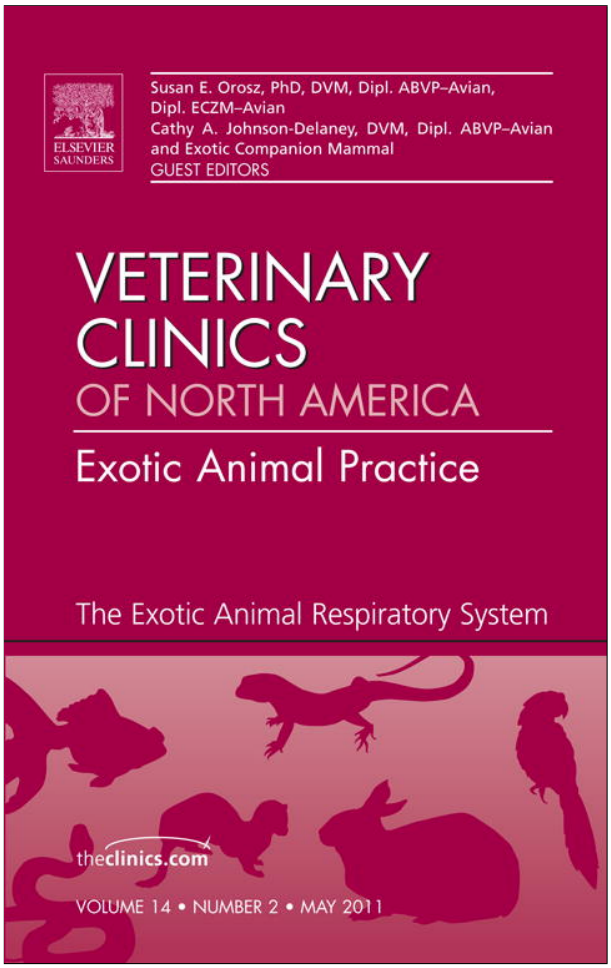

Disorders of the Respiratory System in Pet and Ornamental Fish

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KEYWORDS

• Pet fish • Ornamental fish • Branchitis • Gill
• Wet mount cytology • Hypoxia • Respiratory disorders
• Pathology

Living in an aquatic environment where oxygen is in less supply and harder to extract than in a terrestrial one, fish have developed a respiratory system that is much more efficient than terrestrial vertebrates. The gills of fish are a unique organ system and serve several functions including respiration, osmoregulation, excretion of nitrogenous wastes, and acid-base regulation.[1] The gills are the primary site of oxygen exchange in fish and are in intimate contact with the aquatic environment. In most cases, the separation between the water and the tissues of the fish is only a few cell layers thick. Gills are a common target for assault by infectious and noninfectious disease processes.[2] Nonlethal diagnostic biopsy of the gills can identify pathologic changes, provide samples for bacterial culture/identification/sensitivity testing, aid in fungal element identification, provide samples for viral testing, and provide parasitic organisms for identification.[3–6] This diagnostic test is so important that it should be included as part of every diagnostic workup performed on a fish.

ANATOMY AND PHYSIOLOGY

The respiratory system of most fish species includes the gill arches, 2 opercula, and the buccal cavity, all located in the cranial portion of the body. Most teleosts (including pet and ornamental fish) have 4 respiratory gill arches and 1 dorsally located, nonrespiratory pseudobranch.[1,2,7–9] The arches are supported by a bony skeleton and are protected by a specialized, moveable flap called an operculum. Each respiratory

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arch has 2 rows of gill filaments (or primary lamellae) that project from the arch, like the "teeth of a comb,"[10] with numerous rows of secondary lamellae extending perpendicular from these primary lamellae (**Fig. 1**).[1–3] The secondary lamellae consist of epithelial cells (usually 1 or 2 layers) surrounding a central vascular space, supported by contractile pillar cells.[2,3,10] It is at the secondary lamellae where gas exchange occurs. Comparatively large gills (with a corresponding increased lamellar surface area) can be found in some very active fish (eg, tuna) and fish with a relative tolerance for hypoxia.[11,12] The lamellar epithelium is thin and its external surface is covered with microridges that serve to further increase the surface area for respiration.[2,10,13] A protective biofilm consisting of mucus and other cellular and chemical components covers this epithelium. This layer and the underlying lamellar epithelium are very sensitive to stress and environmental changes.[2]

Blood flow to the gills comes directly from the heart via the ventral aorta. The lamellar blood channels are so small in diameter that erythrocytes move through the capillaries one red blood cell at a time. Because nearly the entire cardiac output enters the gills, traumatic injury to the gills can negatively affect the health of the fish and can be potentially fatal.[1,9,11,14] This is an important consideration when attempting nonlethal gill sampling for diagnostic purposes. A complex system theorized to involve interactions between many hormones and the autonomic nervous system serves to control lamellar perfusion in the gills.[1,10] A counter-current system between the blood flow and water flow creates an efficient transfer of oxygen by passive diffusion from a relatively higher oxygen concentration in the water to a lower concentration in the bloodstream. Video demonstrations of blood flow through the gills can be found on the Internet as a supplement to an article by Evans and colleagues.[15] A small amount of blood is also delivered to a central venous sinus via alternate pathways for delivering nutrients to the gill tissue.[1,2] Abductor and adductor muscles allow the fish to spread the gill filaments and change the flow of water over the gills in times of higher oxygen demand.[2,14,16]

The highly vascular and semipermeable nature of the gills may be exploited as a drug delivery method. In one study, topical gill application of a spawning hormone was used in small tropical fish where injections were not feasible.[17] Another example is the spray delivery of a highly concentrated anesthetic solution to the gills for sedation or anesthesia. This method is used most often in public aquariums for large elasmobranchs, but can be used in a variety of species where immersion in the anesthetic solution is impractical or cost prohibitive.[18]

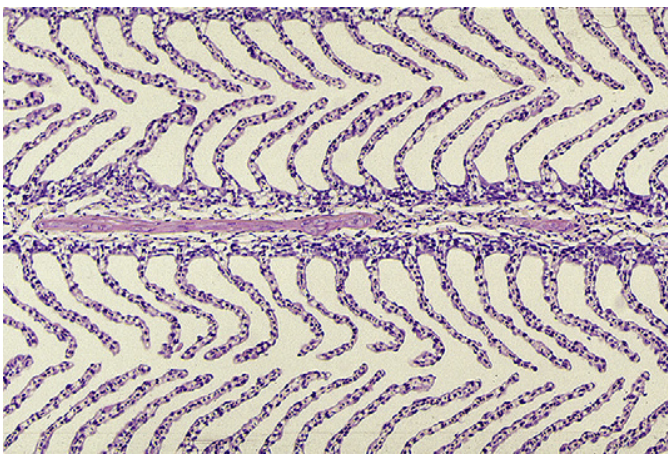

Fig. 1. Histologic view of a normal gill section with secondary lamellae seen extending perpendicularly from primary lamellae (H&E, original magnification ×400).

Air-breathing fish, or labyrinth fish, and some catfish have developed very vascular accessory organs that serve to extract oxygen from the air at the surface of the water.[2,3,19] This ability can help with oxygen extraction from the air when the aquatic environment becomes hypoxic or can aid gas exchange in species that bury themselves in mud during dry seasons.[16,18,20] Air-breathing fish can be obligate air breathers (must have access above the water surface) or facultative air breathers (only breath air when the dissolved oxygen level of the water is very low).[21] Examples of pet fish in this group include bettas (*Betta splendens*) (**Fig. 2**), paradise fish (*Macropodus opercularis*), and gouramis (multiple species). Prolonged and unpredictable induction of anesthesia may occur in these species when using an anesthetic in an aqueous solution.[18]

Respiration in fish is continuous and unidirectional (compared with the bidirectional mechanism in mammals): water flows into the buccal cavity, flows past the gill tissue, and exits via the opercular opening.[2,3,9,14] This type of respiratory cycling is called a dual-phase pump.[9] During the first phase, the mouth is open and the opercula are closed, water enters the expanding buccal and opercular cavities.[3,9] In the second phase, the mouth closes, the opercula open, and the buccal cavity contracts forcing the water to flow over the gills and exit via the opercular opening.[3,9] Some fish species such as tuna use ram gill ventilation (open mouth swimming) as a primary means of respiration instead of the dual-phase pump, whereas other species resort to this method only when swimming rapidly or when in a strong current.[2,9,22] Ram ventilation can reduce the energy expenditure required for respiration.[1,22] Fish can also cough, temporarily reversing the flow of water over the gills.[3,23] Coughing can be triggered by mechanical and chemical stimulation of the gills.[10] An increased coughing rate has been associated with irritant pollution,[10] gill parasitism, bacterial gill disease,[24] and excess mucus[3] and could be a more sensitive indicator than an increased opercular rate of general stress in fish.[23]

RESPONSE OF GILLS TO INJURY AND CLINICAL SIGNS OF GILL DISEASE

Gills are delicate, highly vascularized organs that are always in direct contact with the external environment. Because of this, gills are highly susceptible to invasion by various infectious agents; damage from environmental toxins, irritants, and pollutants; and the adverse effects of stress. Common causes of stress in pet and ornamental

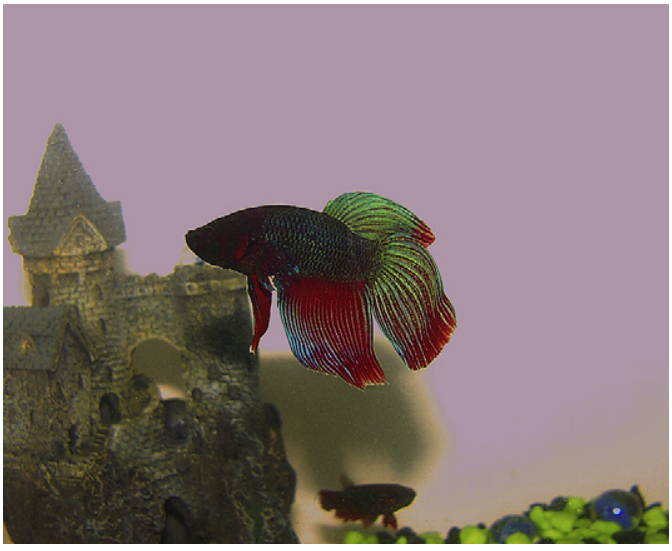

Fig. 2. *Betta splendens* has an accessory organ, the labyrinth organ, for breathing atmospheric air.

fish include capture and handling, overcrowding, poor water quality, overfeeding, inadequate husbandry and maintenance, and interspecies and intraspecies incompatibility. In response to stress, fish undergo a similar stress-induced hormone response as other pet species that includes the release of catecholamines and glucocorticoids.[25–27] Catecholamine-mediated reactions to stress include increased blood flow to the gills in an effort to improve oxygen uptake,[25,27] increased heart and respiratory rates, and altered permeability of the gills.[27] Some of these changes can occur rapidly. It is important to note that functional changes will occur before detectable morphologic changes. For example, hyperventilation induced by hypoxia can occur within seconds.[1] The acute changes induced by hypoxic conditions are an attempt to maintain adequate oxygenation of the fish despite a lowered oxygen concentration in the environment.[28,29] A reflex bradycardia is often observed with hypoxia and may occur in an attempt to reduce blood flow to the gills and favorably increase gas exchange.[29]

Oxygen depletion can be a common occurrence in aquatic environments and is a major cause of stress and hypoxia in fish. In pet and ornamental fish, some situations that cause hypoxia include transport and shipping, as well as plant/algae overgrowth in ponds or aquaria.[26] Clinically, hypoxic conditions can occur as an adverse effect of therapeutic treatments, such as formalin, or after a massive algae die-off following the use of an algaecide. Mechanical failure of aeration equipment, pumps, or filtration equipment can also contribute to hypoxic conditions.[30]

Lesions that can occur in the gills in response to injury include color change because of congestion or thinning of blood, diffuse or focal necrosis, telangiectasia (dilation of the terminal blood vessels), edema, hemorrhage, lamellar thickening and fusion, epithelial hypertrophy and hyperplasia, and increased mucus secretion.[2,8,13,26] Increased mucus production may initially be beneficial, as the mucus may bind some toxins found in the water or prevent some pathogens from attaching to the gill.[8] Most of these can be seen grossly or during wet mount cytologic examination. To complicate matters, many gill lesions seen on histopathological examination can also be a result of artifacts from processing and improper fixation of specimens or poor euthanasia technique.[2,13]

One interesting response to environmental hypoxia has been reported in the Crucian carp (*Carassius carassius*), the goldfish (*Carassius auratus*), the mangrove killifish (*Kryptolebias marmoratus*), and the giant Amazonian fish (*Arapaima gigas*).[31] In these species, secondary lamellae are normally located inside a cellular mass, effectively reducing the respiratory surface area.[26,31] During times of environmental hypoxia, the interlamellar cell mass recedes, exposing the lamellae, and thus increasing the efficiency of oxygen uptake via the increased functional area.

Most clinical signs of injury to the gills are a reflection of hypoxic environmental conditions or a relative hypoxia and can be readily observed without direct manipulation of the fish. **Table 1** lists common, nonspecific clinical signs that can be associated with diseases and injury to the gills. It is important to remember that the gills perform more than just a respiratory function, so diseases that affect the gills can also cause clinical signs related to the disruption of osmoregulation, acid-base imbalances, and nitrogenous waste accumulation in the body.

DIAGNOSTIC EVALUATION OF RESPIRATORY DISEASE

All evaluations of clinically ill fish start with a good history and physical examination. Historical questions should be broad in nature and investigate life support systems (eg, filters, water and air pumps) and maintenance, husbandry and management

Table 1
Clinical signs associated with diseases of the gills[a]

Clinical Sign	Description	Comments
Abnormal dark coloration	Darker than normal for particular species, reproductive status, and color phase	General stress Systemic disease Poor water quality
Coughing	Rapid opercular flaring that moves water in a reversed directional flow over the gills	Occasional coughing is normal, increased rate can be indicative of disease Exposure to gill irritants Gill parasitism Stress
Flared opercula	Opercula open, exposing the gill tissue	Severe hypoxia, usually agonal Goiter, pharyngeal foreign body
Flashing	Rubbing the body on bottom of tank or pond exposing the ventral aspect (a "flash" of pale color of the ventrum)	Parasitism Poor water quality
Gasping	Exaggerated and repeated opening and closing of the mouth	Hypoxia Relative hypoxia (anemia, nitrite poisoning) Poor water quality Many gill disorders
Gathering at water inflow sites/facing into water current	Congregation at areas of moving water-waterfalls, water inflow pipes, water fountains, and so forth	May be an attempt to find increased oxygen concentration or increased water flow over the gills
Gilling	Increased opercular rate	Nonspecific sign of stress Hypoxic or conditions that create a relative hypoxia
Jumping	Jumping out of the water Desiccated fish may be found on the floor or edge of the pond	Startled fish Poor water quality (low oxygen, low pH) Parasitism toxic or caustic substances
Mucus production increased	The increased mucus layer can impart a hazy or dull appearance to the gills	May see trailing strands of mucus exiting the operculum
Piping	Open mouthed gulping at the air: water interface	See also "Gasping" Do not confuse with normal bubble nest building seen in fish such as bettas

[a] These signs are not pathognomonic for gill disease and can be present in many diseases of fish.

practices, number of fish and variety of species, recent changes or treatments, presence of new fish, and the duration and course of disease.[3,4,32,33] Life support system failures including the misuse of ozone can contribute to or be a direct cause of respiratory disease in fish.[3]

Examination should first include observation of the live fish without any direct handling of the affected fish. Clinical signs as listed in **Table 1** may be observed. Physical examination of the fish follows observation. Rinsed, powder-free nitrile, vinyl, or latex gloves should be worn to reduce any damage to the fish's skin and to reduce the risk of zoonotic disease transmission (**Fig. 3**).[34] If the examination takes more

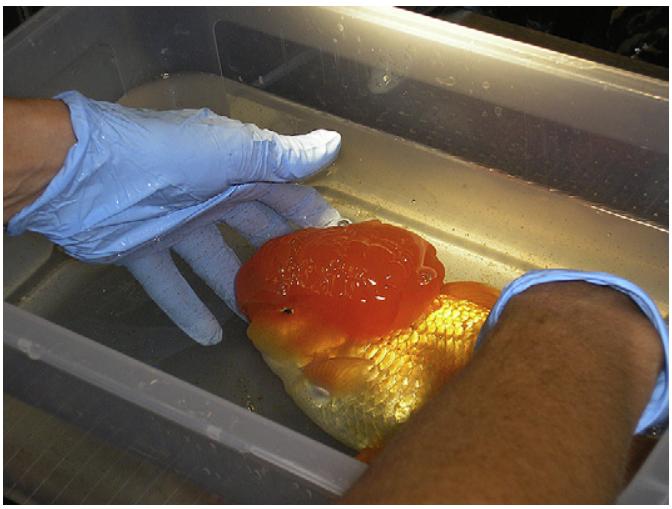

Fig. 3. Handling fish with gloves reduces damage to the fish's slime coat and protects the practitioner from zoonotic disease transmission.

than a few minutes, the fish should periodically be placed in water or should be irrigated with water to prevent desiccation of the external surfaces and gill tissue. Fish will usually resist manipulation of the opercula and gills for examination purposes so sedation or anesthesia is recommended unless the patient is considered a high-risk candidate for anesthesia. The opercula and surrounding tissue should be carefully examined for any lesions. Abnormal examination findings that may be seen on gross examination of the gills include focal, patchy, or diffuse necrosis; focal or diffuse color changes; increased mucus production; missing sections of gill filaments; the presence of multicellular parasites or nodular lesions; and focal or diffuse swelling of the gill (**Figs. 4** and **5**).

Water quality testing should also be performed for the complete evaluation of sick fish. There are many environmental abnormalities that can lead to respiratory distress. General testing parameters should include dissolved oxygen (DO), ammonia, nitrite, nitrate, and pH. Salinity measurements should be added for general testing of marine systems. Further testing can include tests for the presence of heavy metals (including copper) and chlorine/chloramine.

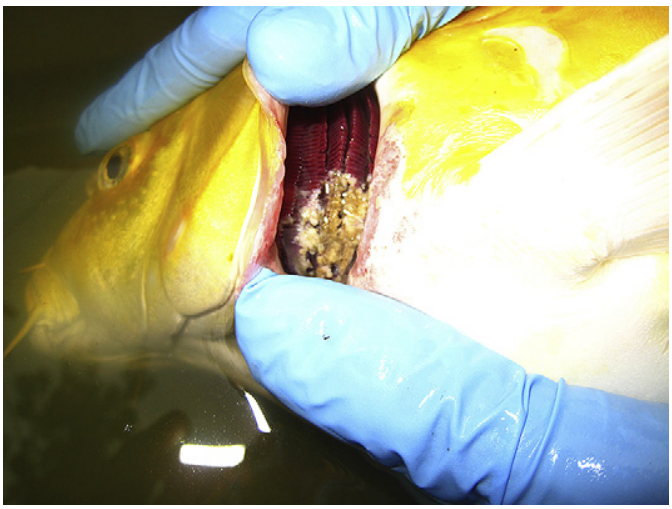

Fig. 4. An area of focal necrosis in the gills owing to a predation attempt on a koi (*Cyprinus carpio*).

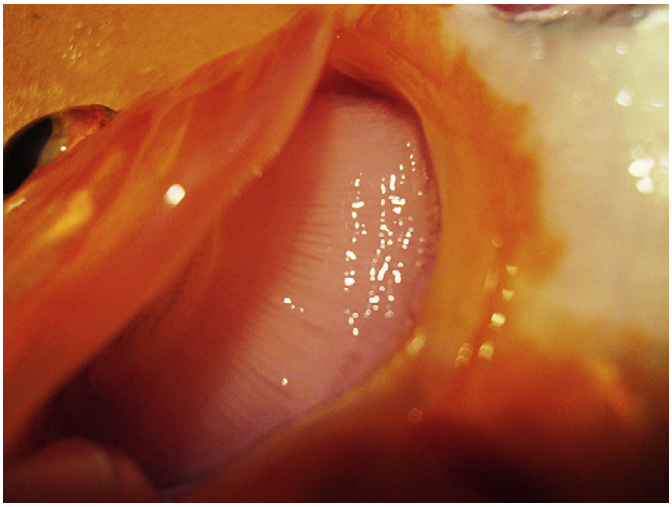

Fig. 5. The extreme pallor of these gills from a goldfish (*Carassius auratus*) is suggestive of anemia.

Diagnostic testing, including wet mount cytologic preps of the gills, are also considered part of the minimum database in the evaluation of sick fish. The gill biopsy is essential for any case of respiratory disease and should be evaluated in all but the most seriously morbid cases. A gill biopsy allows for evaluation of gill morphology and for the presence of parasites. Only a very small piece of gill tissue needs to be sampled during a nonlethal examination. Hemorrhage is not usually a concern unless a large amount of tissue is removed or the patient is a mature or gravid fish.[3–6]

Gill biopsy for the wet mount examination procedure is completed as follows[3–6] (**Figs. 6** and **7**):

1. Place a drop of water from the aquarium or pond on a clean slide.
2. Using gloved hands, gently lift the operculum of the sedated or anesthetized fish to reveal the gills. Gill rakers should be examined carefully during the physical examination, as many larger parasites will lodge in these areas.
3. With fine scissors such as iris tenotomy or suture removal scissors, snip a tiny section from the distal end of a few primary lamellae.
4. Place the gill tissue in the water drop on the prepared slide and place a coverslip over the tissue.
5. Examine the unstained slide immediately for evidence of parasites, fungi, or bacteria.

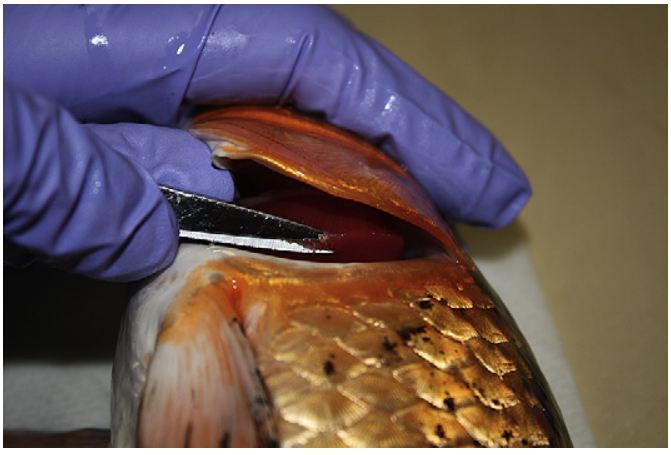

Fig. 6. A small section of gill tissue is removed for a wet mount preparation.

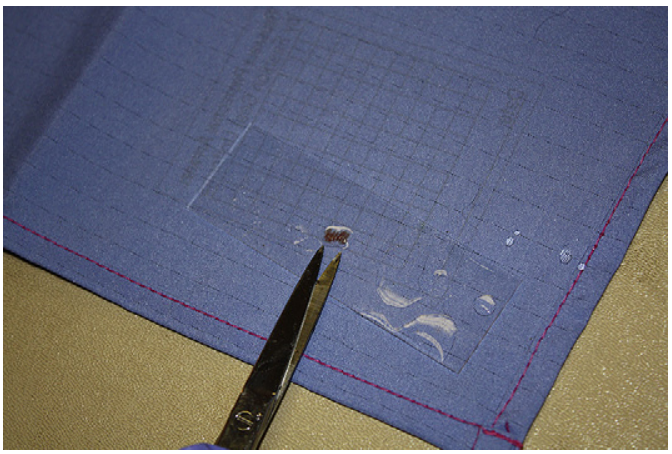

Fig. 7. A tiny piece of gill tissue is placed in the water drop for a wet mount examination.

6. The sample can also be examined for gill architecture. Gill pathology such as hyperplasia, hypertrophy, necrosis, lamellar fusion, excess mucus and telangiectasia may be observed.
7. In extremely large fish, one may need to scrape only the surface of the gills.

Another nonlethal technique for evaluation of the gills is with the use of rigid endoscopy. The scope is placed under the operculum and the practitioner can visualize the gills in situ as water flows over them.[35] With the magnification of the endoscope, the erythrocytes can be observed moving through the secondary lamellae and it may also be possible to see some external gill parasites.[35]

Hematological parameters associated with prolonged or severe hypoxia (and subsequent respiratory disease) include polycythemia, polychromasia, and anemia.[36,37] Serum biochemical changes that may be observed during hypoxia include increases in ammonia, potassium, calcium, magnesium, and phosphate.[37] An increased hematocrit has been reported in obligate air-breathing fish.[38] Hypoglycemia and a reduction in serum lipid values have also been reported with hypoxia.[37] An elevation of blood urea nitrogen (BUN) has been associated with copper-induced gill damage irrespective of any renal damage.[38,39] It is important to note that interpretation of hematological parameters and serum or plasma chemistry values can be difficult because not many profiles have been established for the multitude of species kept as pet and ornamental fish. In addition, interpretation of hematological and chemistry information is further complicated by alterations that may occur in the values owing to handling, stress, environmental temperatures, sex, breeding status, diet, use of anesthesia, and overall water quality.[5,36,37]

If a necropsy is performed, large sections of a gill arch can be removed and placed in formalin or other fixative for histopathology or other media for purposes of viral testing or bacterial culture and identification.

COMMON DISEASES OF THE GILLS

Infectious Diseases

Parasitic diseases

Parasitic diseases are the most common infectious diseases diagnosed in fish. The most common method of introduction is failure to quarantine and treat new additions to a pond or aquaria. Most of the parasites listed in the following paragraphs do not specifically target the gills but can be found in numerous locations on the body. An advantage to colonizing the gill epithelium is the protection offered to the easily damaged tissues by the opercula. Clinical signs suggestive of parasitic infestations

of the gills include flashing, increased mucus production, ulcerations and scale loss, and other general signs of dyspnea (see **Table 1**). A more complete description of parasitic diseases in fish can be found in a previous edition of the *Veterinary Clinics of North America, Exotic Animal Practice* series.[40]

Ichthyophthirius multifiliis, a holotrichous ciliated protozoan, is the most common parasite of fish and infects a large number of freshwater fish in a wide range of aquatic environments. "Ich," also known as "Ick" or "white spot disease," has a direct, complex life cycle that is temperature dependent. *Cryptocaryon irritans* is the marine version of this parasite. The disease derives its common name from the typical 1.0 mm white nodules (0.5 mm nodules for *Cryptocaryon*) seen distributed over the skin and gills of the fish (**Fig. 8**). Both parasites cause nearly identical pathology and clinical signs. At water temperatures typical of what can be found in tropical fish aquaria (25°C/77°F), the parasite can complete its life cycle in 3 to 6 days.[40] The life stage found on a fish, the trophont, is embedded in the epithelial tissues of the gill and is protected from external treatments.[40] Only the free-swimming life stage, the theront, is susceptible to treatment. Clinical signs are typical of external parasitism and include flashing, excess mucus production, cutaneous lesions, frayed fins and tail, dyspnea, lethargy, osmoregulatory insufficiency, and death.[24,40–42] Diagnosis is made by examination of a wet mount cytology preparation of the skin and gills. The parasite is easily identified by the characteristic slow, rolling motion and unique shape of its nucleus. The nucleus of *Ichthyophthirius* has a horseshoe shape, whereas the nucleus of *Cryptocaryon* is lobulated with 4 beadlike segments (**Fig. 9**).[40]

Trichodinid parasites are commonly diagnosed ciliated protozoans of both marine and freshwater fish species that primarily parasitize the skin and gills. These parasites are often seen in conditions that promote high levels of organic debris in the water, overcrowding, and poor husbandry practices.[40,43] Clinical signs are those typical of external parasitism and diagnosis is made by examination of a gill biopsy wet mount preparation. The circular, ciliated parasite with a characteristic internal circular denticular ring moves rapidly in the wet mount preparation, in a rotating whirling pattern, and has been described as a "scrubbing bubble" or flying saucer (**Fig. 10**).[40,43]

Chilodonella sp (*Brooklynella* sp is the marine counterpart) is another ciliated protozoan parasite that can be found on the gills of fish. Observation of the flattened parasite with several distinct bands of cilia in a wet mount cytologic preparation is diagnostic. Gill pathology seen with this parasitic infestation includes hyperplasia and fusion of the lamellae.[40]

Fig. 8. A goldfish (*Carassius auratus*) infected with *Ichthyophthirius multifiliis*.

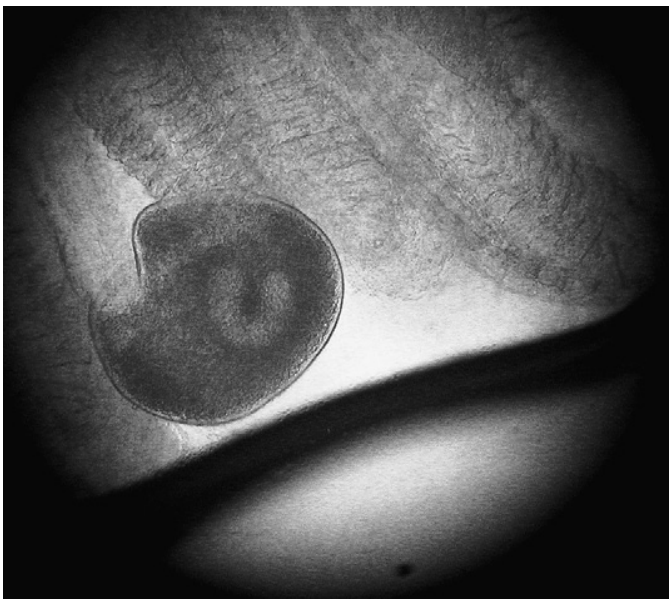

Fig. 9. A wet mount prep showing *Ichthyophthirius* in the gill tissue, magnification ×40.

Sessile or sedentary ciliates of clinical importance in causing respiratory disease in fish include *Epistylis* and *Capriniana*.[4,24,40] These ciliates are usually found on fish held in ponds where large amounts of organic debris are present.[40] *Epistylis* appears as a gelatinous to white cottony mass on the operculum and other locations. Wet mount cytology will help differentiate this ciliate from *Saprolegnia* (a fungal cause of gill disease) and *Flavobacterium columnare* (a bacterial cause of gill disease).[24,44] *Capriniana piscium* has a predilection for gill tissue and causes severe respiratory distress in affected fish by mechanically blocking gill tissue.[24,40]

Ichthyobodo sp (previously known as *Costia*) can cause severe respiratory distress and heavy mucus production when it colonizes gill tissue. The flagellated protozoan is approximately equal in size to a red blood cell and demonstrates a flickering-type motion in wet mounts. The parasite may be seen concurrently with other diseases causing debilitation in the fish, including koi herpesvirus (KHV) infections and bacterial sepsis. Two other parasitic flagellated *Cryptobi*a species, *C branchialis* and *C agitans*, have been reported to specifically colonize gill tissue.[24,40]

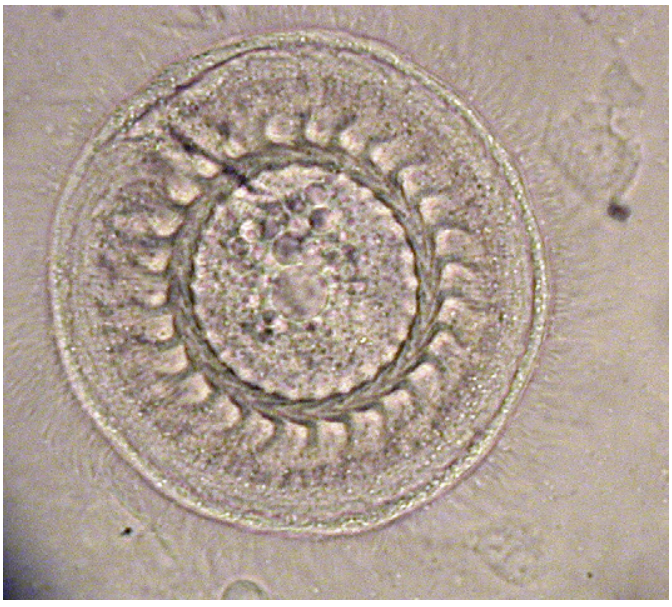

Fig. 10. *Trichodina* sp as seen in a wet mount prep, original magnification ×40.

Another group of protozoan parasites that causes respiratory disease includes the parasitic dinoflagellates, *Amyloodinium ocellatum* (marine) and *Piscinoodinium* sp (freshwater). Both have life cycles similar to *Ichthyophthirius* and only the free-swimming life stage is susceptible to treatment. Disease caused by these organisms is referred to as "velvet" or "rust" because of the characteristic gross appearance of the affected fish. Infestation of the gills can cause severe pathology, including edema, hyperplasia, inflammation, hemorrhage, and necrosis of the gill filaments.[4,24,40]

Monogenean flatworms, are a frequent finding in the gills of freshwater and marine pet and ornamental fish species. The most common genera infecting freshwater pet fish are *Dactylogyrus*, the oviparous "gill fluke" (**Fig. 11**), and *Gyrodactylus*, the viviparous "skin fluke." Neither parasite is location specific and can be found both on the skin and gills of affected fish. The author has seen heavy *Dactylogyrus* infestations most often on imported fancy goldfish and *Gyrodactylus* in other species, such as koi and discus (*Symphysodon* sp). The capsalid marine flukes, *Benedenia* sp and *Neobenedenia* sp, can be found colonizing the skin and gills of affected fish. Clinical signs of monogenean infestations are nonspecific and can range from mild flashing and coughing to heavy mucus production, lethargy, secondary bacteremia, cutaneous lesions, and death.[4,24,40–43] Diagnosis is most often made by examination of a gill biopsy wet mount preparation. Marine capsalids may also be seen after a freshwater dip, a common procedure used in quarantine protocols of newly acquired marine fish. The pale-colored parasites dislodge from their attachments on the fish and can be seen in the fish's water when viewed against a dark background.[40]

Other parasites that have been reported to cause gill problems include the parasitic intracellular microsporidians that can cause respiratory disease in fish, including *Pleistophora* sp, *Glugea* sp, and *Heterosporis* sp. Diagnosis is made with histopathology and wet mount examination of the lesions. The myxosporean, *Myxobolus intrachondrealis*, has been identified in the gill cartilage of the common carp, *Cyprinus carpio*.[45] Other *Myxobolus* sp may be identified as a cause of disease of the gills and associated structures.

Larval digenetic trematodes such as *Neascus* sp and *Clinostomum* sp may also infest the gill tissue. Mild infestations cause minimal pathology but a heavy infestation

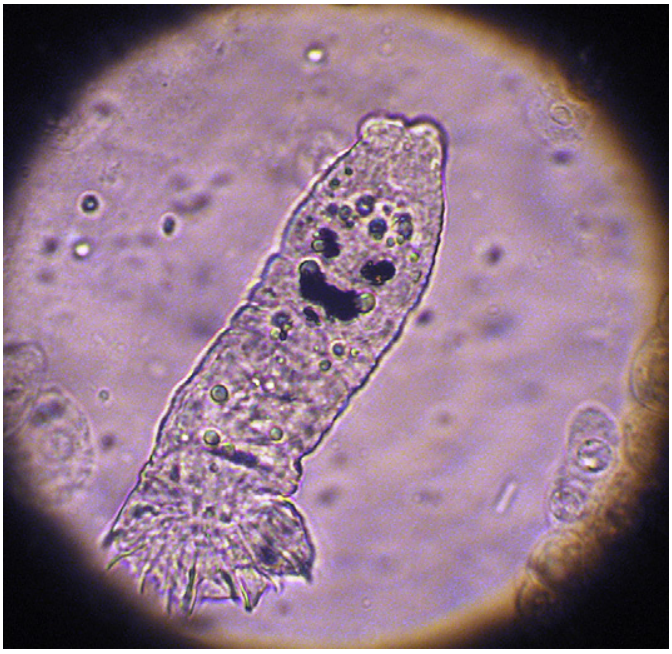

Fig. 11. *Dactylogyrus* sp ("gill fluke") as seen in a wet mount prep, original magnification ×40. Note the characteristic black eyespots and posterior holdfast organ of this species.

can lead to tissue damage and secondary infections.[40,41] Crustacean parasites that may be seen on the gills include *Lernaea* sp (ie, anchor worm), *Argulus* sp (ie, fish louse), the copepod *Ergasilus* sp in freshwater systems, and the isopod *Gnathia* sp in marine systems.[3,40,41] Leeches may also occasionally be found attached to the opercula and gill tissue of pond-reared fish (**Fig. 12**).[3]

Bacterial diseases

Bacterial diseases in fish are fairly common and outbreaks are usually a secondary complication of immunosuppression after stressful events. Pathogenic bacteria that are isolated from gill lesions are frequently organisms that are ubiquitous in the environment.[40,46] Probably the most common bacterial disease of freshwater fish is *Aeromonas hydrophilia*, a Gram-negative bacterium that causes a disease syndrome commonly known as "motile *Aeromonas* septicemia," "red sore disease," "hemorrhagic septicemia," or "ulcer disease."[47] This pathogen initially affects the gills and skin of the fish, then progressively becomes a septicemia infecting many internal organs. The clinical manifestations in the gill may include pale-colored gills to focal or widespread hemorrhagic and necrotic areas of the gills.

Another bacterial disease of the gills, commonly termed "bacterial gill disease," is caused by *Flavobacterium branchiophilia* (a species previously placed in the genus *Cytophagus*, then *Flexibacter*).[47] This Gram-negative bacteria predominantly affects the external surface of the gill causing the number of epithelia and mucus cells to increase. The space between the lamellae becomes filled in with proliferative cellular tissue, which reduces the surface area of the gill and consequently reduces the ability of the gill to allow diffusion of gasses across the tissue. Necrosis of the gill tissue will

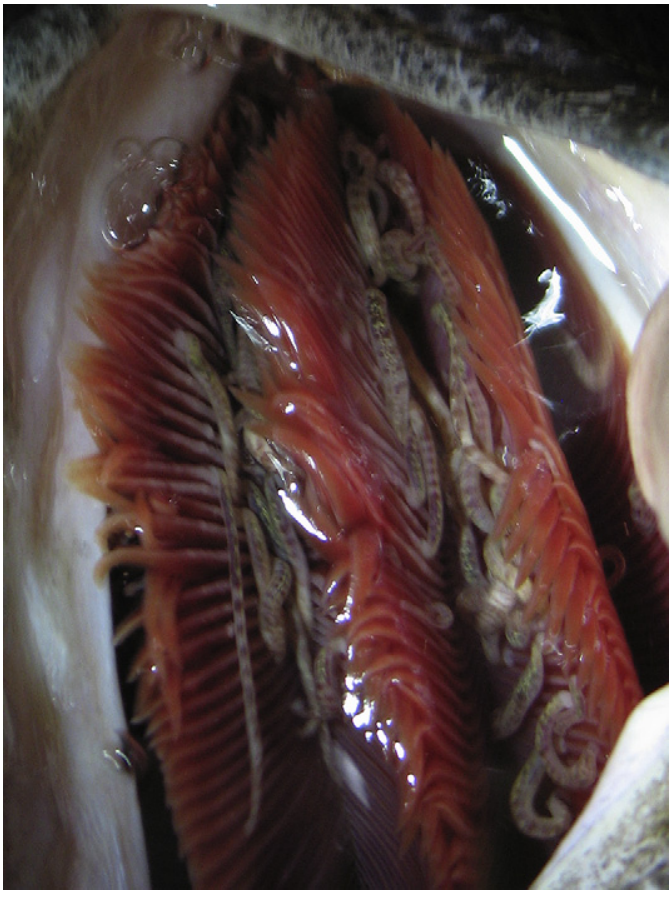

Fig. 12. Leeches (species unknown) found on the gills of a wolf eel (*Anarrhichthys ocellatus*). (*Courtesy of* Dr T. Miller-Morgan.)

sometimes occur in severe cases. Basically, fish find it hard to breath and often face the water current to maximize water flow through the oral and branchial cavities. Numerous scenarios can lead to this bacteria becoming established in a population of fish, such as overcrowding, elevated metabolic waste products, accumulation of organic material in the water column, and increased water temperatures. Another bacteria in this group, *Flavobacterium columnare*, can also affect the gill, causing a disease known as "columnaris" or "cotton-wool" disease. This bacterium colonizes the surface of the gill where it often imparts a distinctive yellowish color to the gill. In wet mount preparations, the bacteria are commonly observed in characteristic haystacklike clusters of filamentous, flexing organisms. This pathogen causes necrosis of the cells at the distal end of the gill filament and progresses to involve the entire filament. Like *F branchiophilia*, predisposing factors include crowding, poor water quality, and increased water temperatures.

Chronic mycobacteriosis may also manifest as an infection of the gills.[48] This can be seen grossly as small white nodules among the gill filaments, in wet mounts as discrete granulomas within the gill tissue, or in histopathology as granulomas with acid-fast bacteria (**Fig. 13**). This bacterial group (*M marinum, M fortuitum*, and so forth) is an important zoonotic pathogen in fish, and pet fish clients should be made aware of the potential human health hazard.[34,48]

Viral diseases

Viral diseases of fish typically produce nonspecific local and general clinical signs and lesions. The most common method of introduction into a naïve population is by failing to isolate and quarantine new animals at viral permissive water temperatures. Most viruses have a specific water temperature range required for spreading the infection to new fish. There are no specific treatments indicated for viral disease outbreaks, although increasing the water temperature outside the specific viral range has been associated with abatement of clinical signs in cases of goldfish herpesvirus and KHV.[49,50] Surviving fish should not be considered "cured" because they can become latent carriers and spread the disease by shedding the virus, infecting naïve fish. Viral diseases of importance that are known to cause lesions in the gills include goldfish herpesvirus (Cyprinid Herpes Virus 2 [CyHV-2], herpes viral hematopoietic necrosis [HVHN]), KHV (Cyprinid Herpes Virus 3 [CyHV-3], carp interstitial nephritis), *Megalocytivirus,* and Spring Viremia of Carp (SVCV). Viral hemorrhagic septicemia (VHS) has been isolated from many species of marine and freshwater fish. Although generally

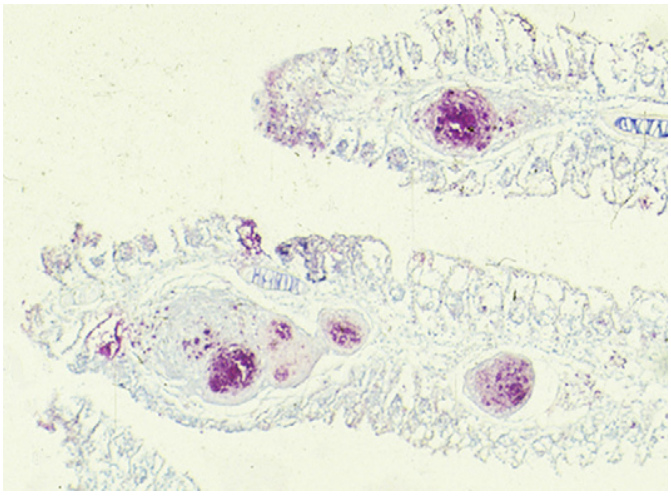

Fig. 13. Histologic section of branchial mycobacteriosis (acid-fast stain, original magnification ×400) showing several granulomas.

not a differential for disease outbreaks in ornamental fish populations, the virus has anecdotally been reported in koi but has not been confirmed.[49]

Goldfish herpesvirus disease outbreaks can occur in all varieties of goldfish and have been detected in the United Kingdom, the United States, Taiwan, Japan, and Australia.[50] Outbreaks generally occur in water temperatures higher than 15°C and can cause acute mortalities, from 50% to 100%.[49,51] Goodwin and colleagues[50] reported that high mortalities were most often associated with shipping stress and acute temperature drops. In addition to the common clinical signs of lethargy and anorexia, affected fish often have multifocal pale, patchy gill lesions. Necropsy reveals splenomegaly with white nodules in the splenic tissue, ascites, and swollen kidneys.[49–51] A quantitative polymerase chain reaction (PCR) test developed by the University of Arkansas is available for confirmation of the disease.[49,52]

KHV has been associated with massive fish mortalities in naïve populations. The virus shows a global distribution and has been found in wild common carp populations in addition to koi, the ornamental variety of *Cyprinus carpio*. Most infections occur in water temperatures between 16°C and 28°C following exposure to an infected fish.[49,53] Failure to quarantine new additions at viral permissive water temperatures is the most common historical finding. Concomitant infection with parasites and secondary bacterial and fungal infections are common. Clinical signs include respiratory distress (piping, gasping, elevated opercular rate), cutaneous ulcerations and hemorrhages, loss of scales, enophthalmos, lethargy, and anorexia. Typical gill lesions include widespread patchy necrosis, areas of hemorrhage, swelling, and excessive mucus (**Fig. 14**).[49,54] The virus survives in water, mud, and feces of infected fish for prolonged periods.[49,55] Draining and drying ponds for several weeks in addition to cleaning and disinfecting tanks and equipment has been recommended. Diagnosis can be made by history, observation of the clinical signs, and the use of PCR testing, virus neutralization tests, or virus isolation.[49,54] A commercial enzyme-linked immunosorbent assay (ELISA) test that detects antibodies to KHV is available to aid in the identification of clinical carriers but false negatives can occur if the fish is tested early in the course of the disease.[49] This test may be combined with quarantine at permissive temperatures to screen for uninfected or KHV carrier fish. An effective vaccine is currently being investigated by several researchers. Goldfish housed with KHV-infected koi have tested positive via PCR for KHV, making them and other species potential vectors in spreading the disease.[49,56,57]

Fig. 14. Gills from a koi infected with Koi herpesvirus. Note the focal areas of necrosis and hemorrhage.

Megalocytivirus is a genus of iridoviruses that has been shown to cause systemic disease in many species of marine and freshwater fish, including several ornamental species commonly kept as pets. Clinical signs are nonspecific and may include lethargy, anemia, hemorrhage of the gills, and high mortalities.[49,55] Large, basophilic cytomegalic cells can be observed on microscopic examination of the gill and other organs.[58]

There is no specific treatment for viral diseases. Improving husbandry practices and water quality, treating secondary or concurrent diseases, and reducing stress may result in a favorable outcome in some infections. Prevention is best and can be achieved through screening suppliers of fish, antemortem testing of fish for exposure when such tests exist, quarantine of all new introductions, and practicing good biosecurity techniques.

Fungal diseases

Fungal pathogens of the gill are generally considered to be opportunistic infections and are usually associated with adverse environmental conditions, poor aeration, or physical trauma. However, fish exposed to intensive culture situations, poor nutrition, temperature shock, external parasites, spawning activity, or other activities causing immunosuppression are generally more susceptible to fungal infections. Most fungal pathogens of pet and ornamental fish belong to the Class Oomycetes in either the genera *Saprolegnia* or *Aphanomyces*.[59,60] The fungal mass is composed of branched, nonseptate fungal hyphae and accumulated debris from the water that becomes trapped in the fungal hyphae. The characteristic gross pathology observed with this type of infection is a white to gray cottonlike mycelial mass on the surface of the gill. This is often associated with an underlying focal, superficial erosion of the gill epithelium. Sometimes these fungal pathogens, especially *Aphanomyces* sp, may become more aggressive and invade the deeper tissues of the gill. When this happens, there is usually no grossly visible external cottony growth on the surface of the gill, as the hyphae penetrate into the dermis, underlying muscle, and viscera of the fish.

In addition, 2 species of the genera *Branchiomyces (B sanguinis, B demingrans)* are considered primary pathogens of gill epithelial tissue. These fungal infections, commonly called “gill rot” or “branchiomycosis,” are usually associated with poor water quality conditions or poor feeding practices (**Fig. 15**). Infections of the gill may present as hemorrhagic swellings and progress to necrosis of large areas of the gill owing to infarction of local blood vessels. Infections of the gill may also invade other tissues of the branchial cavity such as the pseudobranch and thymus.

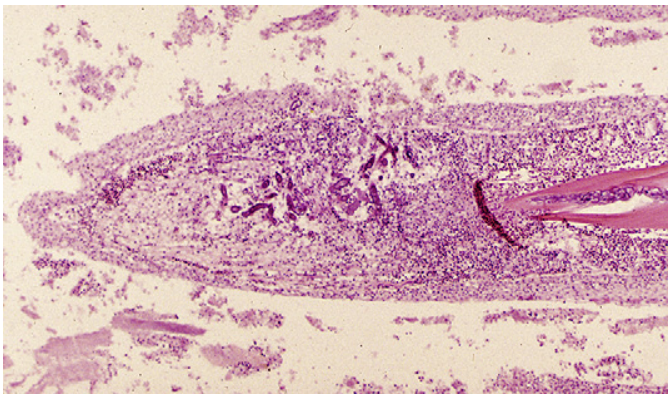

Fig. 15. Histologic section (hematoxylin-eosin stained, original magnification ×400) of gill tissue infected with *Branchiomyces* sp showing general swelling and loss of normal filament architecture. The hyphal elements (dark pink structures) of the fungus can be seen in the center of the affected tissue.

Noninfectious Diseases

Environmental problems

Many different environmental issues can negatively affect gill morphology and function. Water quality problems such as ammonia, nitrite and nitrate toxicity, supersaturation of gases, and turbidity can significantly reduce the ability of gasses, ions, and minerals to diffuse across the gill surface. The water quality toxicities and turbidity chemically and physically irritate the gill tissues and cause them to become hyperplastic. As the epithelial tissues of the gill become thickened, diffusion across the gills becomes more difficult, affecting oxygen uptake and carbon dioxide and ammonia excretion. Thus, the fish not only becomes hypoxic, but also hyperammonemic and acidotic with resulting homeostatic imbalances. The 3 major water quality toxicities (ie, ammonia, nitrite, and nitrate) are difficult to distinguish either grossly or histologically (**Fig. 16**), but all can be acutely or chronically stressful to fish depending on the concentration and time of exposure and all can be lethal. Wet mounts of gill biopsies may have an increased amount of mucus, a thickened appearance to the gill lamellae, and clubbing and fusion of gill filaments. Additionally, nitrite toxicity, which can sometimes cause methemoglobinemia (ie, brown blood disease), may cause the gills to become a dark red to chocolate-brown color once the methemoglobinemia reaches concentrations of 40% or higher.[61]

Supersaturation of gases in the water column is another problem that can affect the function of the gills. This problem, commonly called “gas bubble disease” manifests as bubbles in the capillaries of the gills and fins, air bubbles in the eye, and raised bubbles in the skin. The elevated gas concentrations above atmospheric levels can be caused by insufficient aeration of water obtained from deep wells or springs; leaks in pumps, valves, or water lines; or sudden water temperature changes. Various gases may be involved, although elevated nitrogen concentrations seem to be the most common, and may be both chronically stressful and acutely lethal to fish. Wet mounts of gill biopsies will reveal air bubbles within the lumen of the capillaries, which prevents normal flow of blood though the vessels (**Fig. 17**).

Water pH parameters outside an optimal range (species dependent) can cause changes in the gills and increase the toxicity of some pollutants in the water.[8,61,62] A low pH causes increased mucus production and changes typical of a stress-induced response in the gills. Acidic water also favors higher toxicity of heavy metal pollutants.[8,61] High water pH can cause hypertrophy of the goblet and epithelial cells in the gills leading to excessive mucus production and swelling.[8,61]

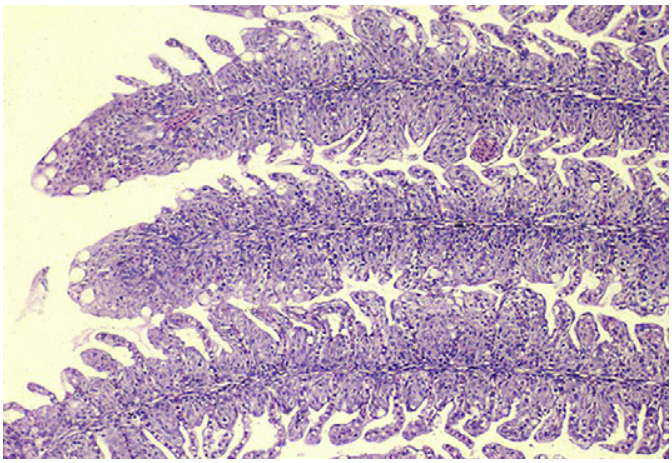

Fig. 16. Histologic section from the gill of a fish with water quality (ammonia or nitrite) toxicity. Note the thickened appearance, lamellar fusion, and hyperplasia (H&E, original magnification ×40).

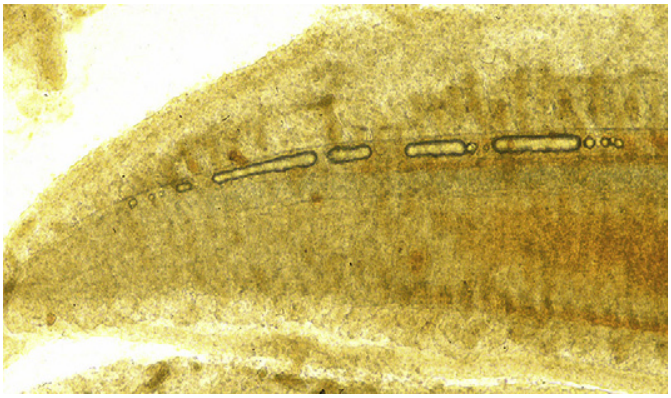

Fig. 17. Gas bubble disease. Note the air bubbles found in the capillary lumen of a secondary lamella caused by supersaturation of gases in the water column (original magnification ×100).

The gills are a major site for damage caused by pollutants in the water. Contaminants and/or toxins that can cause gill damage and subsequent signs of respiratory distress include chlorine; heavy metals, including copper, iron, zinc, mercury, and aluminum; formalin; potassium permanganate; and detergents. Heavy metals contamination of the environment results in structural damage of the gill epithelium.[8] Chlorine is found in many municipal water supplies where it has been added for its disinfectant properties.[63,64] Chlorine is toxic to fish and can result in severe pathologic changes in the gill tissue. Lesions commonly seen include epithelial lifting, hypertrophy, hyperplasia, lamellar fusion, excess mucus production, and necrosis.[3,63,65] The most common finding is failure to use a dechlorinator when adding water, setting up a new system, or performing a water change in aquaria and ponds. Diagnosis is made on historical findings, physical examination findings, and water testing for chlorine.

Copper is a heavy metal that can be introduced into systems as a therapeutic agent, algaecide, decoration, or it may exist as a component of the plumbing system. Copper toxicity results in edema of the gills, in addition to immunosuppression and liver and kidney damage. Measurement of toxic levels of the free copper ion in the water with field test kits can support a diagnosis. Copper is more toxic when used in systems with low alkalinity and low pH.[62,64] Zinc toxicity may also occur when fish are housed in galvanized tubs or in display ponds where the public can toss coins into the water. Zinc and copper toxic effects are additive.[62,64] Other heavy metals that cause gill pathology include aluminum (when present in acidic water it can cause gill necrosis) and iron and manganese (where high levels of each can favor iron and manganese oxide precipitates depositing on the gills, causing lamellar fusion and necrosis).[62,64] A sample of water can be submitted to an environmental lab for heavy metal testing for definitive diagnosis, although this testing can run several hundred dollars.

Potassium permanganate, a common water treatment used to treat pond fish, can also cause precipitation of manganese oxides when used in systems with a high water pH. Another water treatment and therapeutic agent, formalin, can cause gill irritation and reduce dissolved oxygen content in ponds. Formalin is more toxic in aquaria and ponds with soft, acidic water and at high temperatures.[64] Species vary in their sensitivity to formalin.

Nutritional diseases

Suboptimal nutrition can also affect the gill. A pathologic condition (ie, nutritional gill disease) caused by a deficiency of pantothenic acid in older feed has been reported

to affect the gills of catfish and trout.[66,67] Gross lesions included hyperplasia of the proximal portions of the gill filaments causing the gill filaments to become swollen and bulbous. The resulting clubbing and fusion of the gill filaments interferes with gaseous exchange and the fish become lethargic and anorexic. Dry feeds that have been stored for extended periods of time or exposed to high temperatures are usually the cause of the problem. Fortunately, the condition appears to be reversible with the use of fresh feed, although recovery may be a gradual process. Although this specific problem has not been reported in pet fish, it is assumed that the same type of pathology would occur if pet or ornamental fish were fed a diet deficient in pantothenic acid.

In addition to being nutritionally deficient in some cases, fish food can also become contaminated with various toxic agents including mycotoxins, pesticides, and herbicides, leading to toxicities in fish. For example, aflatoxin, a mycotoxin produced by *Aspergillus* sp, has been shown to cause necrotic changes in the gills.[68]

Neoplastic diseases

Neoplasia of the gills and the surrounding tissue is a relatively rare occurrence.[3,69] The consequence to respiration depends on the location and size of the lesion, degree of invasiveness, and/or mechanical interference of respiration. Papillomas, squamous cell carcinomas, chondromas, branchioblastomas, and pseudobranchial adenomas have all been reported to occur in multiple fish species.[3,69,70] Thyroid hyperplasia can cause compression of the gills and distension of the opercula.[4] **Fig. 18** shows a koi, *Cyprinus carpio*, with a neoplastic mass in the gill cavity.

Miscellaneous diseases and conditions

There are several other situations that can involve the gills and affect respiration and disease susceptibility. Missing or deformed opercula can be a result of a genetic defect, traumatic injury, and deficiencies of Vitamins A and C (**Fig. 19**).[3,71,72] In one article, osseous metaplasia caused swelling of the gills in a goldfish, *C auratus*.[73] Many species of ornamental fish root in the pond or aquaria substrate. Occasionally, a piece of substrate can become lodged in the oral cavity or pharyngeal region causing signs of respiratory distress owing to interference of the normal gill and opercula function (**Fig. 20**).[3]

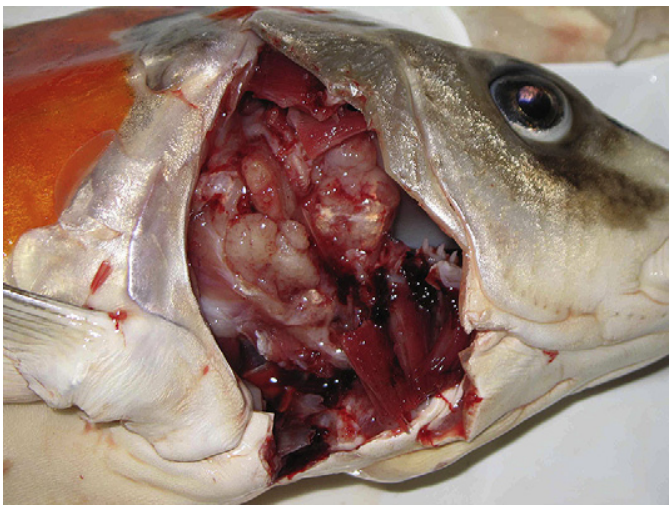

Fig. 18. A neoplastic mass in the branchial cavity of a koi (*Cyprinus carpio*). (*Courtesy of* Dr T. Miller-Morgan.)

Fig. 19. Opercular defect in a koi (*Cyprinus carpio*).

TREATMENT OF RESPIRATORY DISORDERS IN FISH

Treatment of respiratory disorders requires an accurate diagnosis and understanding of the role any environmental disorders may have. Inaccurate diagnoses can delay appropriate treatment and risk increasing morbidity in the population. Many treatments used to treat ornamental and pet fish are not approved by the US Food and Drug Administration, thus the client should be informed of any known adverse effects, and consent to treat should be obtained before instituting therapy. Water or immersion-type treatments should be closely monitored for any adverse reactions in the fish or the biofiltration system. Some treatments also have the potential to cause gill irritation or toxicity. In the treatment of parasitic diseases, it is recommended not to bypass the filtration system because of the possible hidden presence of life stages "hiding" in the filtration system. Supplemental aeration is recommended whenever possible to maximize respiratory efficiency. When fish have been removed to a newly set-up hospital or isolation tank for treatment, there is a possibility of water quality abnormalities developing from a nonactivated filter that can lead to a worsening of the clinical signs. Routine water quality monitoring is needed in these systems.

Medications commonly used in the treatment of diseases of the gills can be found in **Table 2**.

Fig. 20. Piece of gravel removed from the pharynx of this goldfish exhibiting respiratory distress.

Table 2
Formulary for the treatment of selected respiratory diseases in fish[a]

Condition(s)	Treatment[a]	Dosage(s)	Comments
Bacterial diseases			
	Amikacin[40,72]	5 mg/kg IM q 12 h	Has not been studied pharmacokinetically in pet fish, used frequently by hobbyists
	Aztreonam[40,72]	100 mg/kg IM, ICe q 48 h	Used by koi hobbyists
	Ceftazidime[73]	20–30 mg/kg IM q 72 h	Not studied pharmacokinetically in pet fish Used commonly by fish veterinarians
	Enrofloxacin[35,73–75]	5–10 mg/kg IM, ICe q 48–72 h 5 mg/kg PO every 24–48 h 2.5 mg/L × 5 h every 24–48 h	Only studied pharmacokinetically in koi and pacu, but used commonly by fish veterinarians in many species Resistance becoming an issue in bacterial infections in koi
	Florfenicol[76–78]	Red Pacu: 20–30 mg/kg IM q 24 h Koi: 25 mg/kg q 24–48 h; shorter half-life in three-spot gourami may necessitate more frequent dosing 50 mg/kg PO q 24 h in koi; shorter half-life in gourami may necessitate q 12-h dosing Minimal absorption as bath treatment in koi[77]	Studied pharmacokinetically in red pacu, koi, and three-spot gourami Available in food as a veterinary feed directive (VFD) (Aquaflor) with specific legal constraints. Cannot be prescribed for extralabel use
	Hydrogen peroxide 35% (35% Perox-Aid)[78,79]		FDA approved for use for bacterial gill disease and columnaris disease in specific species
	Oxytetracycline[35,80–82]	7 mg/kg IM q 24 h 1.12 g/lb food/d for 10 d 750–3780 mg/10 gallons for 6–12 h, repeat daily for 10 d (dose will depend on hardness of water) 50%–75% water changes between treatments[35]	Studied pharmacokinetically in red pacu Increased Ca and Mg inactivate, not useful in marine systems as bath treatment Available as a medicated food, Terramycin 200 for fish Water can become yellow with use of oxytetracycline Bacterial resistance common

	Sulfadimethoxine/ ormethoprim[40,72,82]	50 mg/kg/d for 5 d	Not useful as bath treatment Not studied pharmacokinetically in pet fish Medicated feed available (Romet B, Romet-30)
Fungal diseases			
	Formalin[73,78,83,84]	(See section on parasites for dosing information)	
	Hydrogen peroxide 35%[78,79]	Egg treatment: 500–1000 mg/L dip for 15 min	A trial bioassay is recommended before widespread use
	[a]Malachite green[44,58,73,84–86]	1–2 mg/L 30–60-min bath treatment 0.1 mg/L prolonged immersion 0.5 mg/L 60-min bath 0.3 mg/L 24-h bath 100 mg/L topical application	Carcinogenic, teratogenic, respiratory poison, and stains objects Can be toxic to gill tissue Reported toxicity in eggs near hatching, small marine fish, young fry, some tetra, catfish, scaleless fish, loach species, and plants Toxicity enhanced with warmer water temperatures and low pH Can be toxic when combined with formalin Use zinc-free solution Remove from water with activated carbon Rinse after topical application
	Sodium chloride[58]	10–50 g/L for 1–2 min bath	Kills the infectious zoospore, not the fungal hyphae on the fish
Parasitic diseases			
Freshwater external protozoan parasites	Sodium chloride solution ("saltwater," hypersalinity treatment)[40,87]	3–6 g/L prolonged immersion 10–30 g/L dip (5–10 min or until fish is stressed)	Can also reduce osmoregulatory stress Dip is often used in quarantine protocols
Freshwater external protozoan and crustacean ectoparasites Fungal diseases (saprolegniasis)	Formalin (37% formaldehyde)[73,78,83,84]	0.125–0.25 mL/L Bath q 24 h × 2–3 days for up to 60 min 0.015–0.025 mL/L (15–25 ppm) prolonged immersion, every 2–3 d	Carcinogenic, human health concerns *Depletes oxygen*, additional aeration required Some fish very sensitive Not for use in stressed fish

(*continued on next page*)

Table 2
(*continued*)

Condition(s)	Treatment[a]	Dosage(s)	Comments
	Formalin (cont.)		Toxicity enhanced with low water pH and low alkalinity Do not use if white precipitate forms Contraindicated >27°C (80°F) Toxic to invertebrates
External protozoan, dinoflagellate, monogenean, and crustacean pathogens of marine and freshwater fish	Hydrogen peroxide 35%	50–100 mg/L for 30–60 min for 3 consecutive days	Investigational claims can be evaluated at: http://www.fws.gov/fisheries/aadap/summaryHistory11-669.htm (AADAP = Aquatic Animal Drug Approval Program)
Marine protozoan ectoparasites and some monogenean infestations	Freshwater (hyposalinity)[87]	Dip (duration in minutes)	Not effective against all protozoans A common quarantine procedure
Marine protozoan ectoparasites and dinoflagellates	Copper[40,84]	0.2 mg/L free copper ion prolonged immersion 100 mg/L bath	Not recommended for freshwater systems Bound to inorganic compounds Toxic to invertebrates Elasmobranchs may react adversely Copper levels should be monitored Solubility affected by pH and alkalinity Immunosuppressive and toxic to gill tissue

External infections ectoparasites (protozoan, monogeneans)	Potassium permanganate[40]	2 mg/L prolonged immersion 5–20 mg/L 1-h bath	Inactivated by organic compounds in water Caustic Toxic in high pH water Stains Can be toxic in some fish species Can cause blindness in humans (powder) Watch for signs of stress in patients with use Safer products are available
Monogenean	Closantel 5 mg/mL and mebendazole 75 mg/mL (Supaverm, Janssen Animal Health)[40,59]	1 mL/400 L bath treatment	Caution-Koi ONLY! Fatal to goldfish. Not studied pharmacokinetically in fish Not FDA approved, not commercially available in the United States Used by hobbyists
Monogenean	Praziquantel[35,40,73]	2–10 mg/L bath or prolonged immersion once weekly for 3–6 treatments	Treatment frequency and duration is determined by species of monogenean and water temperature
Dinoflagellates	Chloroquine[40]	10 mg/L prolonged immersion	Affects only the infectious dinospore stage Toxic to bacteria and invertebrates
General treatments			
	Sodium chloride	1 g/L prolonged immersion	Reduction of stress, nitrite toxicity
	Water changes		Indicated for environmental toxins (ammonia, copper, nitrite)

Abbreviations: FDA, Food and Drug Administration; ICe, intracoelomic; IM, intramuscularly; PO, by mouth; q, every.

[a] Some compounds should not be recommended for use by a veterinarian but are included here for completeness and because of their frequent use by laymen.

SUMMARY

The respiratory organ of fish is the gill. In addition to respiration, the gills also perform functions of acid-base regulation, osmoregulation, and excretion of nitrogenous compounds. Because of their intimate association with the environment, the gills are often the primary target organ of pollutants, poor water quality, infectious disease agents, and noninfectious problems, making examination of the gills essential to the complete examination of sick individual fish and fish populations. The degree of response of the gill tissue depends on type, severity, and degree of injury and functional changes will precede morphologic changes. Antemortem tests and water quality testing can and should be performed on clinically affected fish whenever possible.

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