Bacterial and Parasitic Diseases of Pet Fish

Helen E. Roberts, DVM[a,*], Brian Palmeiro, VMD, DACVD[b,c], E. Scott Weber III, VMD, MSc[d]

KEYWORDS

• Pet fish • *Aeromonas* • *Ichthyophthirius*
• Wet-mount cytology • Ornamental fish

BACTERIAL DISEASE IN PET FISH

Bacterial disease is extremely common in ornamental fish and is most frequently associated with bacteria that are ubiquitous in the aquatic environment acting as opportunistic pathogens secondary to stress. Less commonly, bacterial disease is caused by primary or obligate pathogens. Most bacterial infections of fish are caused by gram-negative organisms and include the genera *Aeromonas*, *Citrobacter*, *Edwardsiella*, *Flavobacterium*, *Pseudomonas*, and *Vibrio*.[1–3] Bacterial disease in fish is complex and involves the interplay of various factors including the environment, the host (immune system function, host susceptibility, etc.), and pathogen-specific factors such as virulence. Stress can result in immune suppression and is critical in the pathogenesis of bacterial disease in fish with poor environmental conditions as the most common stressor involved in precipitation of bacterial disease. Water quality should routinely be assessed when investigating any disease outbreaks in aquatic organisms. Major bacterial pathogens in fish can be divided into the following four major groups[1] and one minor newly emerging group of pathogens:

Ulcer forming or systemic, gram-negative bacteria. This group includes bacteria in the genera Aeromonas, Vibrio, Edwardsiella, Pseudomonas, Flavobacterium, and others. This is the most common group of bacterial pathogens that affect fish.

External, gram-negative bacteria. This group of bacteria most commonly causes external infections. Some of these bacteria may also cause systemic infections.

[a] Aquatic Veterinary Services of WNY, PC, 5 Corners Animal Hospital, 2799 Southwestern Blvd., Suite 100, Orchard Park, NY 14127, USA
[b] Pet Fish Doctor, 645 Pennsylvania Ave, Prospect Park, PA 19076, USA
[c] Veterinary Referral Center, Malvern, PA 19355, USA
[d] Companion Avian and Exotic Animal Service, Department of Aquatic Animal Health, University of California, Medicine and Epidemiology, 2108 Tupper Hall, Davis, CA 95616, USA
* Corresponding author.
E-mail address: nyfishdoc@aol.com (H.E. Roberts).

Vet Clin Exot Anim 12 (2009) 609–638
doi:10.1016/j.cvex.2009.06.010 **vetexotic.theclinics.com**
1094-9194/09/$ – see front matter

Included in this group are Flavobacterium columnarae, Flexibacter maritimus, yellow-pigmented bacteria, Cytophaga spp, and others.

Systemic, gram-positive, rapidly growing bacteria. These bacteria generally cause systemic infections and include Streptococcus spp and related species.

Slow-growing, acid-fast bacteria. These bacterial pathogens cause systemic, chronic, granulomatous disease. The most common pathogens in this group are Mycobacterium spp.

Newly emerging intracellular rickettsial pathogens.

Clinical signs of bacterial disease may be peracute (mortality without gross evidence of disease), acute, or chronic and varies with the particular pathogen and various host related factors. With misuse of antibiotics, antibiotic resistance is becoming more important in treating bacterial diseases in fish. This article discusses specific bacterial diseases in fish including clinical presentation, diagnostics, and treatments.

ULCER-FORMING AND SYSTEMIC INFECTIONS CAUSED BY GRAM-NEGATIVE BACTERIA

This is the most common group of bacterial pathogens that affect fish and includes bacteria in the genera *Aeromonas*, *Vibrio*, *Edwardsiella*, *Pseudomonas*, *Flavobacterium*, and others. Clinical signs of ulcer-forming and systemic infections caused by gram-negative bacteria include lethargy, anorexia, abnormal swimming patterns or spinning, hemorrhagic lesions on the skin, ulcerative skin lesions, abdominal distension, ascites, abnormal position in the water column, exophthalmia (“pop-eye”), skin darkening, gill necrosis, and mortality.[1–3] With gill involvement, respiratory signs such as increased opercular rate, piping (gasping for air at the water surface) and respiratory distress may be seen.

Motile Aeromonad Septicemia

Motile aeromonads are the most common bacterial pathogens of fish and may result in a syndrome called motile aeromonad septicemia (MAS). MAS is most commonly caused by ubiquitous aquatic bacteria of the *Aeromonas hydrophila* complex, including *A hydrophila*, *A sobria*, and *A caviae*. *A hydrophila* is the most common isolate and is more commonly isolated from freshwater than marine fish. MAS is almost always secondary to an underlying stressor and is most commonly found in conjunction with eutrophic water quality conditions. In fishponds, this aeromonad is commonly isolated from clinically ill fish in the warmer months of the year. Common clinical signs include cutaneous hemorrhages and ulcers that can be deep through the dermis to connective tissue and muscle, visceral hemorrhages, edema, dropsy or ascites, and exophthalmia.[1–3]

*Ulcerative Dermatitis in Koi (*Cyprinus carpio)

Ulcerative dermatitis (UD) is a multifactorial syndrome seen in koi (*Cyprinus carpio*) and related cyprinids such as goldfish (*Carassius auratus*) that results in ulcerative skin lesions. Clinical signs include raised or erythematous and missing scales, and ulcers that extend from the skin into the underlying musculature; in severe cases, bone may be exposed or penetration into the coelomic cavity may occur. Progression to septicemia can also occur resulting in clinical signs such as those seen with MAS. Osmotic distress due to loss of epidermal integrity may result in fluid retention, exophthalmos, and dropsy. **Fig. 1** exhibits the typical ulcers in a koi with UD. Various bacterial pathogens have been isolated from these cases including *A salmonicida* and *A hydrophila*.[1,2,4] *A salmonicida* can be difficult to culture as it is fastidious and quickly overgrown by other rapidly growing bacteria such as motile aeromonads.[1] In a recent

Fig. 1. A koi with ulcerative dermatitis exhibiting typical cutaneous ulcers. Note exposure of underlying musculature and peripheral annular rims of hemorrhage surrounding the ulcers.

abstract, *A salmonicida* DNA was detected by way of polymerase chain reaction (PCR) in 77% of koi with ulcerative skin lesions.[5] Although not always the case, UD lesions caused by *A salmonicida* have been diagnosed by one of the authors (ESW) more in autumn and winter, when water temperatures are cooler. Numerous pathogens have been isolated from these cutaneous lesions by way of sterile-swab and sterile-tissue cultures including *Aeromonas* spp, *Pseudomonas* spp, *Citrobacter* spp, *Chryseobacterium* spp, *Delfia* spp, and *Shewanella putrefaciens*.[6]

Causes of UD in koi can be divided into predisposing, primary, secondary, and perpetuating factors (**Table 1**).[7]

Table 1
Predisposing, primary, secondary, and perpetuating causes of ulcerative dermatitis in koi

Predisposing	Primary	Secondary	Perpetuating
Chemical stressors: poor water quality or other undesirable environmental conditions	Ectoparasites	Secondary bacterial invaders—motile aeromonads, *Pseudomonas*, *Flavobacterium*, *Citrobacter*, etc	Fibrosis and scarring, granulation tissue, tissue weakening
Physical stressors: shipping, overcrowding, aggression, etc	*Aeromonas hydrophila* complex	*Saprolegnia*	Recruitment of inflammatory cells and enzymatic tissue degradation
Poor husbandry: filtration, tank or pond design, nutrition, etc	*Aeromonas salmonicida*	Parasites that invade damaged epidermis—nematodes, flukes, *Trichodina*, *Ichthyobodo*, etc	Osmotic stresses due to lack of epidermal integrity
—	Trauma	—	Septicemia
—	Koi Herpes Virus	—	—
Physiologic stresses	—	—	—
Genetic factors	—	—	—
—	—	Algae	—

From Palmeiro BS. Bacterial Diseases. In: Roberts HR, editor. Fundamentals of ornamental fish health. Wiley-Blackwell. In Press; with permission.

In most cases, many of these factors interact together to result in clinical disease, emphasizing the need for a thorough diagnostic evaluation. Systemic antibiotics are often used in the treatment of this condition. Prolonged bath immersion treatment with salt at 0.1% to 0.3% is typically used to decrease osmotic stresses. In some cases, debridement of the ulcer and removal of necrotic tissue or scales may be necessary. Various topical treatments such as silver sulfadiazine can also be helpful. No treatment should be initiated without assessing and addressing water quality concerns. More information on treatment can be found under treatment of systemic or ulcer gram-negative infections below.

Aeromonas salmonicida

A salmonicida is a nonmotile species of *Aeromonas* that causes a chronic-to-subacute bacterial infection resulting in cutaneous ulcerations of the skin. There are three reported subspecies of *A salmonicida* including *salmonicida*, *achromogenes*, and *masoucida*.[3] The subspecies *salmonicida* is usually associated with systemic infections and is the causative agent of furunculosis in salmonids.[3] The atypical subspecies *achromogenes* is more commonly associated with ulcerative skin lesions in nonsalmonid species such as goldfish, common carp, and eels. It is also the causative agent of carp erythrodermatitis and has been implicated as the causative agent of UD in koi. *A salmonicida* is considered an obligate pathogen of fish, but carrier fish can occur.[3]

Pseudomonas *spp*

Most Pseudomonal-associated septicemia cases in ornamental species are due to *P fluorescens*.[2] Infections are more common in warmer water temperatures and are typically secondary to environmental stressors,[2] although one author (ESW) has diagnosed an infection in a cold marine Atlantic cod. There is still disagreement among fish health specialists if *Pseudomonas* represents a primary or secondary pathogen or a nonpathogenic environmental contaminant.

Vibriosis

Bacteria of the genus *Vibrio* are ubiquitous in the marine and estuarine environment and most commonly cause disease in marine fish. In a survey of 129 cases from tropical marine fish, nearly 50% had either *Aeromonas* or *Vibrio* infections.[8] Species that have been shown to cause disease in fish include *V anguillarum* (most common), *V salmonicida*, *V ordalii*, *V alginolyticus*, *V parahaemolyticus*, *V vulnificus*, and *Photobacterium damselae* subspecies *piscicida*. *V anguillarum* and *V ordalii* are the two most commonly identified from marine ornamentals.[2] Clinically, vibriosis is very similar to motile aeromonad septicemia and results in hemorrhagic septicemia with cutaneous hemorrhages and ulcers.[2] As with other bacterial infections, underlying environmental stressors are often present.

Photobacterium damselae *subspecies* piscicida

One common species recently classified in the *Vibrio* family causing pathology in marine tropical fish is *Photobacterium damselae* subspecies *piscicida.* It is found in a variety of freshwater and marine tropical and temperate species. It can manifest in an acute outbreak with varying mortality, or the disease can be marked by an insidious chronic form with pseudotubercle formation in the kidney and spleen with internal necrosis.[9] This disease was formerly known as piscine pasteurellosis. Outbreaks are associated with high temperatures and can cause catastrophic losses in several species of marine fish. This disease has been treated using various oral antibiotics.

Edwardsiella tarda

Edwardsiella tarda is a gram-negative rod associated with septicemia with or without ulceration in a wide variety of freshwater and marine species.[10] Disease associated with *E tarda* often occurs during the summer in ponds. Reports of disease in ornamental fish have been reported in coral reef grunts and squirrelfish, and a keyhole angelfish.[8] This bacterium can infect all poikilothermic vertebrates and is zoonotic. In disease outbreaks, antibiotics are commonly added to feed.

DIAGNOSIS OF GRAM-NEGATIVE ULCER FORMING OR SYSTEMIC INFECTIONS

Diagnosis is based on history, clinical signs, and bacterial culture or sensitivity. Samples should be submitted to laboratories that are familiar with culturing aquatic pathogens. Most fish pathogens are best cultured at room temperature (22–25°C), not 37°C, which is the common protocol in mammalian microbiology laboratories. A nonselective agar such as blood agar is a good medium for the growth of most fish pathogens.[3] In marine fish, a medium with 1% to 2% sodium chloride may be needed. In some cases, selective specialized media can be used to enhance the growth and isolation of certain pathogens.

The organ of choice for bacterial culture (**Fig. 2**) in systemic infections is the posterior kidney.[1–3] Dorsal and ventral approaches to the posterior kidney have been described.[2] Other organs that may be cultured include the brain (especially when neurologic signs are present), liver, spleen, and anterior kidney. Blood culture has been described as a nonlethal test in cases of systemic bacterial infections. A recent report described good correlation between blood culture results and posterior kidney tissue cultures in a small population of fish.[11] Blood cultures were obtained aseptically from the caudal vein; samples were incubated in brain heart infusion broth and then transferred to blood agar plates.[11] When culturing cutaneous ulcers, tissue cultures obtained aseptically are preferred to superficial swabs as secondary pathogens commonly invade ulcerative lesions. When culturing, samples should be obtained from the leading edge of the ulcerative lesion. Taking a small biopsy for histology or cytologic staining is also recommended to help support microbiologic findings. *A salmonicida* can be very difficult to culture as it is quickly overgrown by less fastidious secondary pathogens. Molecular techniques (such as PCR, ELISA, and immunostaining) can be used to illustrate the presence of *A salmonicida* or other pathogens in ulcerative skin lesions or various tissue samples.[5]

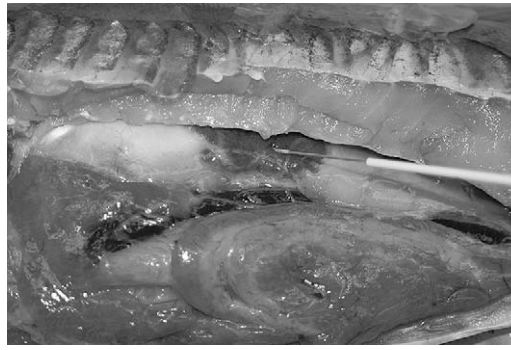

Fig. 2. Bacterial sampling (culture) from the posterior kidney.

TREATMENT OF GRAM-NEGATIVE ULCER FORMING OR SYSTEMIC INFECTIONS

Treatment of gram-negative ulcer forming and systemic infections in fish should always involve a thorough analysis of the environment, and improvement of any poor environmental or husbandry-related problems or other related stressors. The mainstay of treatment for gram-negative systemic or ulcerative disease in fish is antimicrobials. Antimicrobials can be administered parenterally, orally, or as a bath treatment. Parenteral administration of antibiotics is the most effective method to achieve therapeutic blood levels that exceed the minimum inhibitory concentration for aquatic pathogens. Ideally, antimicrobials are selected based on culture and antibiotic susceptibility tests (disk diffusion, automated-broth microdilution techniques) as antimicrobial resistance is common in aquatic bacterial pathogens. Empiric first choice antibiotic should be effective against gram-negative bacteria. Few antimicrobials have been studied pharmacokinetically in ornamental fish. The antibiotics studied in ornamental fish include enrofloxacin, florfenicol, and oxytetracycline. **Table 2** lists common antimicrobials used in pet fish, including dosing information when applicable.

Injectable antibiotics are typically given as intramuscular or intracoelomic injections. Intramuscular injections are most commonly given in the dorsal epaxial musculature. Intracoelomic injections can be given in the scaleless region at the base of the pelvic fin. **Fig. 3** illustrates an intracoelomic injection in a koi.

Oral antibiotics are most commonly used when treating large numbers of fish and when injections are not practical. Antibiotics are either mixed with the feed or top-dressed on the feed using a binding agent such as canola oil. Gel-based diets (such as those by Mazuri Purina Mills, Grey Summit, Missouri) offer the practitioner a convenient and palatable diet in which oral antibiotics can easily be mixed. Oral antibiotics can also be administered by way of oral gavage tube.

Administration of antibiotics in the water is commonplace in the aquarium industry and numerous antibiotics are available over the counter to fish hobbyists. Problems associated with use of bath antibiotics include limited absorption or insufficient dose, damage to the biofilter and development of bacterial resistance.[1] Pharmacokinetic data for bath antibiotics is generally lacking in ornamental fish; absorption is likely greater in marine fish because of increased water consumption. Bath antibiotics should be limited to cases of external infections (such as columnaris disease and ''fin rot'') and in fish that are anorexic.

EXTERNAL GRAM-NEGATIVE BACTERIAL INFECTIONS

Bacterial diseases caused by gram-negative bacteria that are typically more limited to the skin include columnaris disease and fin rot. Columnaris disease is caused by *Flavobacterium columnare* and results in cottony proliferative lesions on the skin and fins.[1] Common locations for the lesions include perioral, periocular, fins, dorsum, and tail regions. The synonym ''cotton-wool'' disease describes the fluffy white cotton-like masses, patches, or plaques often seen with *F columnare.* Given this clinical appearance, columnaris disease is often misdiagnosed as fungal disease in aquarium fish; wet-mount examination of affected areas can be used to differentiate between these two conditions and rule out parasitic infestations (**Fig. 4**). *F columnare* may also affect the gills resulting in respiratory signs. A large number of other similar gram-negative rods including *Cytophaga* spp, *Flexibacter* spp, *Flavobacterium* spp, *Sporocytophaga* spp, and *Myxobacterium* spp have been isolated from fish with similar lesions.[2] All these gram-negative bacteria form yellow to orange pigmented colonies and are occasionally collectively referred to as yellow-pigmented bacteria.[5] Columnaris-type infections caused by *F maritimus* have been reported to cause

a marine form of columnaris disease with similar clinical signs to the freshwater counterpart.[2,3]

Wet-mount examination of the skin reveals characteristic long, thin rods with gliding or flexing motion; ''hay stack'' protrusions of rod-shaped bacteria may also be noted.[1–3] Columnaris disease is common in live bearers such as guppies, platies, mollies, and swordtails. Treatment of columnaris disease can be achieved with antibiotic bath treatment with oxytetracycline (see **Table 2** for dosing) repeated daily for 10 days. Other treatment options include potassium permanganate as a prolonged bath, copper sulfate, and diquat herbicide (Reward Syngenta, Greensboro, North Carolina) dosed at 2 to 18 mg/L for four-hour bath immersions.[1] Treatment should be repeated daily for three to four treatments with large water changes after each bath treatment. Systemic antimicrobials may be needed in more severe infections. A recent study found that a single hydrogen peroxide (H_2O_2) treatment of 3.1 mg/L or more for one hour effectively eliminated external bacteria in the green swordtail *Xiphophorus helleri*.[12]

Fin-rot refers to a characteristic necrosis of the fins resulting in an irregular notched to ragged appearance to the fins. Fin rot is usually secondary to underlying stressors and poor husbandry. Several different species of bacteria can be isolated from these lesions in ornamental fish including *Flavobacterium columnare*, *Flexibacter maritimus*, and *Cytophaga* spp.[2] Treatment of this condition involves searching and removing underlying stressors and using antimicrobials as for columnaris disease.

SYSTEMIC, GRAM-POSITIVE, RAPIDLY GROWING BACTERIA

The most common bacteria in this group that cause disease in fish are *Streptococcus* spp; other gram-positive genera that are closely related to *Streptococcus* and cause disease in fish include *Lactococcus*, *Enterococcus*, and *Vagococcus*. Clinical signs are similar to those involving systemic gram-negative infections such as skin discolorations, exophthalmos, ascites, skin ulcerations, and hemorrhages.[2,13] Neurologic signs are extremely common in fish with streptococcal infections; abnormal swimming behavior such as spiraling or spinning is often reported.[13] High mortality may also occur. On gross necropsy, *Streptococcus* can cause granulomas in the kidney, spleen, or liver. Diagnosis is confirmed by culturing *Streptococcus* and related bacteria. In acute outbreaks of streptococcus, kidney cultures are routinely taken. As many cases invade the central nervous system, culturing the brain in suspected cases is critical when neurologic signs are observed. Antibiotics that may be effective against *Streptococcus* and related species include erythromycin (1.5g/lb of food fed for 10–14 days), amoxicillin or ampicillin, and florfenicol.[13] Immunostimulants added to the feed, such as beta-glucans and nucleotides, have been shown to increase survival for *Streptococcus*-infected redtail black shark populations.[14] In addition to being a human pathogen, *S iniae* causes disease in cultured marine and freshwater fish and is difficult to eradicate from hatcheries and grow-out facilities.

SLOW-GROWING, ACID-FAST BACTERIA: MYCOBACTERIOSIS

Mycobacteriosis in fish is caused by nontubercle-forming *Mycobacterium* species that are ubiquitous in the aquatic environment. The two most common species associated with ornamental fish disease are *M marinum* and *M fortuitum*.[2,15,16] *M chelonae* also causes disease in fish (typically cold-water salmonids) but is less commonly reported.[3] Mycobacteriosis is zoonotic and can cause ''fish tank granuloma'' in people. A recent study in zebrafish illustrated that the primary route of infection is

Table 2
Dosage information for common antimicrobials in pet and ornamental fish

Drug	Parenteral Administration	Oral Administration	Bath	Notes
Amikacin	5 mg/kg IM q 12 h[a]	—	—	Not studied pharmacokinetically in pet fish Used commonly by koi hobbyists
Aztreonam	100 mg/kg IM, ICe q 48 h[a]	—	—	Not studied pharmacokinetically in pet fish Used commonly by koi hobbyists
Ceftazidime	20 mg/kg IM q 72h[b]	—	—	Not studied pharmacokinetically in pet fish Used commonly by fish veterinarians
Enrofloxacin	5–10 mg/kg IM, ICe q 48–72 h (koi)[c] 5 mg/kg IM q 48 h (red pacu)[d]	5 mg/kg PO every 24–48 hr (red pacu)[d]	2.5 mg/L × 5 h every 24–48 h (red pacu)[d]	Only studied pharmacokinetically in koi[c] and pacu,[d] but used commonly by fish veterinarians in many species
Florfenicol	Red pacu: 20–30 mg/kg IM q 24 h[e] Koi: 25 mg/kg q 24–48 h; shorter half-life in three-spot Gourami may necessitate more frequent dosing[f]	50 mg/kg po q 24 h in koi; shorter half-life in gourami may necessitate q 12 h dosing[f]	Minimal absorption as bath treatment in koi[f]	Studied pharmacokinetically in red pacu,[e] koi,[f] and three-spot gourami[d]
Kanamycin	—	300 mg/lb food/d × 10d[g] 50 mg/kg/d in feed[a]	50–100 mg/L × 5 hr, repeat every 3 d for 3 treatments[a,g]	Not studied pharmacokinetically in pet fish May cause renal damage
Oxolinic acid	—	150 mg/lb food/d for 10 d[g]	38 mg/10 gallons for 24 h, repeat as needed[g] 95 mg/gallon for 15 min, repeat twice daily for 3 d[g]	Not studied pharmacokinetallly in pet fish May cause lethargy when used as bath treatment, inhibited by hard water

Oxytetracycline	Red pacu: 7 mg/kg IM q 24 h[h]	1.12 g/lb food/d for 10 d[g]	750–3,780 mg/10 gallons for 6–12 h, repeat daily for 10 d (dose will depend on hardness of water)[g] 50%–75% water changes between treatments	Studied pharmacokinetically in red pau[h] Increased Ca and Mg inactivate, not useful in marine systems as bath treatment
Nitrofurazone	—	1.12 g/lb food/d for 10 d[g]	189–756 mg/10 gallons for 1 h, repeat daily for 10 d[g] 378 mg/10 gallons for 6-12 h, repeat daily for 10 d[g]	Not studied pharmacokinetically in pet fish Systemic absorption from bath treatment questionable, best reserved for external infections Carcinogenic Inactivated in bright light
Sulfadimethoxine/ ormethoprim (Romet B Hoffman-LaRoche)	—	50 mg/kg/d for 5 d[a]	Not useful as bath treatment	Not studied pharmacokinetically in pet fish
Trimethoprim sulfa	—	30 mg/kg PO q 24 hr × 10-14 d[a]	20 mg/L × 5 h q 24 h × 5–7d[a]	Not studied pharmacokinetically in pet fish

Abbreviations: ICe, intracoelomic; IM, intramuscular.

[a] Mashima T, Lewbart GA. Pet fish formulary. VCNA Exotic Animal Practice 2000;3(1):117–30.

[b] Palmeiro B and Roberts H. Bacterial disease in fish. In: Mayer J, editor. Clinical Veterinary Advisor: Exotic Medicine. 2009. In Press.

[c] Lewbart GA, Butkus D, Papich M, et al. Evaluation of a method of intracoelomic catheterization in koi. JAVMA 2005;226:784–8.

[d] Lewbart GA, Vaden S, Deen J et al. Pharmacokinetics of enrofloxacin in the red pacu *(Colossoma brachypomum)* after intramuscular, oral and bath administration. J Vet Pharmacol Therap 1997;20:124–8.

[e] Lewbart GA, Papich MG, Whitt-Smith D. Pharmacokinets of florfenicol in the red pacu *(Paractus brachypomus)* after single dose intramuscular administration. J Vet Pharacol Therap. 2005;28:317–9.

[f] Yanong RPE, Curtis EW, Simmons R et al. Pharmacokinetic studies of florfenicol in koi carp and threespot gourami *Trichogaster trichopterus* after oral and intramuscular treatment. Journal of Aquatic Animal Health 2005;17:129–37.

[g] Yanong R. Use of antibiotics in ornamental fish aquaculture. VM-84. Florida Cooperative Extension Service, UF-IFAS. 2006. Available at: http://edis.ifas.ufl.edu. Accessed June 15, 2009.

[h] Doi AM, Stoskopf MK, Lewbart GA. Pharmacokinetics of oxytetracycline in the red pacu following different routes of administration. J Vet Phamacol Therap 1998;21:364–8.

From Palmeiro BS. Bacterial Diseases. In: Roberts HR edition. Fundamentals of Ornamental Fish Health. Wiley-Blackwell. In Press; with permission.

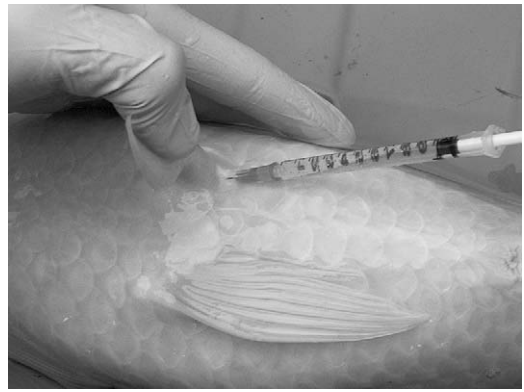

Fig. 3. Intracoelomic injection in a koi. The injection is administered in the scaleless region at the base of the pelvic fin.

through the intestinal tract.[17] Fish can be infected by consuming contaminated feed, by way of cannibalism of infected fish and carcasses or aquatic detritus.[3,15,16]

Mycobacterium spp are ubiquitous in the aquatic environment; a recent report found 75% of water samples from decorative aquaria to be positive for *Mycobacterium* spp.[18] Environmental factors that favor growth of mycobacterium include low dissolved oxygen, low salinity, low pH, warmer water, and high organic loads.[15]

Although mycobacteriosis has been reported in greater than 150 species of freshwater, brackish, and marine species, tropical aquarium fish are most commonly affected.[16] Members of the freshwater families *Anabantidae* (bettas and gouramis),

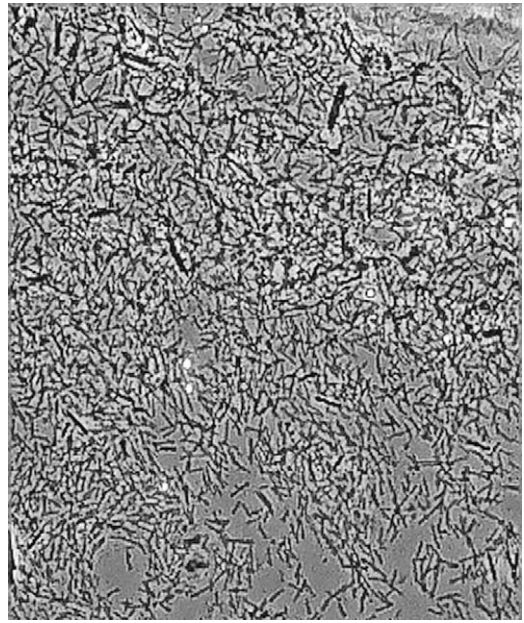

Fig. 4. A wet-mount preparation showing *Flavobacterium columnare*, the etiologic agent of columnaris disease.

Characidae (tetras and piranhas), and *Cyprinidae* (danios and barbs) appear to be particularly susceptible.[16] Also, marine sygnathids are regularly diagnosed with mycobacteriosis on necropsy and histopathology. In cultured and wild finfish, striped bass (*Morone saxatilis*) are commonly affected with the disease, now endemic in the Chesapeake Bay.

Clinical signs of mycobacteriosis are usually nonspecific and can include ulcerative skin lesions, reduced appetite, emaciation, lethargy, exophthalmia (“pop-eye”), swollen abdomen, anorexia, fin or tail rot, and skeletal abnormalities.[1,2] This disease is usually chronic, slowly progressive, and causes low-to-moderate mortalities. Mycobacteriosis is the most commonly diagnosed chronic wasting disease of aquarium fish. **Fig. 5** illustrates a goldfish with dropsy (generalized edema) secondary to systemic mycobacteriosis. On internal examination, granulomas will develop in the liver, kidney, spleen, heart, muscle, gill, and other tissues. Granulomas are typically pale gray to tan but are only visible to the naked eye in more advanced cases.

Diagnosis is based on clinical signs, the presence of granulomas, and the demonstration of acid-fast bacterial rods in tissues. Typical granulomas can be found on light microscopy of internal organ wet mounts (most commonly kidney, spleen, and liver). Granulomas can also be found in skin wet mounts, and less commonly, gill biopsies. **Fig. 6** illustrates the typical appearance of granulomas on light microscopy, with a dark-brown center and surrounding capsule.

When granulomas are found, an acid-fast stain should be performed. Acid-fast stains can be performed on cytologic preparations or histopathological sections. A positive acid-fast stain reveals red-to-pink, rod-shaped bacteria against a light green background. Culture of *Mycobacterium* for definitive species identification can be lengthy and difficult but is best performed on mycobacterial selective media such as Lowenstein-Jensen agar. *M marinum* is classified as a slow-growing mycobacterium, whereas *M fortuitum* and *M chelonae* are classified as rapidly growing.[16] Molecular diagnostics such as PCR are also useful in species identification. Because *Mycobacteria* sp are so ubiquitous in the environment, the gold standard for diagnosis is a positive culture supported by histopathology and PCR identification.

There is no effective cure for mycobacteriosis in fish. In aquaculture, retail, and wholesale situations, depopulation and disinfection is often recommended. Treatment is often different in public aquaria or zoos and private collections, and mycobacteriosis is often managed differently at these institutions and locations. Various antibiotics such as rifampin, erythromycin, streptomycin, kanamycin, doxycycline and minocycline have been suggested as possible treatments, but a clinical cure is unlikely.[1,2,15] Mycobacteria are resistant to many commonly used bactericidal agents at standard

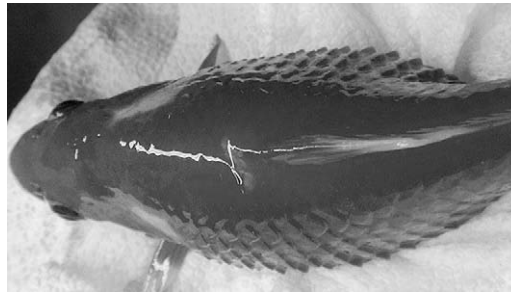

Fig. 5. Dropsy (generalized edema) in a goldfish (*Carassius auratus*) with systemic mycobacteriosis. Note protruding scales and abdominal distension (“pine cone disease”). Also, note exophthalmos.

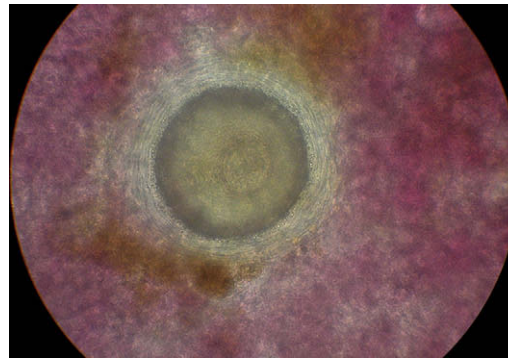

Fig. 6. Granulomas found on internal wet-mount examination of the spleen in a fish with mycobacteriosis. Note dark-brown center with surrounding lighter capsule.

dosage rates, including chlorine bleach and quaternary ammonium compounds.[15] As much as 10,000 parts per million chlorine has been reported necessary to kill mycobacteria.[15] In a recent study comparing efficacy of common disinfectants against *M marinum*, ethyl alcohol (50% and 70%), benzyl-4-chlorophenol/phenylphenol (Lysol) (1%), and sodium chlorite (mixed as 1:5:1 or 1:18:1 [base:water:activator]) were the most effective disinfectants with elimination or reduction of *M marinum* within one minute of contact.[19] Sodium hypochlorite (50,000 mg/L) was moderately effective but required a minimum contact time of 10 minutes to reduce bacterial counts.[19] Disinfectants that were ineffective after sixty minutes of contact time included ethyl alcohol (30%), N-alkyl dimethyl benzyl ammonium chloride (1:256), and potassium peroxymonosulfate-sodium chloride (1%).[19]

OTHER ORGANISMS: *RICKETTSIA*

Intracellular rickettsial organisms are an emerging disease problem in several species of tropical and temperate freshwater fish species.[20] In mortalities of blue-eyed plecostomus catfish, *Panaque suttoni*, shipped from Columbia, Khoo and colleagues[21] identified rickettsial-like organisms from moribund specimens on transport. The most commonly described rickettsial pathology and infection, called piscirickettsiosis, is caused by *Piscirickettsia salmonis* in Chinook salmon. Fish may or may not exhibit clinical signs, and mortality can be as high as 95%.[20] Current antibiotic treatment has not been successful in treating for piscirickettsiosis, necessitating changes in management to control outbreaks and prevent infection.

PARASITIC DISEASES OF FISH

Parasitic diseases are the most common infectious disease seen in pet fish. Fish parasites are a very diverse group and include protozoans, trematodes, turbellarians, nematodes, cestodes, leeches, acanthocephalans, monogeneans, pentastomes, copepods, and crustaceans.[22] Life cycles range from simple and direct, requiring no intermediate host, to complex and indirect, requiring one or more intermediate hosts. Fish may serve as final, paratenic, or intermediate hosts in a parasite life cycle.[22–24] Understanding the life cycles of any diagnosed parasites is critical for effective and successful treatment.[22,23] For example, only the free-swimming life stage (theront) of the common, external, ciliated parasite *Ichthyophthirius multifiliis* are susceptible to chemical treatments.

Introduction of Parasites

Parasites are most often introduced into a naïve, closed population by failing to quarantine and treat infected animals. Other methods of parasite introduction include the addition of live plants without prior disinfection, through fomites (nets, and other commonly shared equipment), by the use of contaminated source water, from wild birds, frogs, and turtles in outdoor ponds, and by way of aerosol droplet transmission between aquaria.[25–28] Standard biosecurity practices including quarantine protocols can reduce the potential for exposure of established populations and allow for treatment of animals in quarantine systems before introduction.

Diagnosis of Parasites in Fish

All sick fish should be evaluated using a standard minimum database that includes a detailed history from the owner, water quality testing, observation for any apparent clinical signs, physical examination, microscopic examination of wet-mount cytology of skin scrapes, gill biopsies, and fecal samples. There are no specific pathognomonic clinical signs for parasitic diseases in fish, although a group of clinical signs can be suggestive. These include: flashing (rubbing on the bottom of the tank or pond, indicative of pruritus); lethargy; cutaneous lesions including scale loss, ulcerations, and increased mucus production; rapid, opercular movements (increased ''gilling''); gasping or piping; weight loss; ''yawning''; osmoregulatory disruption, and deaths.[29]

Most external parasites can be readily identified on direct observation, and wet-mount skin and gill cytology preparations of sedated or anesthetized fish.[22–24,28,29] Internal parasite infections may be identified by wet-mount preparation of fresh fecal samples, gross visualization of the parasite at the vent (eg *Camallanus* sp), evaluation of blood smears, organ squash preparations, histopathology, and necropsy examination.[22–24,28,29] These two procedures are described below.

Skin scraping procedure (**Fig. 7**)

a. Place a drop of water from the tank or pond on a prepared glass slide.
b. A glass or plastic cover slip is gently scraped at a 45-degree angle in a cranial-to-caudal direction in small areas of the body.
c. If lesional skin is present, these areas should be sampled. In fish with no obvious lesions, two to three sites should be sampled. Commonly sampled locations that may yield a high number of ectoparasites are areas that are least hydrodynamic so parasites can best adhere to the skin or gills, including the skin behind the fins, on the caudal peduncle or tail, under the chin and on the ventrum.

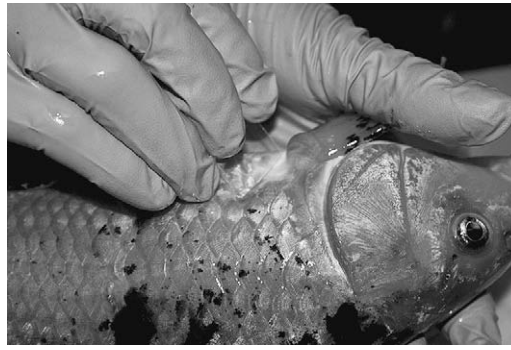

Fig. 7. A skin scrape taken from a koi (*Cyprinus carpio*).

d. Place the cover slip with mucus sample on a slide prepared with a drop of the fish's own water.
e. Starting with the lowest power objective, examine under the microscope for parasites. Identification of parasites is based on observation of their characteristic movements and size. During microscopy, the condenser should be down to improve contrast.
f. The slide is not stained and must be examined immediately. Slides may dry when examined outdoors or in warm or drafty environments. Parasites will become less active and die when left on a slide for a prolonged time, although they may need a few seconds to warm under the microscope's light source in cold temperatures to begin moving for identification.
g. If available, use both light and dark fields to scan the slide.

Gill biopsy procedure (**Fig. 8**)

1. Using gloved hands, the operculum of the sedated or anesthetized fish is gently lifted to reveal the gills.
2. With fine scissors such as iris tenotomy or suture removal scissors, snip a tiny section from the distal end of a few primary lamellae.
3. Place the gill tissue on a slide prepared with a drop of water from the 'fish's own environment.
4. As mentioned above, the slide is not stained and must be examined immediately for evidence of parasites.
5. The sample can also be examined for gill architecture. Gill pathology such as hyperplasia, hypertrophy, necrosis, lamellar fusion, and telangiectasia may be found.
6. In extremely large fish, one may only be able to scrape the gills. Gill rakers should be examined carefully during the physical examination as many larger parasites will lodge in these areas.

Parasites of clinical importance in fish can be divided into seven major groups and will be discussed in detail below.

Protozoan Parasites of Clinical Importance

Ciliated protozoans

Ichthyophthirius multifiliis, "whitespot disease" or "ich," is the most common parasitic disease affecting freshwater fish worldwide.[22,27,30] The parasite can survive in a wide range of temperatures and host susceptibility varies among species with scaleless

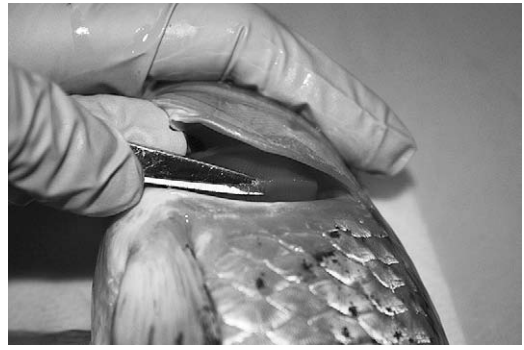

Fig. 8. A gill biopsy being performed on an anesthetized koi (*Cyprinus carpio*).

fish, such as catfish, being particularly vulnerable.[30] Overcrowded systems and poor water quality, which lead to increased stress and reduced immune function in fish, can result in increased morbidity and mortality.[31] Disease caused by *I multifiliis* can present acutely and result in up to 100% mortality.[30,31] The marine counterpart is *Cryptocaryon irritans.* Both have nearly identical pathology and clinical signs. The life cycle of both parasites is direct with a free-swimming infective stage (theront) that is the only stage susceptible to treatment. Trophonts are the encysted feeding stage seen on the host as white nodules. Trophonts break through the epithelium to become encysted tomonts with sticky external capsules that attach to inanimate substrate materials in the environment, including gravel, nets, plants, and so forth.[22,24,27–30,32] These tomonts divide, producing tomites that break through the nodule wall to releasing motile, infective theronts. At 25°C (77°F), infective theronts have 48 hours to find a new host or perish.[24,28,30,32] Upon finding a host, the theront penetrates the epithelium and develops into the ciliated trophont. *Ichthyophthirius* can be transmitted by aerosol dispersion of the infective stage.[26] The life cycle of ich is temperature dependent. It lasts 3 to 6 days at 25°C (77°F), and 10 days at 15°C (59°F). Outbreaks are most common at 15 to 25°C (59–77°F). One difference between marine and freshwater ich is duration of the life cycle. *Cryptocaryon* has a longer life cycle so treatment duration will need to be extended (up to 1 month) when compared with *Ichthyophthirius.*

Clinical signs include white, raised nodules up to 1 mm (0.5 mm for *Cryptocaryon*) on the skin and gills, flashing, increased mucus production, lethargy, dyspnea, secondary bacterial or fungal infections, and osmoregulatory compromise due to the epithelial and gill damage caused by the parasite.[24,27–30,32] Microscopic evaluation of the gills can show hyperplasia, necrosis, excess mucus, and necrosis.[30,32–35] Diagnosis is made by examination of a wet-mount cytology preparation of the skin or gills. Ich is a large parasite, entirely covered in cilia, moves in a characteristic slow-rolling motion, and has a C- or horseshoe-shaped nucleus (**Figs. 9–11**). The nucleus of *Cryptocaryon* differs from *Ichthyophthirius*; it is lobulated with four bead-like segments.[30]

Trichodina and *Trichodinella* spp are other common ciliated parasites found on both freshwater and marine pet fish. There are many species of these parasites, most with a predilection for skin and gill epithelium, but some will parasitize the urinary bladder or oviduct. These parasites are often associated with high levels of organic debris in the

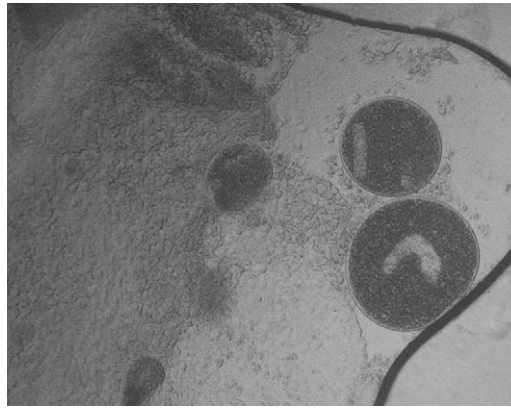

Fig. 9. *Ichthyophthirius multifiliis* as seen on a wet-mount examination of a skin scrape (40× magnification).

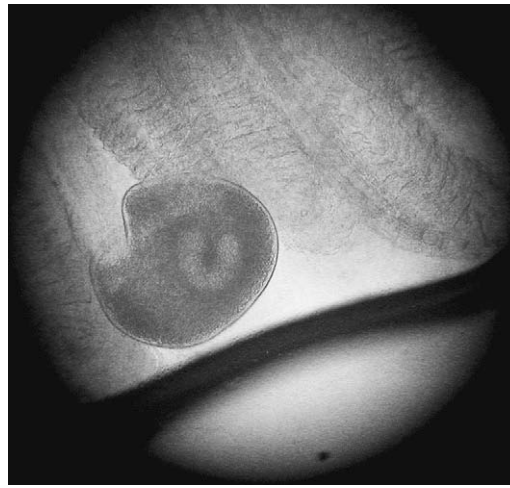

Fig. 10. *Ichthyophthirius multifiliis* on gill prep from a goldfish (*Carassius auratus*). Note the characteristic horseshoe-shaped nucleus (40× magnification).

water, poor nutrition, overcrowding, and subsequent poor water quality.[27,28,36] It is not uncommon to find these parasites on pond fish such as goldfish and koi that exist in ponds with high levels of detritus or organic debris. As with many other protozoan parasites, there is a direct life cycle and reproduction occurs by binary fission. Trichodinids can be introduced by fomites and live plants added to ponds and tanks.

Clinical signs of heavy infestations include flashing, increased mucus production giving a cloudy appearance to the skin, cutaneous hemorrhages, frayed fins and tail, lethargy, and chronic low level mortalities; with severe branchial infestations, respiratory signs may be present.[24,27,29,30,36] Secondary bacterial and fungal infections may occur because of extensive tissue damage. Observation of the circular, ciliated parasite with a prominent internal denticular ring on a wet-mount examination of gills or skin is diagnostic (**Fig. 12**). The parasite has been described as a flying saucer or ''scrubbing bubble''[36] (E.L. Johnson, DVM, personal communication, February

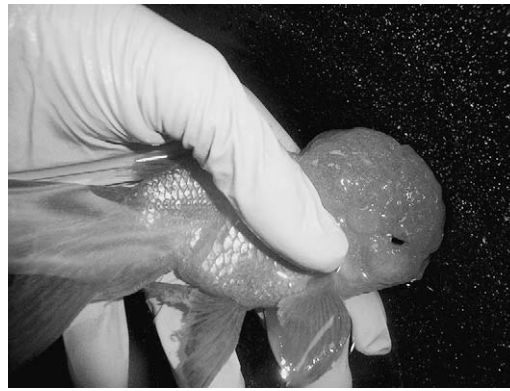

Fig. 11. An oranda affected by *Ichthyophthirius*. Small pinpoint white nodules may be seen on the wen (ornate head growth).

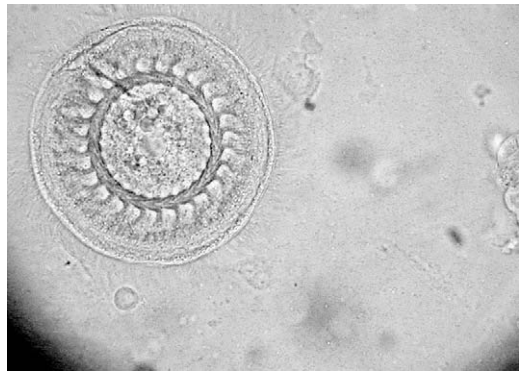

Fig. 12. Trichodina sp on a wet-mount prep from a skin scrape performed on a koi (*Cyprinus carpio*). This koi was being held in poor water conditions at 2°C (36°F).

2009). The motion of this parasite has been described as rotating,[28] scooting,[30] erratic, whirling, and hyperactive.

Chilodonella sp is a ciliated parasite shaped like a heart or an onion, with a flattened appearance. Cilia are located longitudinally on the parasite and may be seen as visible striations. *Chilodonella* can survive a wide variety of temperature ranges, is found worldwide, and can survive in brackish water.[27,30,32] *Brooklynella hostilis* is the marine counterpart named after its discovery at the Brooklyn aquarium. Both parasites are highly pathogenic and severe tissue damage can occur before any gross pathology is visible.[27,30] Clinical signs include respiratory distress (gasping, piping, opercular flaring, increased gilling), clamped fins, a ragged appearance to the skin, excess mucus production, secondary cutaneous ulcers, pathologic gill changes including hyperplasia and fusion of the lamellae, depression, and mortalities.[27–30,32] Diagnosis is based on observation of the parasite on wet mount examinations of skin and gills. *Chilodonella* moves in a gliding motion or circular motion on wet-mount preparation.

Tetrahymena sp (freshwater) and *Uronema* sp (marine) are ciliated parasites that cause external (skin and gill) lesions and internal, systemic infections. *Uronema* infects a wide variety of marine species and over a wide temperature range (8–28°C). External signs include small, white patches on the skin, skin hemorrhages, sloughing, and necrosis, and gill aneurysms.[28,30,32,36] Fish with systemic infections may show nonspecific signs such as anorexia and lethargy. Death can occur rapidly once infection is established. *Tetrahymena*, also known as ''guppy disease'' or ''guppy killer,'' is found most often in guppies, other livebearers, tetras, and cichlids.[28,30] This organism can also be found colonizing organic debris in the water. Poor water quality, bacterial infections, and other stressors may predispose fish to *Tetrahymena* infections.[28,30,32] Clinical signs are similar to *Uronema.* Muscle swelling and periocular lesions can also be seen with *Tetrahymena* infections. Keratitis can also occur with this protozoan and other parasites (*Cryptocaryon*, *Ichthyophthirius*, *Henneguya*, and *Glugea*) due to the close connection of the skin and cornea.[37] Deep or systemic infections carry a poor prognosis. Diagnosis is made with wet-mount examination or histopathology of skin and gill tissue. Deep or systemic infections will require histopathology of the affected organ or tissue.

Sedentary or sessile ciliates are most commonly seen on pond-reared fish (koi, catfish, and goldfish) in water with high levels of organic debris and high levels of suspended solids.[28,30] They also occur as secondary invaders on cutaneous ulcers and

other causes of epithelial damage in other pet fish species. Species most often encountered include *Epistylis* (also known as *Heteropolaria*), *Capriniana piscium* (previously known as *Trichophyra*), *Apiosoma* (**Fig. 13**) (previously known as *Glossatella*), and *Ambiphyra* (previously known as *Scyphidia*).[29,30,32] *Epistylis* produces white, fluffy lesions on fins and tail margins, opercular margins, and oral cavity.[30] These lesions can easily be mistaken for fungal lesions or columnaris disease owing to their similar appearance. *Capriniana* has a predilection for gill tissue and can cause severe respiratory distress by mechanically blocking gill tissue in infected fish.[30] Wet-mount cytology (**Fig. 14**) and histopathology of affected tissues are methods used to diagnose sessile ciliate infestations.

Flagellated Protozoans of Clinical Importance

Parasitic dinoflagellates can be found in both marine (*Amyloodinium ocellatum*) and freshwater (*Piscioodinium* sp) tropical fish. Both parasites share characteristic morphology, are temperature sensitive, and have similar life cycles to *Ichthyophthirius*. Only the free-living dinospore is susceptible to treatment. *A ocellatum* can parasitize both elasmobranchs and teleosts.[30] Similar to *Ichthyophthirius, A ocellatum* has also been shown to be transmissible by aerosol dispersion of water droplets, up to three meters in dynamic airflow systems.[25] For both parasites, the gills and skin are the preferential site of infestation and heavy infestation can result in edematous changes, hyperplasia, inflammation, hemorrhage, osmoregulatory compromise, and necrosis of the gill filaments.[27–30,32] Mortalities, in as little as 12 hours, result from hypoxia, secondary bacterial infections, and osmoregulatory compromise.[27,28,36] In addition to respiratory distress, another clinical sign that may be seen is a dusty, gold appearance to the skin, hence the names ''velvet disease,'' ''gold dust disease,'' and ''rust disease.'' Diagnosis is made by wet-mount cytology or histopathology of the skin and gills. In addition to the introduction of infected fish to a system, fomites and the introduction of ''infected'' water may play a role in transmission.[27]

Ichthyobodo, previously known as *Costia*, is a very small, flagellated parasite (about the size of a red blood cell) of freshwater fish found in a wide variety of species with a global distribution. The parasite can survive a wide temperature range (2–30°C) and has been occasionally on marine fish.[27,30,32] The life cycle is direct and transmission occurs from fish to fish. Mortalities are higher in fry, young fish, and stressed,

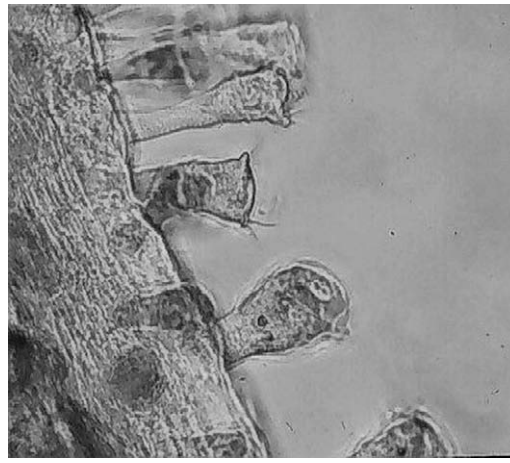

Fig. 13. *Apiosoma* sp Wet-mount preparation.

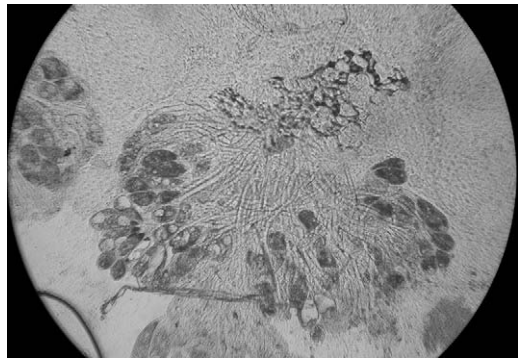

Fig. 14. Sessile ciliate infestation on a wet-mount preparation.

debilitated adult fish. Clinical signs include severe respiratory distress, lethargy, depression, flashing, anorexia, epithelial irritation, heavy mucus production, and deaths. Deaths may occur before any clinical signs. Diagnosis is based on wet-mount cytology. The organism's movement has been described as a ''flickering'' candle, or erratic spiraling. Other flagellates of clinical importance include *Hexamita* sp, *Spironucleus* sp, and *Cryptobia* sp. These parasites are found primarily in the gastrointestinal tract of freshwater fish although there are some species that can be found externally on fish. Of the seven species of *Cryptobia* that have been associated with the gastrointestinal tract of fish, only *C iubilans* is reported to be pathogenic and parasitic. *C branchialis and C agitans* typically parasitize gill tissue.[30,32] Spironucleus has been isolated from lesions characteristic of ''hole-in-the-head-disease'' in discus (*Symphysodon* sp) and angelfish (*Pterophyllum* sp) but the role it plays in this multifactorial syndrome is unknown.[29,36] These flagellates are most commonly found to cause clinical disease in freshwater angelfish, cichlids, and anabantids. Clinical signs of gastrointestinal infections include: severe weight loss, anorexia, lethargy, abdominal distension, mucoid enteritis, mucoid or pale feces, exophthalmos, darkening of the skin, buoyancy disorders, redness at the vent, and deaths (**Fig. 15**).[27–29,30,38,39] Concurrent infections with other parasites, poor water quality and other stressors such as overcrowding are not uncommon and increase morbidity. *C iubilans* induces granulomatous disease, primarily seen in the stomach of affected fish (**Fig. 16**).[40]

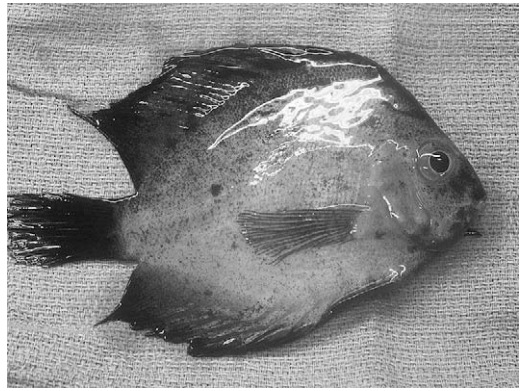

Fig. 15. Thin discus (*Symphysodon* sp) *with a Cryptobia iubilans infection*.

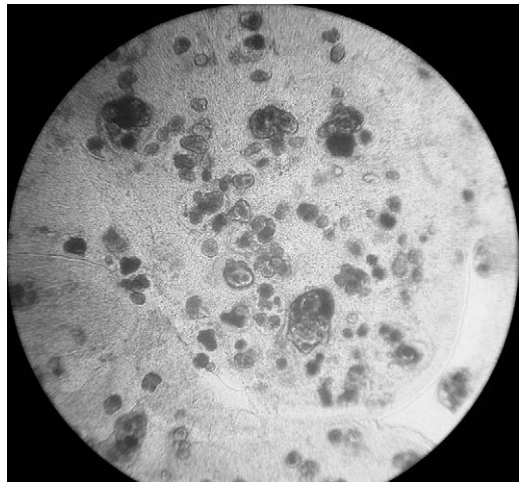

Fig. 16. Wet-mount squash preparation of the stomach wall in a discus with *Cryptobia iubilans*. Note the severe granulomatous gastritis effacing the normal stomach architecture.

Diagnosis of intestinal flagellates can be made by wet-mount fecal examination although an intestinal squash prep or histopathology is more likely to yield a diagnosis. For *Spironucleus* or *Hexamita*, trophozoites are small (12.5–20 μm in length), flagellated, actively motile, with an ellipsoid to pear shape. Trophozoites of *Spironucleus* or *Hexamita* are often localized in the anterior intestinal lumen and therefore may not be present on fecal examination. They have six anterior and two posterior flagella. Species identification and differentiation between *Hexamita* and *Spironucleus* requires electron microscopy. *Cryptobia* is most commonly detected by identifying granulomas in squash preparation wet mounts of the stomach. An acid-fast stain should be performed to rule out mycobacteriosis (another common cause of granulomas in ornamental fish). Motile trophozoites are not commonly seen on wet mounts. When present, flagellated trophozoites are elongate (acute infection) to oval or teardrop shape (chronic infection) with a characteristic slow, undulating movement. The organism has two flagella. Species identification requires electron microscopy.

Microsporidians, Coccidia, Cryptosporidium, and Myxozoans

Microsporidians are intracellular parasites with a direct life cycle and several species have been reported in pet fish. *Pleistophora* is the etiologic agent of ''neon tetra disease,'' causing white cysts (xenomas) to develop in the muscle and other tissues.[28–30] These cysts may be visible below the skin.[32] The xenomas can cause severe deformity of the tissues. Rupture of the xenoma releases spores that are released and ingested by new hosts.[28–30,32] *Pleistophora* also affects angelfish, rasboras, barbs, and tetras. Clinical signs include muscle wasting, erratic swimming behavior, color loss, lethargy, secondary bacterial infections at the site of cyst rupture, and deaths. Diagnosis is made by wet-mount examination of the lesions, squash prep of affected organs, and histopathology. Other Microsporidians occasionally encountered in pet fish species include *Glugea* sp and *Heterosporis* sp. There are no reported effective treatments for Microsporidians; however, toltrazuril (Baycox Bayer Animal Health) has shown some efficacy in experimental conditions.[39]

Coccidia are another group of intracellular parasites that can infect a variety of species of fish. Most species show a predilection for the gastrointestinal system causing emaciation, chronic enteritis, anorexia, mucoid stool, and deaths.[27–30,32] Goldfish are the most common pet species presented with infections in a pet fish-practice setting. Immune suppression due to poor water quality and other environmental disorders may predispose the fish to infection. Other organs that may be affected include the reproductive organs, swim bladder, liver, spleen, and kidney. Diagnosis of intestinal infection can be made by wet-mount examination of a fresh fecal sample or cloacal wash. Histopathology and squash preps of affected organs may also be used.

A few species of *Cryptosporidium* have been identified in teleost fish.[28,40,41] Clinically affected fish may show anorexia, food regurgitation, undigested food passed in the feces, weight loss, and deaths.[28] Much is still unknown with regards to the distribution, pathogenesis, and transmission of this parasite.

Myxozoans are spore producing parasites with complex life cycles and a worldwide distribution that infect a wide variety of cultured and wild fish. Species vary from nonpathogenic to highly pathogenic. Clinical signs will depend on the affected area or organ. Species of clinical importance include *Henneguya* (marine and freshwater), *Hoferellus* (freshwater, polycystic kidney disease in koi and goldfish), *Kudoa* (marine, muscle necrosis), and *Myxobolus* sp. Lesions can range from small, white focal areas on skin to granulomatous masses in the affected tissues. Wet-mount preps, organ-squash preps, histopathology, and special staining may be all required for diagnosis. There is no effective treatment for Myxozoans infections although fumagillin (used to treat a Microsporidean disease caused by *Nosema apis* in honeybees) and malachite green have been tried.[27,39]

Table 3 lists the treatments for the most common protozoans encountered in pet fish medicine.

Monogeneans (flukes) of Clinical Importance

Flukes, or parasitic flatworms, are very common in marine and freshwater pet fish. Most species are found on the skin and gills of affected fish, but a few species can be found on the eye (*Neobenedinia* sp, a marine fluke), body cavity, rectal cavity, ureters, and blood vascular system.[28,30,37] In freshwater fish, the primary species seen are *Dactylogyrus* (the ''gill'' fluke) and *Gyrodactylus* (the ''skin'' fluke). Either can be located on the skin and gills. There is no species specificity seen by either fluke type. Dactylogyrids are egg layers and are often found on imported fancy goldfish, angelfish, and discus. *Gyrodactylus* is a live-bearing fluke. Oviparous monogeneans (Dactylogyridae) release eggs into the water, which hatch into a free-swimming stage (oncomiracidium) that seeks out a fish host. Viviparous monogeneans (Gyrodactylidae) release live larvae that are immediately parasitic.

Clinical signs include flashing, rubbing, gasping, lethargy, ''yawning,'' clamped fins, excess mucus production, secondary cutaneous ulcerations, scale loss, and deaths in severe infestations.[27–30,32] Flukes can predispose fish to secondary ulcers, bacterial diseases, and eventual osmoregulatory compromise due to epithelial damage created by their attachment and feeding behavior.[4] Wet-mount cytology of the gills or skin is used to diagnose monogenean infestations. *Dactylogyrus* can be recognized by the prominent two to four anterior eyespots and a four-pointed anterior end. Gyrodactylids (**Fig. 17**) have no eyespots, and often an embryo is visible inside the fluke. On gill tissue or mucus, either species can be seen anchored and appear to be ''bobbing'' or stretching and compressing its body. Marine species (referred to as capsalids) of clinical importance include *Neobenedenia* sp, *Benedenia* sp, and *Dermophthirius*.[30,32]

Table 3
Antiprotozoal treatments

2-amino-5-nitrothiazol	4.4 mg/gm of food oral treatment	*Cryptobia iubilans*	Not 100% effective, may only reduce infestation[40]
Amprolium	0.63 mL/L of a 9.6% solution given over 2 d in water	Coccidiosis	[27]
Chloroquine	10 mg/L prolonged immersion	Dinoflagellates	—
Copper	0.2 mg/L free copper ion prolonged immersion 100 mg/L bath	Protozoan ectoparasites and dinoflagellates in marine fish	Not recommended for freshwater systems Bound to inorganic compounds Toxic to invertebrates Elasmobranchs may react adversely Copper levels should be monitored daily Solubility affected by pH and alkalinity Immunosuppressive and toxic to gill tissue
Dimetridazole	20 mg/gm of food Oral treatment	*Cryptobia iubilans*	Not 100% effective, may only reduce infestation[40]
Formalin (37% formaldehyde)	0.125–0.25 mL/L Bath q 24h × 2–3 d for up to 60 minutes 0.015–0.025 mL/L (15-25ppm) prolonged immersion, every 2–3 d Change 50% water on nontreatment days	Protozoan parasites, crustacean ectoparasites	Carcinogenic Depletes oxygen, additional aeration required Some fish very sensitive Not for use in stressed fish Do not use if white precipitate forms Contraindicated >27°C Toxic to invertebrates
Freshwater	Used as a dip	Marine protozoan ectoparasites and some monogenean infestations	Not effective against all protozoans A common quarantine procedure

Metronidazole	7–15 mg/L q 24-48h × 5–10 d Prolonged immersion Change 50% water between treatments 25–50 mg/kg (0.25% in food fed at 1% bodyweight/d) for 3 d	Some protozoal flagellates including *Hexamita* and *Spironucleus*	Not very water soluble One oral treatment may be as effective as three water treatments
Monensin	100 mg/kg bodyweight/	Coccidiosis	Experimentally effective[27,30]
Potassium permanganate	2 mg/L prolonged immersion 5–20 mg/L1 h bath	External infectionsectoparasites (protozoan, monogenean trematodes)	Inactivated by organic compounds in water CausticToxic in high pH water StainsCan be toxic in some fish species Can cause blindness (powder)Watch for signs of stress with use Safer products are available
Sodium chloride	3–6 gm/L prolonged immersion 10–30 gm/L Dip (minutes or until fish is stressed)	Protozoan parasites Protozoan parasites	Dip is often used in quarantine protocols
Toltrazuril (Baycox, Bayer Animal Health)	7–10 mg/kg orally q 24 h × 5 d	coccidiosis	Caution with some species

From Roberts H. Freshwater Ornamental Fish. In: Johnson-Delaney C, editor. British Small Animal Veterinary Association Manual of Exotic Pets. 5th edition. BSAVA. In Press; with permission.

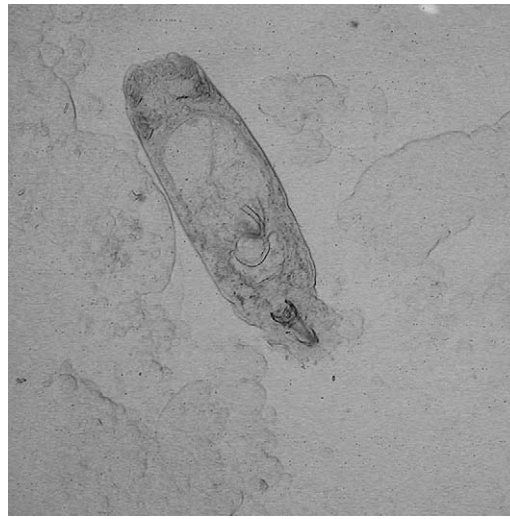

Fig. 17. Wet-mount prep showing a *Gyrodactylus* sp monogenean trematode. An embryo is visible inside the fluke.

Neobenedenia and *Benedenia* are large, and are typified by a large circular opisthohaptor on the posterior end and two smaller suckers on the anterior end. *Benedenia* has two pairs of tightly apposed, curved anchors, whereas *Neobenedenia* has three pairs. These two parasites adhere to the skin, gills, and eyes of susceptible fish, causing significant mechanical damage in large numbers.[42] Treatment methods include the use of praziquantel as a bath or prolonged immersion treatment (2–10 mg/L). The eggs of *Dactylogyrus* sp and marine capsalids are not susceptible to treatment and multiple treatments are required. In addition the eggs of the marine capsalids can be long and have attachments that can adhere to anything in the tank, making treatment and management of these parasites difficult (**Fig. 18**).[42] Other treatment options include organophosphates (to which some fish species are very sensitive), mebendazole, formalin, and potassium permanganate. An ovine anthelmintic, Supaverm (Janssen Animal Health), (closantel 5 mg/mL and mebendazole 75 mg/L) has

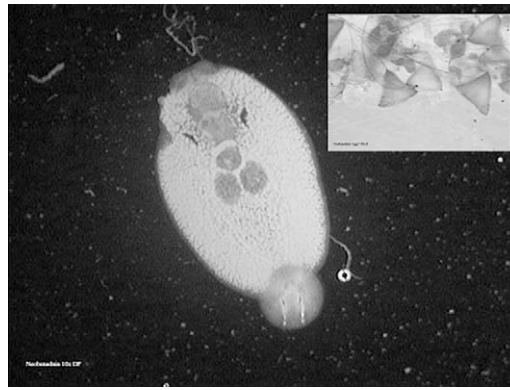

Fig. 18. *Neobenedenia* sp, a marine capsalid fluke, 200× magnification. Inset picture shows eggs with long sticky strands that attach to many surfaces.

been used to treat monogeneans in koi *(Cyprinus carpio*).[43] The dose used anecdotally is 1 mL/400 L. This product has been shown to be uniformly toxic to goldfish (*Carassius auratus*) and 100% mortalities are reported. Anecdotal reports of a death in a discus (*Symphysodon discus*) have also been reported.[43] The authors recommend weekly treatment with praziquantel for 4 to 6 weeks followed by recheck wet-mount cytology after treatment. Formalin is the only US Food and Drug Administration (FDA)-approved treatment for finfish. For marine infections, a freshwater dip in quarantine may be helpful to both diagnose and remove monogeneans.[30,32] This can be done using a dark, five-gallon bucket for freshwater dips and dipping marine fish for 1 to 3 minutes in the bucket. When dips are completed, water in the bucket can be swirled and dislodged capsalid parasites will look like snowflakes swirling in the water. Biologic control has been reported using cleaner fish (French angelfish, neon gobies, and Pacific cleaner wrasse) in marine systems.[30]

Digenean Trematodes of Clinical Importance

Digenetic trematodes are parasites with an indirect life cycle with larval forms (metacercariae) that can cause unsightly lesions on fish but are generally not pathogenic. Severe infections can cause deaths, larval migration may lead to secondary bacterial infections, and ocular lesions may lead to feeding problems. Examples include *Clinostomum* (‘‘yellow grub’’), *Neascus* (’’black spot’’), *Diplostomum* (‘‘eye fluke’’), and *Posthodiplostomum* (‘‘white grub’’). The problem appears in outdoor-raised pond fish such as koi and goldfish, but can be seen occasionally in tropical pet fish kept in aquaria. The life cycle does not typically continue in indoor aquaria unless snails (or other intermediate hosts) are present. **Fig. 19** shows a pond-raised perch with a severe *Clinostomum* infestation.

Turbellarians of Clinical Importance

Turbellarians are a free-living flat worm from the group Platyhelminthes that have been implicated in causing disease in several species of marine tropical fish. Although most turbellarians are commensal organisms, several species have been implicated as pathogens for tropical marine species. The first evidence of pathology noted in Pacific and Caribbean fish, was dubbed ‘‘tang turbellarian’’ in 1981 by Cannon and Lester.[44] Two species were also identified as causing disease in Australian fishes.[44] These

Fig. 19. A yellow perch with a severe ”yellow grub” (*Clinostomum* sp) infestation on the caudal peduncle.

parasites look like small black spots on the skin that can cause damage through grazing on the epidermal surface, or by encysting in the gill filaments. They should not be confused with black spots associated with a Digenean infection. The life cycle for many Turbellarians is direct, with feeding stages on the fish and reproductive stages in the sediment. The most effective control has been through repeated treatments with organophosphates followed by thorough vacuuming of the sediment, and backwashing of the filters. Nonpathogenic free-living turbellarians are often seen in both freshwater and marine aquaria. The presence of large numbers of commensals often indicates a high nutrient load, and can be controlled by increasing water changes and improving environmental quality. Decreasing biologic waste is the key to controlling both pathogenic and nonpathogenic species of turbellarian.

Nematodes of Clinical Importance

Pet fish can be either intermediate or final hosts of nematodes.[39] Nematodes in fish are similar to other animal species and appear as smooth, long worms. Most important are parasitic roundworms of pet fish species that affect the intestinal tract; these include *Camallanus*, *Capillaria*, and *Capillostrongyloides*. Clinical signs can range from none to anemia, lethargy, poor weight gain, failure to thrive, and reproductive problems.[32,45] Other species, such as *Eustrongyloides*, can cause cysts to form in the liver, muscles, and peritoneum.[28,29] *Camallanus* is an ovoviviparous nematode that affects cichlids, guppies and other liver bearers.[32,39] In *Camallanus* infections, the owner may a report a ''red worm'' protruding from the vent of their fish. *Capillaria* sp is most often diagnosed in angelfish, discus, and other cichlids, but can be found in many other tropical freshwater fish. It has a direct life cycle. In a closed aquarium, an infestation of *Capillaria* can spread very rapidly among susceptible fish. A wet-mount examination of fresh feces reveals a typical capillarid egg with bipolar plugs (**Fig. 20**). Diagnosis of nematodes in general is by fecal examination, cloacal wash, squash preps of affected organs, and histopathology. Treatment of nematodes can be accomplished by the use of fenbendazole (25–50 mg/kg in food) or levamisole (1–2 mg/L bath $\times$ 24h). Neither drug is FDA-approved for use in food fish.

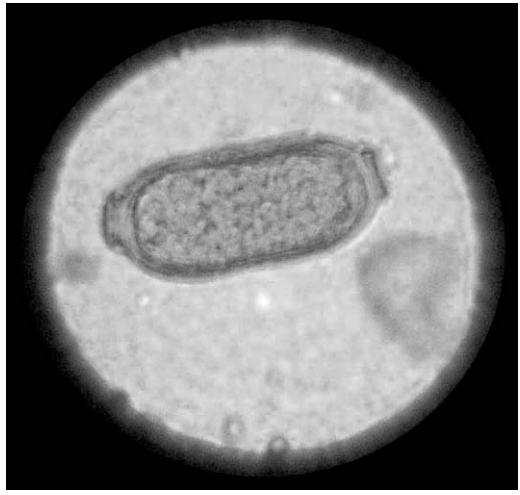

Fig. 20. The classic capillarid egg of *Capillaria* from a discus fecal sample.

Cestodes of Clinical Importance

There are few important cestodes of pet fish. The most detrimental, the Asian tapeworm, *Bothriocephalus acheilognathi*, has been found in koi and other cyprinids (excluding goldfish), channel catfish, and aquarium fish such as discus, livebearers, killifishes, and angelfish.[27,32,39] Clinical signs range from none to lethargy, anorexia, weight loss, chronic intestinal inflammation, intestinal obstruction, and severe mucosal damage. Diagnosis is made by wet mounts of the feces, necropsy examination, and histopathology. Oral praziquantel (not FDA approved) can be used to treat at 50 mg/kg by mouth for one dose, or 5 to 12 gm/kg of feed every 24h $\times$ 2 to 3 days.[32] Treatment is recommended to be given in a separate tank to prevent the dispersion of eggs when the cestode dies.[32]

Crustacean Parasites of Clinical Importance

Parasitic crustaceans are considered ''macroparasites,'' visible to the naked eye. Three genera, *Argulus*, *Ergasilus*, and *Lernaea*, are commonly found in freshwater fish. In marine systems, *Gnathia* sp larval stages are parasitic isopods that can damage host tissue and even kill small fish.[32] *Argulus*, a branchiuran parasite also known as the ''fish louse,'' is a circular, flattened, moving parasite that is commonly seen on koi and goldfish (**Fig. 21**). Fish lice can create damage by their feeding behavior and movement on the fish. *Argulus* may act as a vector for viral diseases such as Spring Viremia of Carp[32] and Koi Herpes Virus. Clinical signs include flashing, agitation, focal red skin lesions, and secondary cutaneous ulcers. Diagnosis is made by gross visualization or wet-mount examination. Anchor worms, *Lernaea* sp, are also easily seen on fish. Females attach under the skin of a fish with an anchoring apparatus; a characteristic forked tail is composed of egg sacs (**Fig. 22**). Anchor worms should be carefully removed manually and the remaining wound treated for any potential secondary bacterial infection. Treatment methods for all crustaceans include chitin inhibitors such as lufenuron (0.1–0.2 mg/L prolonged immersion) and dimilin, organophosphates, formalin dips, and potassium permanganate. The owner should be warned when using a chitin inhibitor that nontarget invertebrate species in the pond such as various insects may also be affected. Water should be carefully disposed of and not allowed into natural waterways.

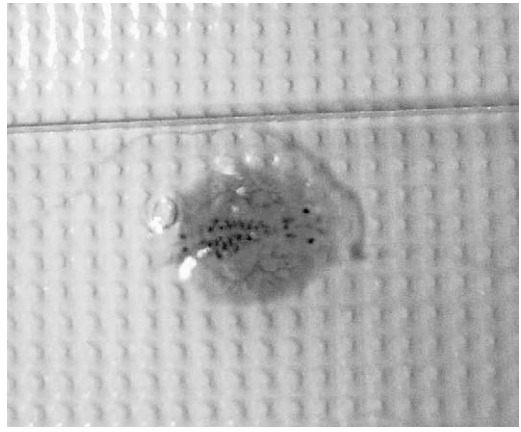

Fig. 21. *Argulus* sp (the "fish louse") on a glass slide.

Fig. 22. *Lernaea* sp (the "anchor worm") on the body wall of a koi (*Cyprinus carpio*).

SUMMARY

Bacterial and parasitic diseases are very common problems in pet fish. Shotgun treatment of fish should never be based simply on gross appearance of clinical signs and lesions without the benefit of diagnostics. Diagnostic testing for bacterial and parasitic diseases is simple to do; many tests can be done on ambulatory visits. Because of unique media requirements and incubation temperatures for some fish pathogens, it is vital to develop a relationship with a diagnostic laboratory that can provide these needs and correctly handle diagnostic samples from aquatic animal patients. When logical treatment strategies are initiated and supported by diagnostic testing, a successful outcome is possible. Identification of the correct pathogens also allows an educational opportunity for discussions on prevention and biosecurity practices with the owners and clients. Also, and, although fish are cold-blooded, there are some bacterial and parasitic pathogens that are zoonotic.

REFERENCES

1. Palmeiro B, Roberts H. Bacterial disease in fish. In: Mayer J, editor. Clinical Veterinary Advisor: Exotic Medicine. Elsevier; 2009, in press.
2. Barker G. Bacterial diseases. In: Wildgoose WH, editor. BSAVA manual of ornamental fish. 2nd edition. Gloucester (UK): British Small Animal Veterinary Association; 2001. p. 185–93.
3. Noga EJ. Fish disease: diagnosis and treatment. St. Louis (MO): St. Mosby; 1996.
4. Hunt CJG. Ulcerative skin disease in a group of koi carp (*Cyprinus carpio*). Vet Clin North Am Exot Anim Pract 2006;9:723–8.
5. Goodwin A, Merry G. Are all koi and goldfish ulcers caused by Aeromonas salmonicida achromogenes? Western Fish Disease Workshop/American Fisheries Society. June 5, 2007.
6. Palmeiro BS, Rankin S. Culture and susceptibility characteristics of ulcerative dermatitis in koi. In press.
7. Palmeiro BS. Bacterial diseases. In: Roberts HR editor. Fundamentals of Ornamental Fish Health. Wiley-Blackwell, in press.
8. Francis-Floyd R, Klinger RE. Disease diagnosis in ornamental marine fish: a retrospective analysis of 129 cases. In: Cato JC, Brown CL, editors. Marine ornamental species: collection, culture and conservation. Iowa State Press; 2003. p. 92–100.

9. Austin B. Bacterial pathogens of marine fish. In: Belkin SS, Colwell RR, editors. Oceans and health: pathogens in the marine environment. New York: Springer; 2005. p. 391–413.
10. Humphrey JD, Lancaster C, Gudkovs N, et al. Exotic bacterial pathogens Edwardsiella tarda and Edwardsiella ictaluri from imported ornamental fish Betta splendens and Puntius conchonius, respectively: isolation and quarantine significance. Aust Vet J 1986;63(5):369–71.
11. Klinger RE, Francis-Floyd R, Riggs A, et al. Use of blood culture as a nonlethal method for isolating bacteria from fish. J Zoo Wildl Med 2003;34(2):206–7.
12. Russo R, Curtis EW, Yanong R. Preliminary investigations of hydrogen peroxide treatment of selected ornamental fishes and efficacy against external bacteria and parasites in green wwordtails. J Aquat Anim Health 2007;19(2):121–7.
13. Yanong RPE, Francis-Floyd R. Streptococcal infections in fish. University of Florida IFAS extension publication #57. 2006. Available at: http://edis.ifas.ufl.edu/pdffiles/FA/FA05700.pdf. Accessed January 10, 2008.
14. Russo R, Yanong RPE, Mitchell H. Dietary beta-glucans and nucleotides enhance resistance of red-tail black shark (*Epalzeorhynchos bicolor*, family Cyprinidae) to *Streptococcus iniae* infection. J World Aquac Soc 2006;37(3):298–306.
15. Francis-Floyd R, Yanong RP. Mycobacteriosis in fish. University of Florida IFAS extension publication # VM-96. 2006. Available at: http://edis.ifas.ufl.edu/VM055. Accessed January 10, 2008.
16. Decostere A, Hermans K, Haesebrouck F. Piscine mycobacteriosis: a literature review covering the agent and the disease it causes in fish and humans. Vet Microbiol 2004;99:159–66.
17. Harriff MJ, Bermudez LE, Kent ML. Experimental exposure of zebrafish, Danio rerio, to Mycobacterium marinum and Mycobacterium peregrinum reveals the gastrointestinal tract as the primary route of infection: a potential model for environmental mycobacterial infection. J Fish Dis 2007;30:587–600.
18. Beran V, Matlova L, Dvorska L, et al. Distribution of mycobacteria in clinically healthy ornamental fish and their aquarium environment. J Fish Dis 2006;9:383–93.
19. Marinous M, Smith S. Efficacy of common disinfectants against Mycobacterium marinum. J Aquat Anim Health 2005;17:284–8.
20. Fryer JL, Mauel MJ. The Rickettsia: an emerging group of pathogens in fish. Emerg Infect Dis 1997;3(2):137–44.
21. Khoo L, Dennis PM, Lewbart GA. Rickettsia-like organisms in the blue-eyed plecostomus, *Panaque suttoni* (Eigenmann & Eigenmann). J Fish Dis 1995;18:157–64.
22. Hoffman GL. Parasites of North American freshwater fishes. 2nd edition. Ithaca (NY): Cornell University Press; 1999. p. 1–539.
23. Roberts RJ. The parasitology of teleosts. In: Roberts RJ, editor. Fish pathology. 3rd edition. London: Harcourt; 2001. p. 254–96.
24. Smith SA. Parasites of Fish. In: Roberts HE editor. Fundamentals of Ornamental Fish Health. Hoboken (NJ): Wiley-Blackwell, in press.
25. Roberts-Thomson A, Barnes A, Fielder DS, et al. Aerosol dispersal of the fish pathogen, *Amyloodinium ocellatum*. Aquaculture 2006;257:118–23.
26. Bishop TM, Smalls A, Wooster GA, et al. Aerobiological (airborne) dissemination of the fish pathogen Ichthyophthirius multifiliis and the implications in fish health management. In: Cheng-Sheng L, Patricia OB, editors. Biosecurity in aquaculture production systems: exclusion of pathogens and other undesirables. Baton Rouge (LA): The World Aquaculture Society; 2003. p. 51–64.

27. Baker DG, Kent ML, Fournie JL. Parasites of fishes. In: Baker DG, editor. Flynn's parasites of laboratory animals. 2nd edition. Hoboken (NJ): Blackwell; 2007. p. 69–116.
28. Stoskopf M. Fish medicine. Philadelphia: Harcourt Publishing; 1993.
29. Reavill D, Roberts HE. Diagnostic cytology of fish. Vet Clin Exot Anim 2007;10: 207–34.
30. Noga EJ. Problems 10–42. In: Noga EJ, editor. Fish disease: diagnosis and treatment. St Louis (MO): Mosby; 1996. p. 75–138.
31. Hadfield C, Whitaker BR, Clayton LA. Emergency and critical care of fish. Vet Clin Exot Anim 2007;10:647–75.
32. Longshaw M, Feist S. Parasitic diseases. In: Wildgoose WH, editor. BSAVA manual of ornamental fish health. 2nd edition. Gloucester: BSAVA; 2001. p. 167–83.
33. Speare DJ, Ferguson HW. Gills and pseudobranchs. In: Ferguson HW, editor. Systemic pathology of fish–a text and atlas of normal tissues in teleosts and their responses in disease. 2nd edition. London: Scotian Press; 2006. p. 24–63.
34. Govett PD, Rotstein DS, Lewbart GA. Gill metaplasia in a goldfish, *Carassius auratus auratus* (L). J Fish Dis 2004;27:419–23.
35. Ferguson HW. Skin. In: Ferguson HW, editor. Systemic pathology of fish—a text and atlas of normal tissues in teleosts and their responses in disease. 2nd edition. London: Scotian Press; 2006. p. 64–89.
36. Weber EPS, Govett P. Parasitology and necropsy of fish. Compendium on Continuing Education for the Practicing Veterinarian 2009;31(2). Available at: http://www.compendiumvet.com/Media/PublicationsArticle/PV0209_WEB_Weber_Parasitology.pdf. Accessed March 26, 2009.
37. Williams CR, Whitaker BR. The evaluation and treatment of common ocular disorders in teleosts. Journal of Exotic Pet Medicine 1997;6(3):160–9.
38. Lumsden JS. Gastrointestinal tract, swimbladder, pancreas, and peritoneum. In: Ferguson HW, editor. Systemic pathology of fish—a text and atlas of normal tissues in teleosts and their responses in disease. 2nd edition. London: Scotian Press; 2006. p. 168–99.
39. Noga EJ. Problems 55–72. In: Noga EJ, editor. Fish disease: diagnosis and treatment. St. Louis (MO): Mosby; 1996. p. 163–205.
40. Yanong RPE, Curtis E, Russo R, et al. Cryptobia iubilans infection in juvenile discus. JAVMA 2004;224(10):1644–50.
41. Ryan U, O'Hara, Xiao L. Molecular and biological characterization of a Cryptosporidium molnari- like isolate from a guppy (*Poecilia reticulata*). Appl Environ Microbiol 2004;70(6):3761–5.
42. Seng LT. Control of parasites in cultured marine finfishes in Southeast Asia—an overview. Int J Parasitol 1997;27(10):1177–84.
43. Marshall CJ. Use of Supaverm for the treatment of monogenean infestation in a koi carp (*Cyprinus carpio*). Fish Vet J 1999;(4):33–7.
44. Cannon LRG, Lester RJG. Two turbellarians parasitic in fish. Dis Aquat Org 1988; 5:15–22.
45. Weber ES. Gastroenterology for the piscine patient. Vet Clin Exot Anim 2005;8: 247–76.