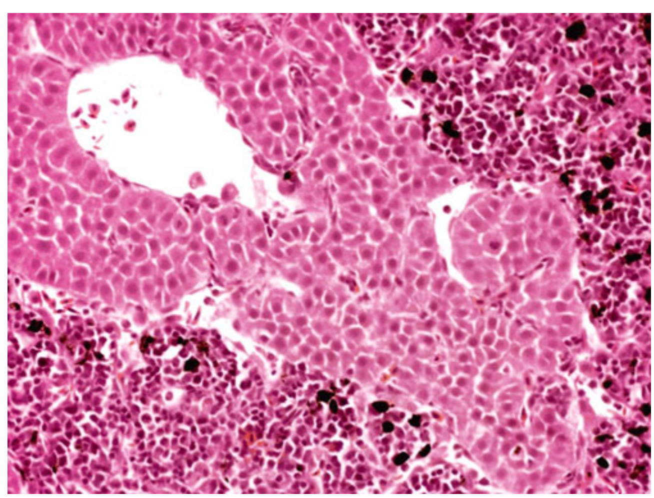

Fig. 2.49 Adrenal cortical tissue in head kidney from rainbow trout. Note melanomacrophages in renal interstitium. Medium power

2.10 Reproductive System

2.10.1 Ovary

The ovaries are paired sac-like organs in the dorsolateral part of the abdominal cavity (Fig. 2.50). They are composed of germinative, stromal, vascular and nervous tissues and suspended in a mesenterium called the mesovary, from the roof of the abdominal cavity. The oviduct is not complete and the ripe eggs are shed into the posterior part of the abdominal cavity before they are funnelled through the urogenital papillae.

In immature fish the ovaries appear as small yellowish or orange spheres. The ovarian follicles line a hollow cavity and ova are passed into this cavity as they mature. The maturing oogonia are surrounded by a single layer epithelial cells and this aggregate of ova and epithelial cells is known as the ovarian follicle. The epithelial cells grow as the ovum develops and are separated by a gradually thickening hyaline capsule called the 'zona pellucida'.

During maturation the oogonia are referred to primary and secondary oocytes. The oocytes enlarge as yolk granules are included into the cytoplasm (vitellogenesis) and the follicular epithelium thickens. With continued oocyte growth the ooplasm becomes impregnated with yolk granules. Several other morphological changes also take place towards the end of the growth phase of the eggs. For example, the eggs become translucent due to the coalescence of yolk globules. At sexual maturation, the eggs may almost fill the abdominal cavity. Examination of ovaries undergoing active oogenesis indicates that oocyte development is not synchronous, i.e. oocytes of varying sizes and in different phases of vitellogenesis are present. Histologically, the ripe egg is characterized by a translucent cell membrane and a distinct animal pole with the nucleus at the micropyle.

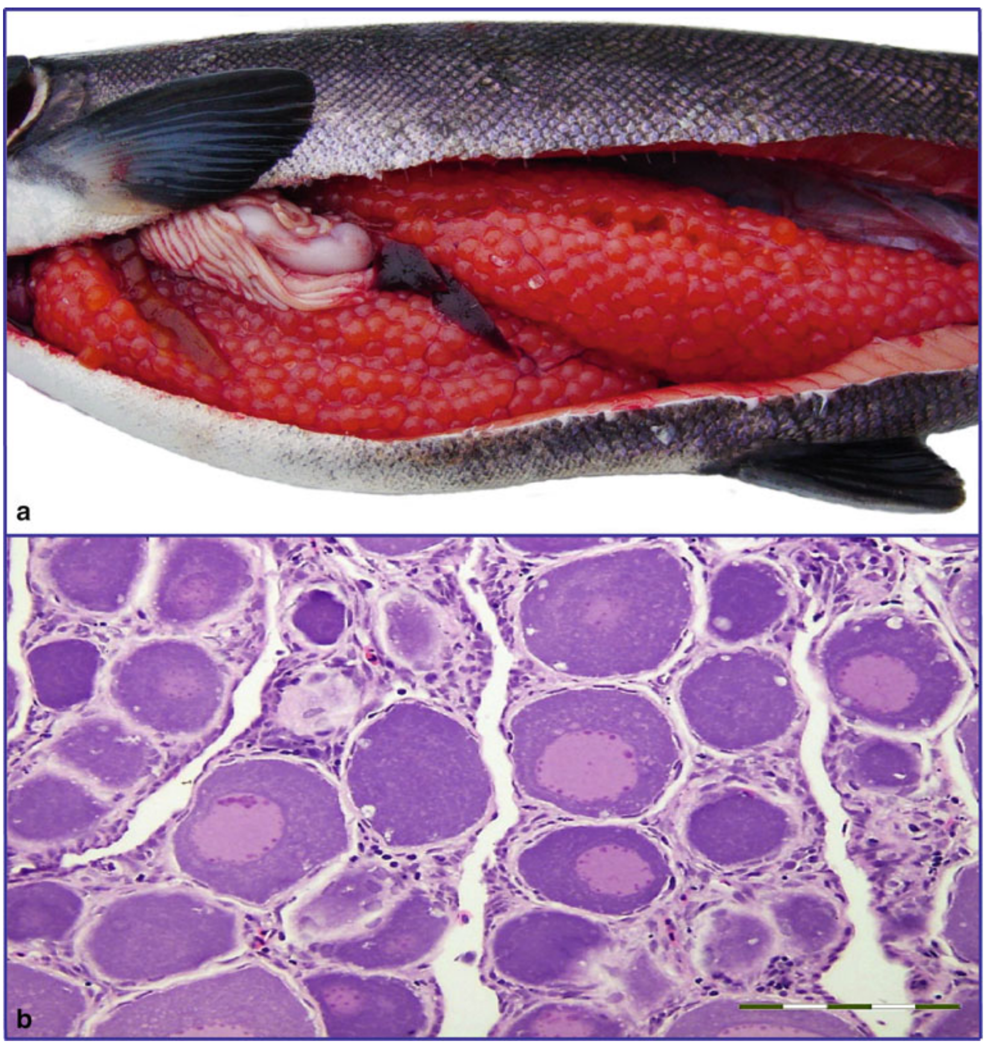

Fig. 2.50 (**a**) Ovaries in mature Atlantic salmon. (**b**). Developing oocytes in a rainbow trout fry. Bar = 100 μm

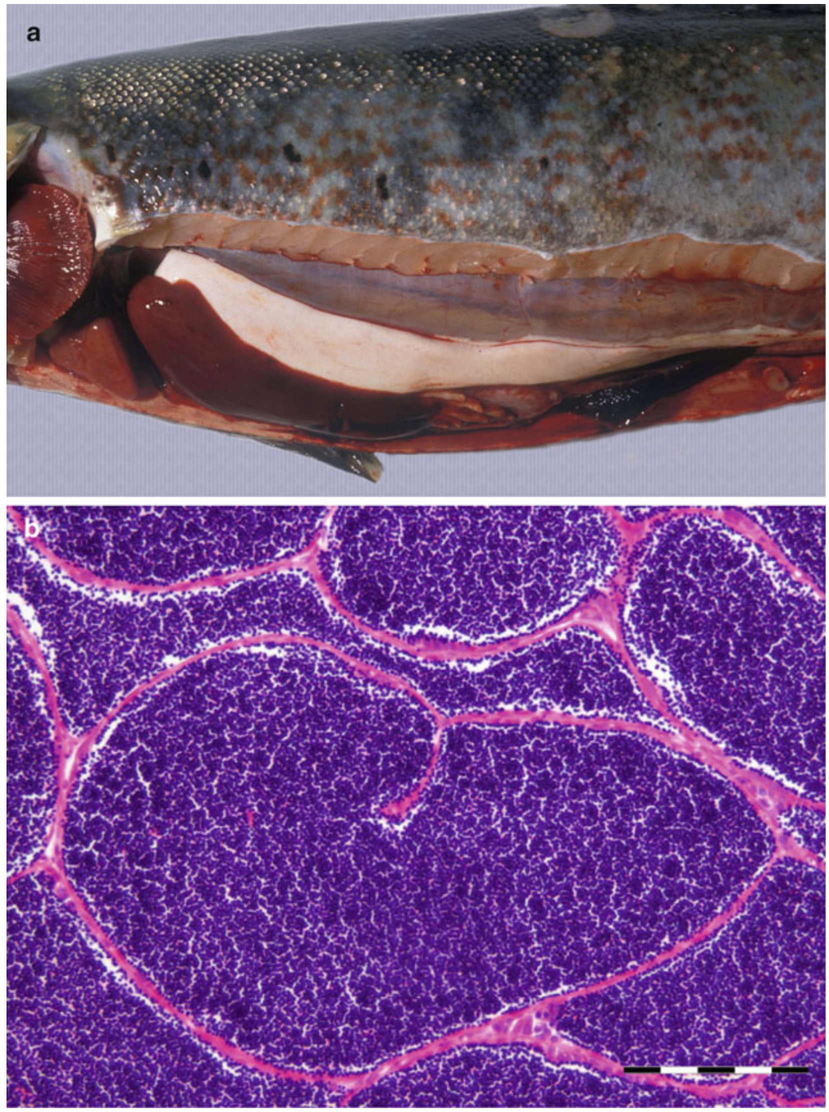

Fig. 2.51 (**a**) Mature testes from Atlantic salmon. (**b**) Lobules packed with spherical spermatogonia of varying diameter

2.10.2 Testes

The testes are a pair of sac-shaped organs surrounded by a capsule of connective tissue suspended from the abdominal roof by a mesenterium called the mesorchium. In juveniles, they are thin strands while they may be large white flabby organs in mature males (Fig. 2.51). The process of maturation of the male gamete involves the multiplication of spermatogonia or sperm mother cells, which develop from the spermatogenic epithelium to form spermatocytes. Many of these cells eventually undergo a meiotic division to become haploid spermatozoon with head, middle section and a long tail. The earliest stages of spermatogenesis are the primordial germ cells. Some of these divide to form primary spermatogonia and then divide to form cysts of spermatocytes, while others remain quiescent. During maturation lobules packed with spherical spermatogonia of varying diameter become evident as the lobules divided into cysts contain spermatogonia in different stages of development. Cells with mitotic figures are apparent. Most cysts contain primary and secondary spermatocytes and a few contain spermatids ready to be discharged into the lumen of the lobule. A main collecting duct termed the 'vas deferens' collects the mature spermatozoa to an excretory meatus at the urogenital papilla.

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