Necropsy Techniques for Fish

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Fish medicine has become more mainstream within the veterinary profession over the past two decades. Aquarium hobbyists, public aquariums, and private facilities are relying more and more on veterinary expertise for fish health problems. Most problems in fish health are related to water quality and other husbandry issues; the necropsy is only one important part of a complete work up. As with necropsy of exotic terrestrial animals, an understanding of the basic anatomy and physiology of a few common fish species can help the practitioner gain confidence when working with more exotic fish. However, because species differences do exist, protocols may require modification. Moribund or fresh dead specimens are best. With fish, perhaps more than any other class of animals, wet mount biopsies of external and internal tissues are very informative. Parasites are an important contributor to disease, and most easily identified alive. The delicate structure of the gill and the rapidity with which it can break down also necessitates careful analysis at the outset. Likewise, other pathology can be identified quickly using wet mounts. Microbiological culture of kidney, brain, liver, spleen, and other affected organs, and histopathology are also important aspects of the fish necropsy. Cultures should be incubated at temperature ranges similar to those for the fish. Certain viruses, including Spring viremia of carp (SVC) and koi herpes virus (KHV–not *Herpesvirus cyprini*, the causative agent of carp pox) are important pathogens that require confirmatory virus isolation and culture. SVC is a notifiable disease.

Key words: Aquarium, fish, necropsy, biopsy, anatomy, pathology.

Fish health is an important and diverse area of opportunity for the veterinary profession. Aquarium fish are the number one pets in the U.S. in private water gardens, stocked with koi and goldfish, and maintain popularity. Public and private aquarium facilities are increasing in numbers. Besides traditional stand-alone public aquaria, large indoor and outdoor exhibits are routinely integrated into offices, restaurant chains, outdoor stores, hotels, and other facilities. Health of breeding populations of endangered species is a concern, and fish are important research animals. Finally, the health of natural fish populations continues to provide information as an indicator of environmental health.

Importance of History and Management

The necropsy can help determine causes of morbidity and mortality in fish. However, more so than in other classes of animals, the importance of history and environmental factors cannot be overlooked.[1-3] The vast majority of disease problems in fish are linked intimately to water quality and management issues, factors that do not always translate into directly observable pathology. Clinicians expecting to determine cause of disease based solely on biopsies or necropsy will be disappointed, as will their clientele. Complete information on the life support systems, water quality, husbandry/general management, the fish, and the problem is critical. The following is not a complete list, but major issues include:

Life Support System (LSS)

Includes the physical system set up including dimensions of the tank(s) or pond (s); filtration components; plants; invertebrates; length of time established; substrate; pipe/system materials; and if an outdoor pond, access to birds and/or predators.

Water Quality

Includes water source (municipal, well, other; water treatment protocols); history and measurements of ammonia, nitrite, nitrate in marine systems, pH, temperature, dissolved oxygen, hardness, alkalinity, salinity and heavy metals and other toxins.

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General Husbandry/Management

Includes maintenance protocols for life support systems (water changes, cleaning, filtration backwash, and component checks); sanitation, disinfection, and quarantine protocols; nutrition (live foods, prepared diet, and storage protocols); chemical/salt usage; general fish keeping/breeding experience of client; and overall expectations/purpose of the fish.

Fish

Includes specific biology and requirements; familiarity of client with species; other fish of the same or different species in community systems and their compatibility; and recent introductions.

Problem

Includes the chief complaint and client's perspective; previous problems; major changes in any of the categories listed above; chemotherapeutants used so far; morbidity/mortality time course; behavioral changes and visible lesions.

Indications for Necropsy

If evaluations of history, life support systems, water quality, and husbandry or management are not adequate to solve the problem, nonlethal techniques including biopsy of skin, fins, and gills; examination of fresh fecal samples; and blood culture may be all the additional information required.[4] However, if a client is interested in a thorough evaluation of a problem, a necropsy is required. Likewise, if a community or population of fish is at risk, necropsies should be performed to fine-tune treatment and prevention.

Importance of Sample Selection

Good sample selection is critical. Moribund fish showing typical disease signs are preferable. If several fish can be killed when evaluating a population, fish should be chosen at different stages, including at least one with early stage disease. Fish with late stage disease often have secondary complications that mask initiating causes.

For large populations of low disease prevalence with mortality as the only sign, random sampling may result in the selection of healthy fish for evaluation. If necropsy samples reveal no significant findings (and environmental and management factors have been carefully evaluated and ruled out), evaluation of fresh dead fish can be helpful. In general, fresh dead fish should have relatively clear eyes, good coloration, red to pink gills, and should not have a bad odor. Because of the rapidity with which dead fish are cannibalized, autolyze, and/or take on secondary organisms (such as fungi, bacteria, and parasites including the ciliates *Tetrahymena* or *Uronema*), the significance of lesions on dead fish should be regarded cautiously.

Although moribund fish are ideal specimens for post mortem work ups, fresh dead, frozen, and formalin-fixed specimens can provide useful information.[1] Fresh dead fish examined immediately or kept in a plastic bag in the refrigerator or on ice for 6 to 12 hours can provide almost as much information as moribund fish.[1] Frozen and formalin-fixed fish are much less informative.[1] Common protozoal parasites and monogeneans often die or leave a dead host. Cultures collected from fish that have been dead in water for as little as thirty minutes to sixty minutes may be contaminated due to decomposition and autolysis.

Basic Fish Anatomy and Physiology

Fish species vary widely in their anatomy and physiology. Several popular fish species from two important families, the Cyprinidae (koi–*Cyprinus carpio* and goldfish–*Carassius auratus*) and the Cichlidae (discus–*Symphysodon* sp. and Oscar–*Astronotus ocellatus*) highlight some of the similarities and differences in gross anatomy. Several good references provide additional species information.[2,5-9]

External Structures/Organs

Skin

The skin consists of a mucus layer, epidermis, and dermis. Epidermal layers contain numerous types of cells including taste cells, alarm cells, pigment cells, and mucus-producing cells. In addition to other functions, mucus acts as a

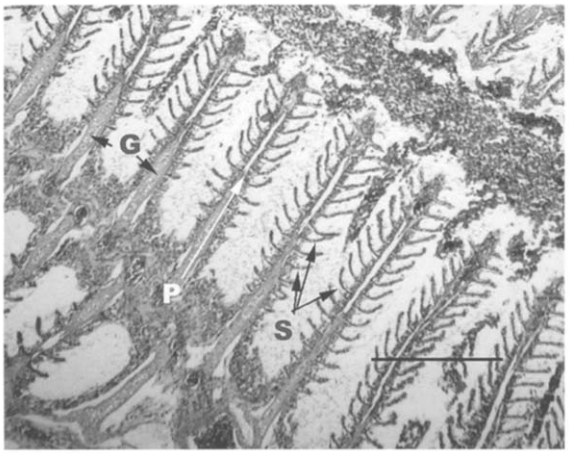

Figure 1. Primary gill lamella (P) are supported by gill cartilage (G); secondary lamella (S) are visible as plate-like structures extending from the primary lamella Bar ~ 250 μm H & E stain.

lubricant to decrease friction in swimming, helps provide a barrier against and prevents the attachment of pathogens, and contains biomolecules important for immune defense. The fish exterior generally contains either 1) scales (originating from the dermal layer and comprised of a type of bone) that are covered by the epidermis (eg, koi, goldfish, cichlids, tetras, and loaches–that have very fine scales); 2) no scales (eg, channel catfish); or 3) bony plates (eg, *Corydoras* sp. catfish, Plecostomus catfish [*Hypostomus* and related genera], seahorses [*Hippocampus* spp.]). The lateral line system, comprised of a series of very small pits, is known as the organ of distant touch. Among other functions, it facilitates schooling behavior. Injury or lesions to the skin can result in significant osmoregulatory compromise and increased chance of infections.

Gill

The primary respiratory apparatus in fish, the gills evolved to maximize extraction of oxygen from water. The gills are also the sites for carbon dioxide and ammonia removal from the body. In most teleosts (bony fishes), there are four gills on each side. Fine structure is comprised of primary and secondary lamellae. (Fig 1) Primary lamellae contain a specific type of cartilage (gill cartilage) for structural support while secondary lamellae branch off the primary lamellae. A grouping of primary and secondary lamella (gill filaments) can be thought of as having a "Christmas tree" (three-dimensional) structure. Because the epithelium on secondary lamellae is one cell thick and the surface area of the gills is large, gill compromise is a constant threat.

Pseudobranch

The pseudobranch is anatomically related to, and resembles the gills, but is not involved with respiration. The pseudobranch is located on the dorsal section of the inner operculum, and may or may not be superficial enough to be seen grossly. In cyprinids, it is deeply embedded. The pseudobranch is a hemibranch, only containing filaments on one side. Proposed functions include provision of gases/oxygen to the eye, osmoregulation, or sensory capability, but these are still debated. Some clinicians believe pathophysiology of the pseudobranch may contribute to idiopathic exophthalmia in some species.

Operculum

The operculum is the gill covering or gill flap which is important for respiration. Oxygenated water passes through the mouth, enters the oral cavity, passes through the pharynx, enters the opercular cavity (where gills are located), passes over the gills, and then out the opercular valve (gill slit). This direction of water movement over the gills is important for gas exchange. The operculum and gill slit are highly modified in some species (eg, seahorses), making ante-mortem biopsies of gills difficult. Rate of opercular movement is used to monitor respiration. The males of some fish (eg, Cyprinids, including goldfish) develop small white dots/bumps known as nuptial tubercles during breeding season on their opercula and anterior pectoral fins.[10]

Finnage

The types of finnage present depend on species, and sexual dimorphism exists in many species (Fig 2). The caudal and dorsal fins are primarily locomotory. The dorsal fin acts as a stabilizer, and in some species, may have venomous spines. The pelvic fins are also stabilizers and, in some fish, such as angelfish and gouramis, are sensory. The adipose fin, or "fatty" fin, present in some fish groups, including most tetras, is located behind the dorsal fin. The anal fin

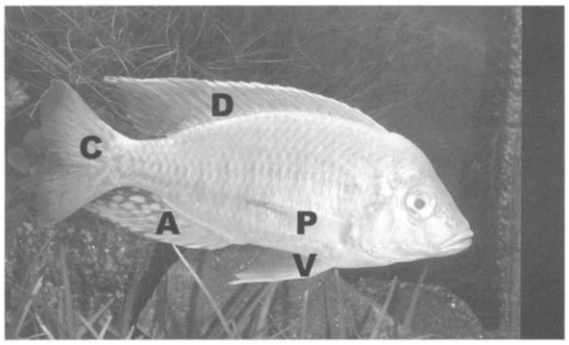

Figure 2. Fin types in an African cichlid. A: anal; C: caudal; D: dorsal; P: pectoral; V: pelvic.

is a stabilizer but has become a modified male reproductive intromittent organ in the Poeciliidae family (swordtails, guppies, mollies, platys) and some other groups. Pectoral fins of some mature males may have nuptial tubercles.[10]

Mouth

The location of the mouth on the head (ie, more ventral, terminal, or dorsal); presence of jaw teeth and/or pharyngeal teeth (teeth located in throat region of many types of fish); and overall structure of the oral cavity can differ significantly, depending on type of food/prey eaten. Some species, including many African cichlids, arowana (*Osteoglossum* and *Scleropages* spp.), and jawfish (*Opistognathus* spp.) use their mouth to brood developing eggs or fry. The mouth may also be used for nest building and for territorial or breeding displays or fighting.

Vent/anus/genital pore

If the intestinal tract, urinary tract, and gonadal ducts empty into a common chamber (cloaca), then the common opening to the outside is known as the vent. If the intestine/digestive system opens out separately, this opening is known as the anus, and the reproductive opening is known as the genital pore. Some fish species can be sexed based on location and form of these structures.

Eyes

Because fish are aquatic, they lack tear glands and most lack movable eyelids. Muscles attached to the spherical lens move it toward or away from the retina for accommodation. The lens refracts light by means of successive differentially dense layers. Fish eyes are particularly prone to external injury and physiopathological or infectious processes leading to exophthalmia or buphthalmia.

Nares

The nares are the "nostrils" of fish and are olfactory in function.

Internal Structures/Organs

Brain

The central nervous system. Squash preps of the brain are not typically informative but are important for culture and histopathology.

Muscle

Muscle of fish is similar to that of other vertebrates. There are three major types: skeletal ("filet" muscles), smooth (eg, gastrointestinal tract musculature), and cardiac (heart).

Swim bladder

Also known as the gas or air bladder, it is important for maintaining neutral buoyancy in water and, thus, conserving energy. In some species, it may also be used for sound production or respiration. Swim bladders are either physostomus (connection to the esophagus is patent, and air moves in through this opening) or physoclistous (there is no connection, and air enters via a vascular rete, or "gas gland"). In Cyprinids such as koi and goldfish, it is bi-lobed (anterior and posterior chamber, separated by a sphincter) and easily removed still inflated from the body. The caudal chamber connects to the esophagus.[2,5] In other species, such as discus, an intimate association with the lateral body wall, makes it difficult to dissect out without puncturing it. Depending on species, it may be more translucent or somewhat opaque, thick or thin. It is usually located beneath the kidney.

Kidney

Usually red/brown in color, kidney functions include hematopoiesis, excretion, and immunity. Melanomacrophage centers (MMCs), black or brown clusters of cells, are commonly seen in

the kidney, spleen, and/or liver, depending on species. MMCs are believed to play a role in immune function and degradation of senescent cells. The kidney is an important part of the reticuloendothelial system (RES), common to all vertebrates. The RES functions to remove particulate matter, senescent cells, and infectious agents from the body. The kidney lies ventral to the spinal cord and dorsal to the swim bladder in most species. The kidney can be divided into two anatomic and physiologic sections in most fish. The anterior is primarily hematopoietic and the posterior is primarily excretory.

Liver

The liver functions are similar to those in other vertebrates. In most teleost species, this organ is more properly called the hepatopancreas (contains exocrine pancreatic tissue which can also be found in other areas in many fish). Coloration can vary from pale tan to brown to red, depending on species, diet, nutritional status, and reproductive status. It is often one of the largest/most visible organs in the anterior portion of the coelomic cavity. In some species such as koi and goldfish, the liver is intimately intertwined with the intestinal tract.

Spleen

The spleen is important for immune function (MMC's, RES) and hematopoiesis. It is the smaller, dark red organ frequently located close to the stomach and may be triangular, oval/rounded, or more elongate in shape.

Heart

Located in the pericardial cavity (separate from the coelomic cavity), in the throat region, caudal and ventral to the gills. The heart is two-chambered (atrium, ventricle), but contains a thin walled sinus venosus leading into the atrium, and a thick, elastic bulbus arteriosus, leading out of the ventricle. It is typically not very informative on squash preps but important for histopathology. The heart is also part of the RES.

Gonad (ovary and/or testes)

Sexual reproduction in fish is diverse and many species exhibit sexual dimorphism. Size and structure of the gonad and developmental stage of oocytes can vary greatly depending on species, age, reproductive status, and season. During breeding periods, size of the gonad, increases greatly and often fills the coelomic cavity, displacing other organs. Most common freshwater aquarium species, and many marine species, are dioecious (separate sexes). Many common marine fish species are protandrous (male first, then female) or protogynous (female first, then male) hermaphrodites. The females of some species, such as the common live-bearing Poeciliids (swordtails, guppies, and related groups), hold sperm in their ovaries for various lengths of time. One common error is mistaking sperm for flagellates, especially since the testes are closely apposed to the intestine at the vent/anus. In general, sperm are much shorter lived than flagellates on a slide, and will usually die within a minute or two.

Stomach

The stomach is typically thicker walled than the intestine, and gastric glands/mucosa are distinct compared with intestinal tract lining on wet mounts and histology. A true/distinct acid stomach is not present in Cyprinid species such as goldfish and koi. It is very important to examine the stomach in cichlids for the presence of parasites and/or granulomas.

Intestinal tract

Usually thinner walled than the stomach, it is typically shorter in carnivores (eg, Oscars) and longer in omnivores (koi, goldfish) and herbivores (eg, plecostomus).

Clinical Observations

Clinical observations of live fish before necropsy can help identify clinically diseased animals and pinpoint location of lesions. Sick fish typically isolate themselves and are darker or lighter than their conspecific tank mates. Other aberrant behaviors, such as piping at the surface, may indicate gill pathology; flashing (scratching) may indicate skin irritation and/or parasitism; aberrant positioning may indicate swim bladder disease or gastrointestinal disease; and spinning or aberrant swimming may indicate neurologic disease.[8,11]

Necropsy Equipment and Supplies

General Supplies

A compound microscope, a dissecting microscope or magnifying lamp (helpful for small species) a pathology report form, latex gloves, scissors (small (ophthalmic), medium, and/or large, with blunt tips and with pointed tips), scalpel, rat tooth forceps, microdressing forceps, necropsy tray/table (depending on size of fish), slides, cover slips, microscope, paper towels, needles/syringes (for blood sampling), dechlorinated freshwater (or salt water) for wet mounts, tricaine methanesulfonate (TMS or MS-222) (Finquel®, Argent, Redmond, WA; or Tricain-S®, Western Chemical, Ferndale, WA, USA), and sodium bicarbonate (baking soda) are general supplies for performing necropsy. More specific equipment may be required for larger fish or certain species (for example, larger sturgeon may require a drill, dremel tool, or saw to cut through the neurocranium to culture the brain).

Microbiological supplies

Culture swabs (for submission to a microbiology laboratory), or microbiological media (tryptic soy agar (TSA) with 5% sheep's blood plates; Sabouraud's dextrose agar or other media as indicated), sterile loops, sterile gauze, alcohol burner, glass jar/container for instruments, alcohol pads, matches or barbeque grill lighter are the basic microbiological supplies.

Histopathology

Ten percent neutral buffered formalin (for light histopathology); Trump's solution (for electron microscopy); specimen jars are the basic histopathology supplies.

Wet Mount Evaluation

Use of MS-222 before biopsy of external structures has been shown to result in significant loss of some parasites from the body.[12] Before euthanasia by lethal dosing with MS-222, biopsies of the skin, gills, and fin should be evaluated. With experience, the clinician can do this quickly and with minimal distress to the animal by holding the fish in the folds of a net and exposing each area to be sampled one at a time. Latex gloves should be worn to avoid additional damage to the skin and to avoid accidental removal of external parasites or lesions. Also, be gentle when using a net to avoid accidental removal of external parasites from the body. Covering the eyes of the fish can help decrease stimulation and reduce movement. Any abnormalities including areas with discoloration, increased mucus, hemorrhages, erosions, ulceration, masses, or parasites should be sampled as described in the following section.

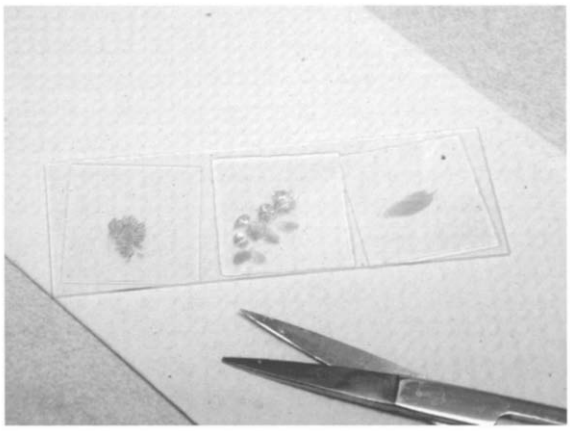

Figure 3. Gill, skin, and fin clips on a slide. Small samples are more easily examined.

Thorough evaluation of wet mounts is a mandatory part of a good necropsy. Many important findings, including external and internal parasites, are best observed and quantified in this manner.

Slides are prepped to receive biopsy samples by placing three small drops of water equidistant on a slide. This will allow for three different biopsies to be evaluated per slide. Once taken, each biopsy should be placed on one of these drops of water with a cover slip. (Fig 3) For external (skin, gill, and fin) samples, it is important to use dechlorinated freshwater (do not use distilled water) for freshwater fish. Use salt water for saltwater fish. For internal samples, freshwater should suffice for both freshwater and marine fish species. If the clinician is not yet comfortable identifying organs on wet mount, slides should be labeled with a marker according to the organ to reduce confusion. In most instances, only a small section (2 to 3 mm^3) of organ is necessary. Larger sections of fins can be examined. Excess tissue leads to thick preps that

are dark and difficult to examine. Larger fish often have more fibrous tissue in their internal organs making squash preparations a bit more difficult.[1]

During microscopic examination of squash preps/wet mounts, the clinician should use a methodical search pattern. A quick scan at 40X (low power) will help with orientation and preliminary identification of areas of interest. Manipulation of the condenser to decrease the aperture will help increase contrast and simplify observation of smaller structures/parasites, as will the use of phase contrast. Most parasites and lesions of interest should be visible at 40x-100x power. However, at 100x, only the movement of some smaller parasites (such as the flagellates *Spironucleus* and *Ichthyobodo*) may be discerned, with more structures visible at 400x. Microsporidian parasites are also very small (approximate range, 1 to 5 × 2 to 8 μm),[13] and although their cysts (sporoblastic vesicles) or xenomas are visible at higher powers, individual spores, which are nonmotile, are more clearly seen at 200 to 400x. *Flavobacterium columnare*, the cause of columnaris disease, is a long, rod-shaped Gram-negative bacteria that can often be found flexing in haystack formations.[1,2] These are most commonly appreciated at 200 to 400x.

Skin, Gill, and Fin Biopsy

Skin

Using a cover slip or the back of a scalpel blade, gently scrape any areas that appear abnormal (increased mucus, erosions, masses) (Figs 4 and 5). In addition, scrape areas that are either softer, have fewer scales, and/or experience reduced water flow (such as areas on the head, ventrum, and behind the pectoral fins). These areas have a greater chance of harboring parasites. Scrapes made near the vent/anus may result in expression of sperm in mature males, and should not be confused with flagellates.

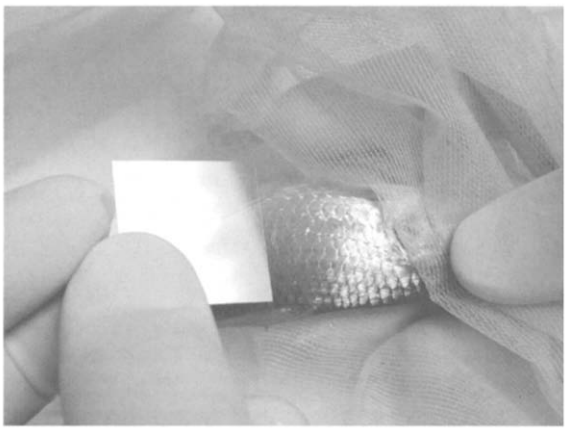

Figure 4. Skin scrape using cover slip.

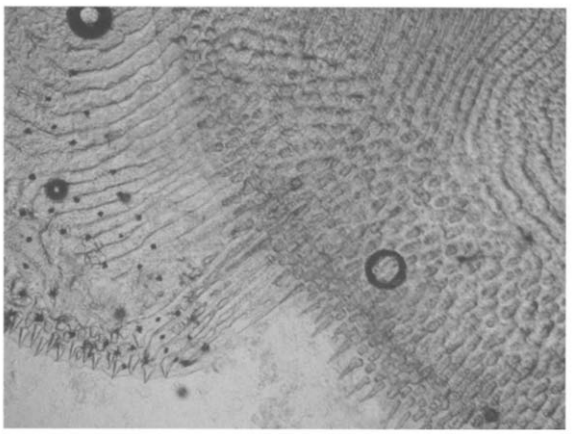

Figure 5. Normal scales and mucus from skin scrape. 100x. Note growth rings on ctenoid type scales ("teeth" or comb located at bottom margins of the scale; most visible near bottom of figure), and pigment cells.

Gills

Lift the operculum (the scissor blades can be used as a lever to do this or forceps can be used) and cut a small section of the gill tips (Fig 6). If there are any specific areas that appear abnormal, target these areas. After the fish has been euthanized, a section of the gill arch (base of gill where filaments connect) should be examined by wet mount and saved in fixative, especially if areas of the gill distal to this section appear pale or necrotic. At least one fungus, *Branchiomyces*, may be found more commonly at this location if present.[14] Because of the gill's delicate structure and location, gill pathology is often the first sign of poor water quality, bacterial disease, or parasitism.

Fins

Cut a small section from the dorsal fin, caudal fin, pectoral fin, and anal fin, and examine each. (Fig 7) For better evaluation of dark, pigmented

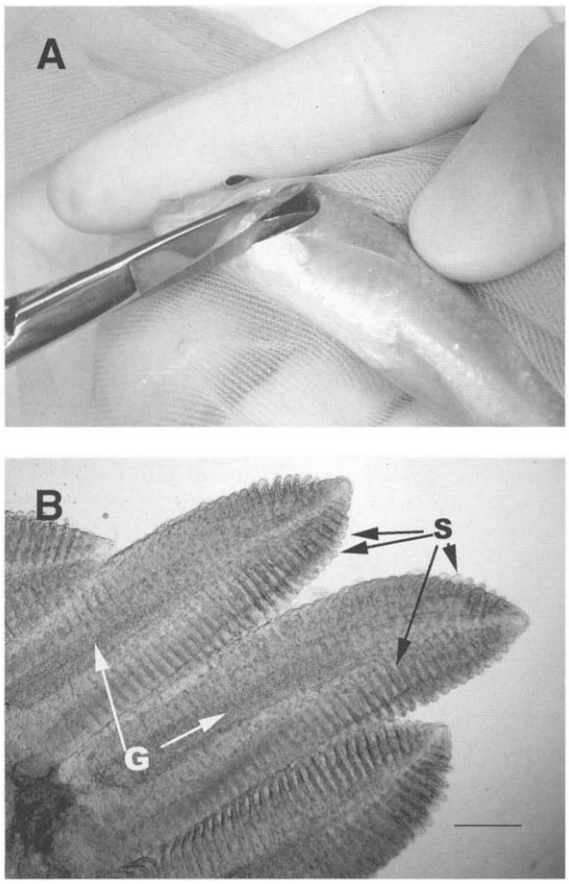

Figure 6. A) Gill biopsy. Scissors are used to open the operculum and cut the tips of the primary lamella; do not cut the gill arch at this step. B) Gill wet mount: Primary lamella are supported by gill cartilage (G). Secondary lamella (S) are shown. Bar ~ 250 μm.

skin or fins, the clinician should flatten the sample completely, increase microscope lighting (but keeping the condenser stopped down for contrast), and focus up and down through several different planes to look for parasite movement. The novice may mistake pigmented cells for pathology. Check along the edges of dark fins, skin, or scales, and in the water immediately surrounding these areas.

Parasites in Fish

Understanding fish parasites can appear to be a daunting task. External parasitism is a common cause of "flashing" (fish rubbing against something in their environment) and parasites are frequently encountered during necropsy. Many important groups are uncommon in other classes of animals. The following is a very brief discussion of a few important groups, and should only be considered a starting point. For comprehensive coverage including pictorials, refer to several excellent texts.[1,2,8,13,15,16] It behooves the fish clinician to understand the significance and life cycle of common parasites.

The most common group, the ciliated protozoans, includes *Ichthyophthirius multifiliis* (white spot, Ich) (Fig 8) in freshwater fish and *Cryptocaryon irritans* in marine fish (marine Ich). Both species have a fairly broad host range, can multiply rapidly, from one to several hundred in one generation, and require multiple treatments. Consequently, observation of just a few of these on a fish is cause for concern. Other ciliates, such as *Trichodina* and *Epistylis* (and other stalked ciliates), can be used as an indication of water quality conditions, since both are more common in highly organic waters.[1,2,16]

The flagellated protozoans (including *Ichthyobodo necator*, found externally, and *Spironucleus vortens* and *Cryptobia iubilans* (Fig 9), both found in the gastrointestinal tracts of cichlids, are among the smallest of the parasites of fish. For example, *Ichthyobodo necator* is approximately 10 × 5 μm and consequently, is much more easily identified initially by movement. All can cause significant damage to their hosts, although granuloma formation is associated specifically with *Cryptobia iubilans*.[17,18] The dinoflagellates *Amyloodinium* and *Piscinoodinium* (Fig 10) are nonmotile, oval to rounded, granular, brown/yellow/or green, and can also be a cause for alarm. Their life cycles are similar to those of the

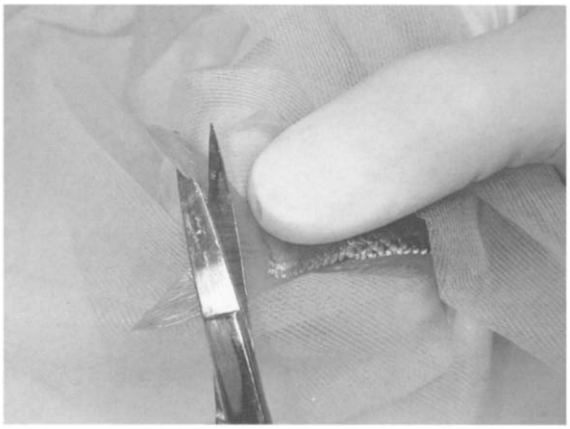

Figure 7. Fin biopsy. Only a small section is required for examination (target lesions).

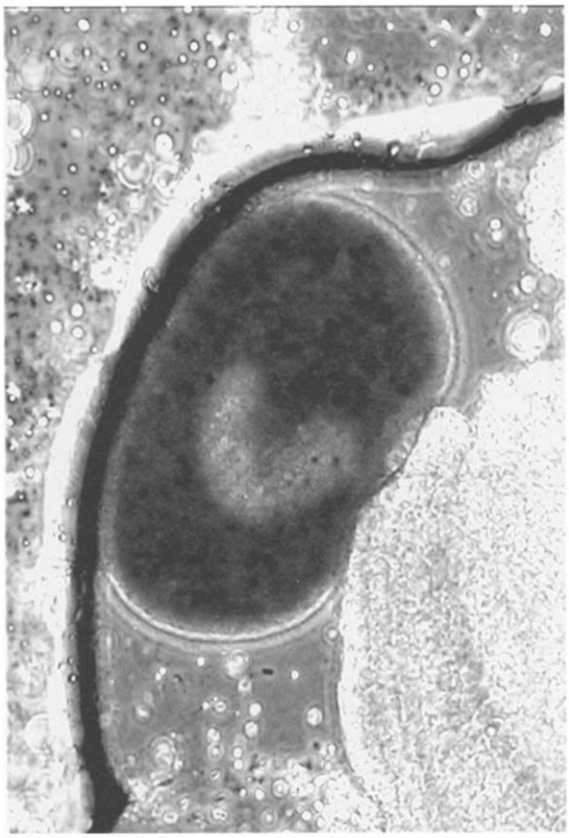

Figure 8. *Ichthyophthirius multifiliis.* 100x. Ciliated protozoan, causative agent of freshwater "Ich" or white spot. *Ichthyophthirius multifiliis* is one of the largest protozoan parasites of fish. Note U-shaped macronucleus.

"Ichs" and one parasite can become hundreds in one generation.

The monogenes (Fig 11) are flatworms with a direct life cycle that can cause significant damage to skin, fins, and gills. Although many require a microscope for visualization, some are large enough to see unaided. Egg-laying species are more problematic because eggs are resistant to treatment.

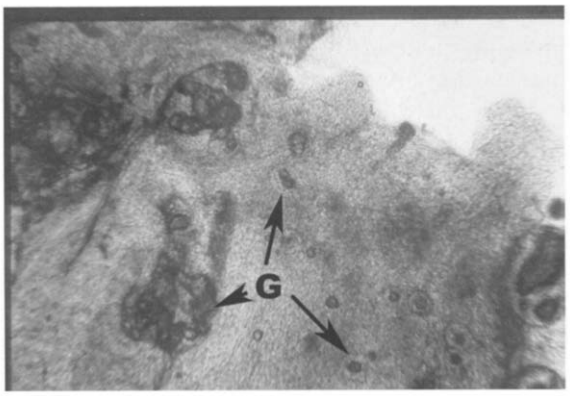

Figure 9. Stomach squash/wet mount from an African cichlid. 40x. Note granulomas (G) caused by *Cryptobia iubilans.*

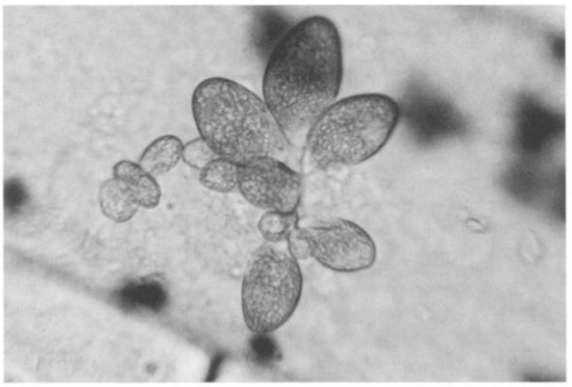

Figure 10. *Piscinoodinium* on a fin clip. 100x. Note granular appearance, and oval/tear drop shape. Parasite is nonmotile.

The microsporidians and myxozoans are two nonmotile, spore-forming groups that are typically found within granular-appearing cysts. These can be found anywhere in the body. Any cysts that appear to have granular structure (and do not contain a worm) should be squashed to rule out one of these two groups. Individual microsporidians are very small (requiring high power magnification) and have a "wooden shoe/clog" appearance (with one structure, the posterior vacuole, resembling the opening to

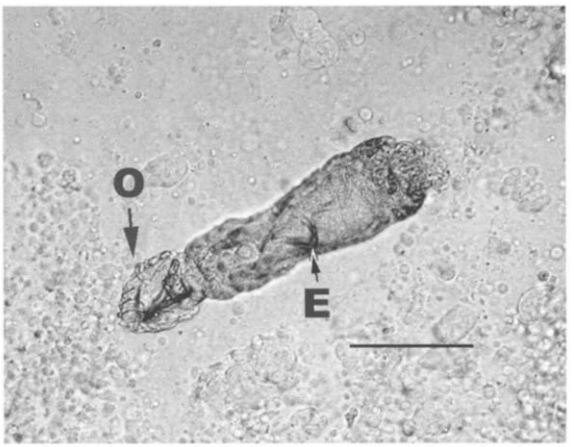

Figure 11. Monogene (*Gyrodactylus* sp.). *Gyrodactylus* is live bearing, as evidenced by the hooks located on the embryo (E). The opisthaptor (O) contains numerous hooks and anchors. Bar ~ 100 μm

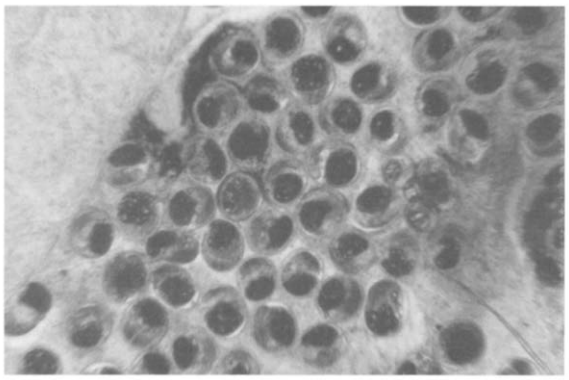

Figure 12. Digenean trematodes: encysted metacercarial stages, coelomic cavity. 100x. Fish was clinically normal. Digenes in the gills can be more problematic, if numerous.

the shoe). Myxozoans are larger (6 to 65 μm), and more varied in shape. Different species contain 2 to 6 polar capsules. Because microsporidians, in general, have a direct life cycle, and most myxozoans studied have an indirect life cycle, it is critical to distinguish between the two.

Digenean trematodes are flatworms with an indirect life cycle that, in freshwater fish, typically involves a bird and a snail as intermediate hosts (although more complicated life cycles exist). The life stage most commonly found in fish is the encysted metacercaria (Fig 12). Cysts are typically oval to round, and differ somewhat in appearance from one species to another. These can be found in almost any organ, and become a problem if they interfere with organ function (such as overabundance in gill tissue). Some wild specimens can have tens or hundreds of digenes in their coelomic cavity, with no apparent ill effects. Adult digenes are less common, but may be found in fish. One distinguishing feature of digenes is the presence of both an oral (or anterior) and ventral sucker on their body.

Nematodes can be found in the gastrointestinal tract or other organs as adults or encysted juveniles. Cestodes are less commonly found.

Lernaea (anchorworm– resembles one or several small, long filaments hanging from the fish) and *Argulus* (fish louse) are two common crustacean parasites, and can cause problems in many species including goldfish and koi, but both are easily identified and visible grossly.

Euthanasia

After skin, gill, and fin biopsies have been taken, provide a lethal dose of MS-222 to the fish. One recommendation[19] is for a dosage rate of 50 to 250 mg/L, buffered with sodium bicarbonate (baking soda) at a two-part sodium bicarbonate to one part MS-222 ratio. Fish should be kept at the dosage rate that results in anesthesia within 60 seconds[1] and should be kept there for approximately 10 minutes after breathing (cessation of opercular movement) has occurred. However, in practice, some species may require quite a bit more than 250 mg/L. As much as 600 to 800 mg/L or more may be required to produce a surgical plane of anesthesia within 60 seconds (eg, for labyrinth fishes such as gouramis). At these much higher doses, the fish are usually dead within several minutes.

The lethal dose can be administered as a bath or administered via squeeze bottle to one set of gills (left or right), avoiding dripping into the other side (in case additional sampling or histopathological sections are required). The fish should be still, no longer responsive to stimuli, and have lost the ocular righting reflex (eyes are in normal position, parallel to body, regardless of position of the body—the clinician may have to gently press the eye parallel to check).

External Examination

After the fish has been euthanized, a quick, but complete external gross examination should be performed. Record a description of lesions including size, location, and number. Deformities should also be noted. Additional lesions should be sampled and wet mounted and examined microscopically after microbiological samples have been taken. Lateral, dorsal, and ventral view line drawings of a fish on the pathology report can be helpful for reference of lesions.

Skin/Fins/Gills/Operculum

Examine these structures for erosions, hemorrhages, parasites, fungi, and microscopically for microbubbles (indication of supersaturation), telangectasia (ballooning of capillaries in the gills—evidence of high ammonia or toxins), and other masses. Sampling method may also

artificially induce telangectasia. Brownish or dark brown gills may indicate nitrite toxicity while pale white or pink gills indicate severe anemia in live fish. Be cautious, however, when evaluating fish found dead. Gills degrade and turn white rapidly. Be sure to examine the inner surfaces of the opercula.

Mouth

Check external and internal (oral cavity, pharynx) regions for lesions/parasites.

Vent/Anus/Genital Pore

Check each (if applicable, depending on the species) for swelling, redness, and protrusions (eg, nematodes).

Eyes

Examine for cloudiness, hemorrhages, increased mucus, exophthalmia, endophthalmia, and buphthalmia. If indicated, scrape for external parasites.

Muscle

Areas that appear discolored, hemorrhaged, have masses, or are otherwise abnormal should be thinly filleted and wet mounted for examination, following microbiological sampling.

Microbiological Sampling

After the external examination, the brain, kidney, liver (and spleen, if possible) should be cultured for bacteria. Other internal organs, the coelomic cavity, or coelomic fluids may also warrant culture. Culture of ulcers may be useful and helpful, but requires a great deal of skill and luck to isolate and differentiate primary initiating bacteria from secondary invaders or waterborne contaminants.[1] Some disease-initiating bacteria grow much more slowly than secondaries or contaminants, and may quickly over run the plate. Most common bacterial pathogens of fish will grow on TSA with 5% sheep's blood. There are some exceptions, including *Flavobacterium columnare,* which requires Ordal's media. Fungal cultures are useful for positive identification of external or internal fungal infections, although typical water mold infections can be tentatively diagnosed based on microscopic evaluation and clinical appearance. Corn-meal agar for Oomycetes ("water mold" fungi) and Sabouraud dextrose agar for non-Oomycetes is recommended. For specific procedures, consult a fungal or general microbiology laboratory, or refer to appropriate fish health texts.[1,2,16]

Before culturing the brain, use an alcohol pad to disinfect the dorsal part of the head, midline, but slightly caudal to the eyes. The site can be wiped dry with a sterile gauze pad, or allowed to evaporate. After the alcohol has evaporated, a small, sharp, sterile scalpel blade (ethyl alcohol dipped, flamed, and cooled) is used to cut through the top of the head into the brain case. Frequently a small amount of cerebrospinal fluid will leak out. The cut area can then be sampled with a fine, sterile inoculating needle or swab and streaked onto a TSA with 5% sheep's blood plate or sent to a laboratory. Certain bacteria, such as *Streptococcus* sp., can be readily isolated from brain tissue.

The kidney can be approached dorsally, ventrally, or laterally. The ventral approach is preferred if liver and spleen will also be sampled. For the dorsal approach the dorsal fin should first be cut off with scissors, and the dorsal area of the fish wiped with isopropyl alcohol, especially around the area to be cut. Flaming the wiped area with a barbeque lighter or allowing it to evaporate will help prevent excess alcohol from entering the cut section. The mid-region of the insertion of the dorsal fin is a good landmark for the cut in most fish, although some species, including swordtails, *Corydoras* catfish, and plecostomus catfish, have the majority of their kidney located anteriorly (slightly caudal and dorsal to the opercular cavity). A sterilized pair of scissors (ethyl alcohol dipped, flamed, and cooled) is used to cut through skin and muscle to the kidney (Figs 13 and 14).

The spinal column should be severed during the dorsal approach, since the kidney is directly beneath the spine. Blood will ooze out of the area, an indication that the kidney and near by vessels have been cut. A sterile loop should then be inserted into this area and streaked onto a TSA with 5% sheep's blood plate, or a culture swab should be used to remove a sample. Care

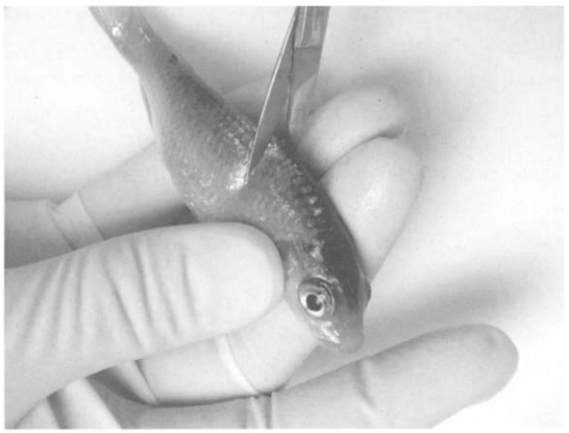

Figure 13. Kidney culture, dorsal approach. The cut should be around the level of the lateral line, only to the area immediately ventral to the spine.

should be taken to avoid cutting too deeply and into other organs, especially the stomach and/or intestine, as contamination will result. The cut should be made at the level of the spine (the lateral line can be a good approximate landmark).

In some species, visualization of an intact swim bladder is a good indication that the gastrointestinal tract has not been breached. In other species, cutting the swim bladder using this approach is unavoidable, but not a problem if the cut remains dorsal to the other organs.

For the ventral approach, the fish should be placed in right lateral recumbency, and the lateral body should be wiped down with alcohol. A precise, shallow incision with a sterile scalpel blade should be made caudal to the operculum, to serve as an opening for blunt dissection scissors to enter and cut out an area of the body wall. This step will expose most of the coelomic cavity, swim bladder, and kidney. Cut from the insertion point dorsally and caudally, and then from the same point, toward the ventrum and caudally. Care should be taken to avoid cutting into any organs, especially the intestines/anal area, to prevent contamination. The swim bladder (or the air space of the swim bladder for those species with delicate bladders) is moved to the side (or deflated) and the kidney (visible as a red or dark red area immediately below the spine) is punctured with a sterile scalpel. A sterile culture swab or inoculating loop is then inserted to take the sample. (Fig 15) After all cultures have been taken, this window should be extended cranially, including cuts through (or removal of) the cleithrum (crescent-shaped, flat bone caudal to the operculum, that supports the shoulder girdle) to expose the anterior section of the coelomic cavity, as well as the pericardial cavity and heart.

The lateral approach is similar to the ventral approach except that instead of completely opening up a window into the body cavity, a scalpel is used to cut a small slit directly into the swim bladder and kidney, just big enough for insertion of a sterile swab or loop. This approach

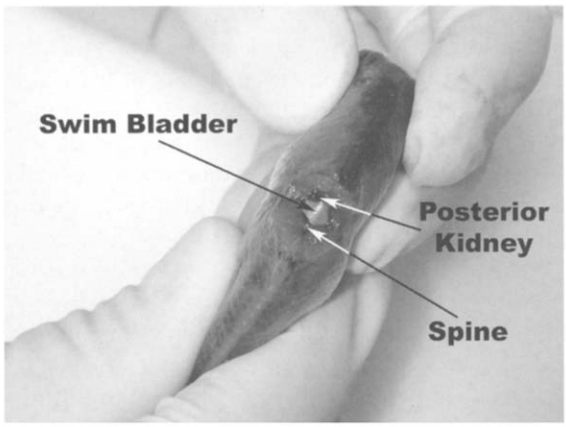

Figure 14. Kidney culture dorsal approach. The cut is directly below the spine, without cutting into the swim bladder. The posterior kidney and spine are exposed.

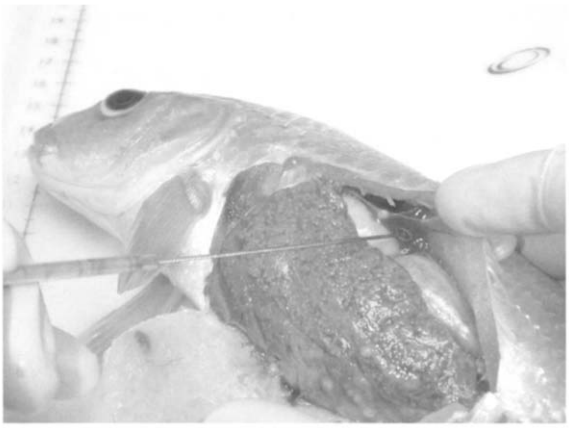

Figure 15. Ventral approach, female koi. This koi is full of eggs. Care must be taken to avoid contamination by contact with the gastrointestinal tract. Note the koi's saddle shaped area of posterior kidney, located at the junction of the anterior and posterior chambers of the swim bladder.

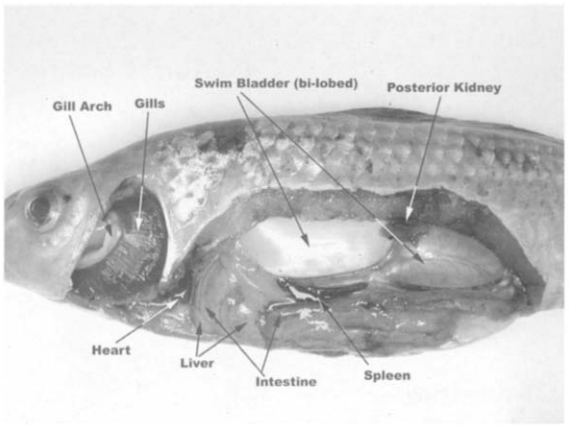

Figure 16. Koi, internal organs. Egg mass has been removed. Note intact, bi-lobed swim bladder, and saddle shaped section of posterior kidney. Liver is intimately intertwined with the intestine, there is no stomach, and the spleen is a dark red elongate organ.

assumes a good understanding of anatomy of the species examined.

After the kidney of the fish has been aseptically sampled, the liver should be stabbed with a sterile scalpel and the cut section sampled. If contamination of the outer portion of the liver is suspected, the outer surface can be seared with the flat end of a heated scalpel blade before stabbing. Splenic samples can be more challenging. For larger specimens, the sample can be taken as per the liver, with careful deflection of overlying viscera. For smaller spleens, a very small piece can be crushed and pulled out with a sterile pair of forceps and this sample then streaked onto a plate or placed on a swab.

Culture of ulcers can be complicated. One good recommendation is to culture early lesions by placing a 1 μL sterile loop into the leading edge of the lesion (aseptically removing scales at the leading edge to increase likelihood of culturing initiating pathogens) and place on to a small 4 mm^2 area of a culture plate. Next, use a sterile Mini-Tip Culturette (Becton-Dickinson, Sparks, MD, USA), to swab the inoculated area onto half the plate. Finally, pull the streak across one quarter of the plate, and then across the last quarter. This should help isolate individual colonies.[1] Externally found saltwater bacterial species may require marine agar, or blood agar with additional salt, for growth.

Cultures should be incubated according to normal temperature ranges for the species examined (25 to 30°C for most warm water species). If mycobacterial infections are suspected, an appropriate mycobacterial media such as Lowenstein -Jensen media or Dorset's egg agar should be used (in addition to TSA with 5% sheep's blood agar for other bacteria). Some rapid, less fastidious mycobacterial strains will grow on blood agar in 4 to 7 days.

Internal Examination

After cultures have been taken internal organs should be examined grossly and microscopically by means of wet mount preparations. As described previously, differences among species, and even among varieties can be striking, so the clinician should be familiar with normal anatomy of some common species as a starting point. Koi (Figs 15 and 16) and fancy goldfish (Fig 17) are very popular water garden species and can be quite expensive. Oscar (figs 18, 19, 20) and discus (Fig 21) are two very popular cichlid species that often foster strong owner-pet bonds.

Small sections (approximately 1 cm^3) of each organ described in the "Basic Fish Anatomy and Physiology" section, as well as specific lesions, should be fixed in 10% neutral buffered formalin for histological preparation and examination. (The heart, brain, skin, muscle, eye, opercula and pseudobranch are often forgotten, so be sure to include samples of each of these.) Samples for electron microscopy (EM) should

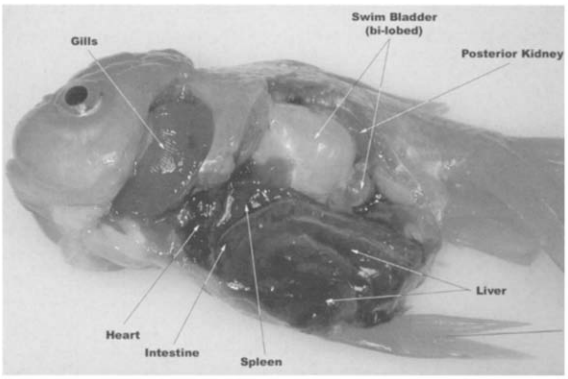

Figure 17. Oranda (fancy goldfish), internal organs. Note intimate attachment of liver with intestine. Also note small posterior chamber of the swim bladder relative to the anterior chamber. This is genetic in this variety, and, along with the body shape, makes the fish prone to buoyancy control problems. Goldfish also lack true stomachs.

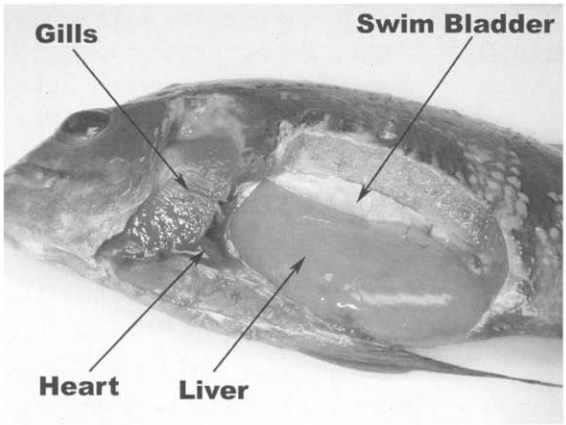

Figure 18. Oscar (cichlid), internal organs, level one. Note large liver.

be saved in fixative recommended by the processing laboratory. Trump's fixative is good for most EM studies.

Operculum

Note if pseudobranch is grossly visible/superficial, and general appearance.

Swim Bladder

Examine for thickening, hemorrhages, necrosis, fungi (some are pigmented), and parasites.

Kidney

Anterior and posterior sections should be squashed separately. The anterior section can look very much like the spleen on wet mount examination. Tubules should be seen on posterior sections, and both sections often have MMCs. Check for uniformity, granulomas, and parasites.

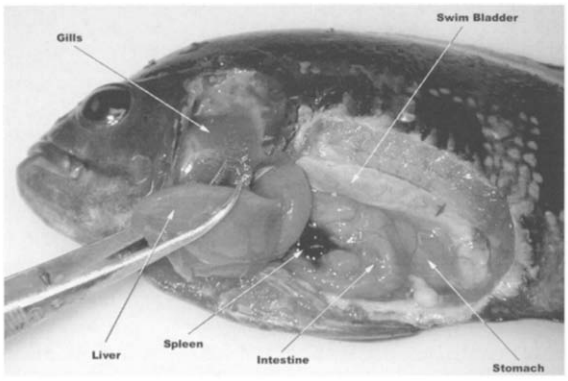

Figure 19. Oscar (cichlid), internal organs, level two, with liver reflected. Note thick walled swim bladder, spleen. Stomach and intestine are surrounded by fat.

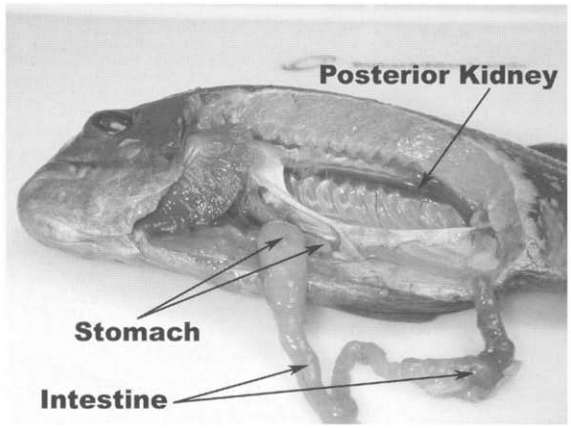

Figure 20. Oscar (cichlid), internal organs, level three, digestive tract exposed, swim bladder deflected. Note relatively short gastrointestinal tract length. Also note posterior kidney.

Liver

Check for color, uniformity, fat content (does it float in 10% buffered formalin?), MMCs, parasites, and granulomas.

Gall bladder

Located near or within the liver, it contains greenish to yellowish fluid that is normally clear.

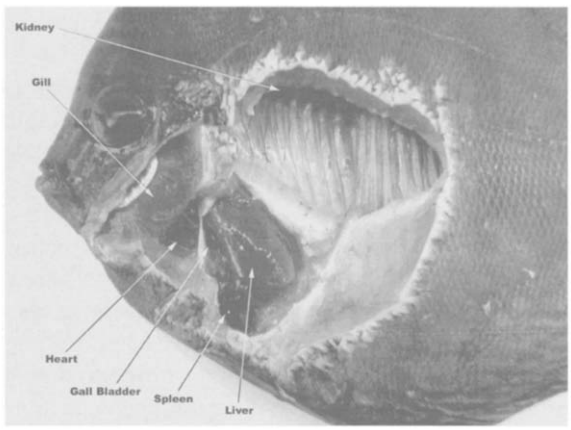

Figure 21. Discus (cichlid), internal organs. Note large cavity where swim bladder was located, dorsally. Kidney is visible. Also note location of heart and relatively small area occupied by viscera.

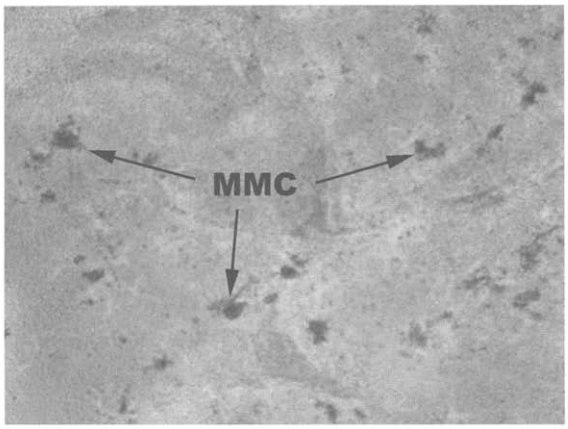

Figure 22. Koi, spleen, wet mount. MMC = melanomacrophage centers. 40x. Note absence of clear/onion skin appearance to area immediately surrounding dark MMCs (cf. Figure 24)

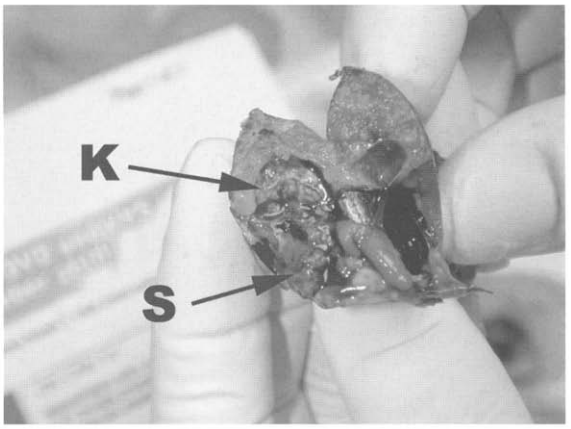

Figure 23. Tilapia, cut section. Note grossly visible granulomas/nodules in kidney (K) and spleen (S). One of the major rule-outs is mycobacteriosis.

It can be relatively large, especially if fish is anorexic.

Spleen

Check for color, size, uniformity, MMCs, and granulomas.

Gonad

Note, by presence of eggs or testes, whether the fish is female or male. Check gonads for size, maturity, granulomas, or hardened structures (in females, degenerating eggs/"egg-binding"). Note if sperm are present, active, and motile.

Stomach

Check for parasites and granulomas. (Remember, true stomach not present in koi, goldfish, or other cyprinids).

Intestinal tract

Check for the presence of food or fluid, hemorrhages, ulcers, and parasites (especially nematodes and flagellates).

MMCs (Fig 22) should not be confused with granulomas (Figs 23 and 24), which are typically more "rocky" or sharp edged in appearance and surrounded by a clear/onion skin area, or with oocytes (Fig 25). Granulomas in fish, as in other animals, are an attempt to wall off an irritating or undesirable (pathogen) or foreign body. Granulomas are commonly associated with certain bacterial, fungal, and parasitic infections, although these infections may occur without chronic, granulomatous lesions. Bacteria that often cause granuloma formation include *Mycobacterium* sp., *Nocardia* sp., and rickettsia-like organisms.

Parasites that can cause granuloma formation include *Cryptobia iubilans* in cichlids, nematodes, and digenean trematodes. Foreign bodies, such as scales, spines, or plant material can cause granulomas as well. Ovaries that are attempting to reabsorb over ripe eggs can have a granulomatous-type reaction.

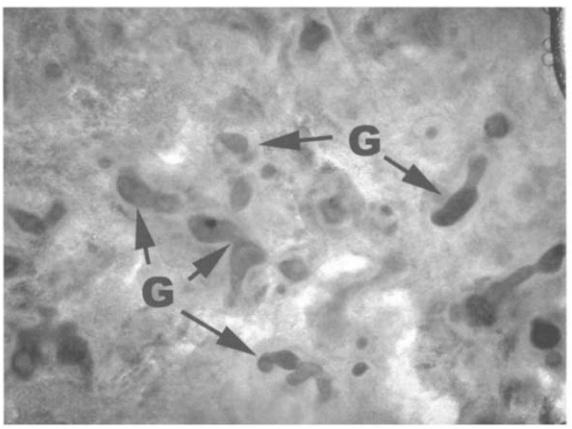

Figure 24. Tilapia, spleen, wet mount, granulomas (G). 40x. Note clear area/halo/onion skin appearance around "rock-like," irregularly shaped dark structures. Granulomas are often brownish in color.

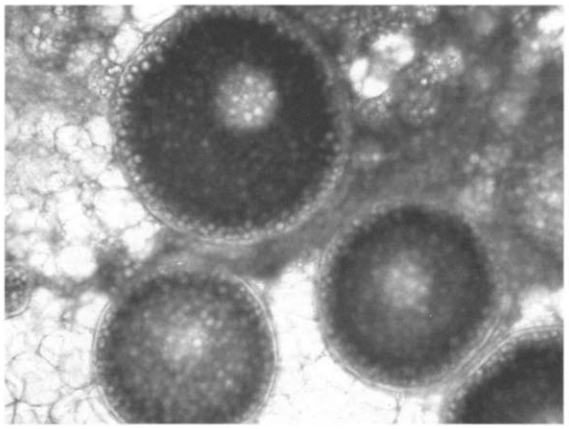

Figure 25. Koi, oocytes. 40x. Oocytes in various stages of maturity, from the ovary of an adult female. Note the relatively rounded shape, and grainy appearance in the large, mid to late stage maturity eggs. Immature oocytes are much smaller and have clear cytoplasm.

Virology

Viruses in ornamentals are not well studied. Because of the lack of necessary cell lines for virus isolation and research, diagnosis of a viral disease is often based solely on history, clinical signs, rule out of other causes, and presence of clear histopathologic lesions and electron microscopic (EM) visualization of viral particles within these lesions. Some viral diseases, such as lymphocystis (caused by an iridovirus) and koi pox (caused by a *Herpesvirus cyprini*), have somewhat pathognomonic lesions, and may only require histology or virus isolation for absolute certainty. Certain viruses in fish are considered of concern by the international community. Spring viremia of carp (SVC), recently reported in the U.S., is a notifiable disease of koi and several other related species and requires isolation and culture for verification.[20-22] There are only three laboratories in the U.S. that are presently USDA accredited to test for SVC (see appendix 1). Clinical signs of SVC are nonspecific and include darkening, external hemorrhages, exophthalmia, ascites, pale gills, and a protruding vent. Edema and generalized inflammation and petechiae may be present in multiple organs internally including the swim bladder. Splenomegaly may be apparent. Concurrent infection with *Aeromonas* sp. or other bacteria may lead to inaccurate diagnoses.[20-22] Contact your state veterinarian and one of these local laboratories for proper sample submission protocols if you suspect SVC. Koi herpes virus (KHV—not the same virus as the etiologic agent of carp pox) is also a very pathogenic virus with high associated mortalities that should be isolated and cultured for positive diagnosis.[23] The only consistent sign in fish with KHV is pale or irregularly colored gills.[23]

Summary

The mastery of necropsy techniques implies an understanding of pathological findings. There are a number of excellent references that should be a part of any fish veterinarian's library.[1,2,8,15,16] Water quality problems are always high on the differential list, at the very least as a contributing factor. Dissolved oxygen problems, excess ammonia and nitrite, improper or fluctuating pH or temperatures, and supersaturation can all lead to acute or chronic disease. Some of these problems can manifest themselves in gill, skin, or fin pathology. Introduction of toxins, including chlorine and copper, and improper use of chemotherapeutants can also result in visible pathology.

Infection by bacteria and parasites is often secondary to husbandry or management problems. External examination, microscopic evaluation, and a good history and assessment of husbandry practices are often enough to determine course of action.

Although ornamental fish virology is still in its infancy, viruses in some species, such as koi, have gained more research attention. Clinical signs of disease and pathology associated with SVC and KHV are not specific and virus isolation is required. SVC is a notifiable disease in the U.S.[22] For other viruses, presence of histopathologic lesions and visualization of viral particles in these lesions using transmission electron microscopy is corroborating evidence but not definitive.

Appendix 1: USDA approved diagnostic laboratories capable of testing for spring viremia of carp.

1. University of Arkansas-Pine Bluff, Cooperative Extension Program, PO Box 4912 or 1200 University Drive, Pine Bluff, AR 71611, Phone: (870) 543-8537.
2. Pennsylvania Animal Diagnostic Laboratory

System, State Veterinary Laboratory, 2305 North Cameron Street, Harrisburg, PA 17110, Phone: (717) 787-8808.

3. Washington Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Washington State University, PO Box 647034, Pullman, WA 99164-7034, Phone: (509)335-9696.

Acknowledgments

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