C4

Bacterial Diseases

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Introduction

Most bacterial diseases of fish are due to opportunistic infections by ubiquitous bacteria. Most are Gram-negative bacilli. The chapter starts with a general overview. Subsequent sections include *Aeromonas*, *Vibrio*, *Edwardsiella*, *Flavobacterium*, *Yersinia*, *Streptococcus*, *Renibacterium*, *Mycobacterium*, *Nocardia*, *Francisella* spp., epitheliocystis, and piscirickettsial-like organisms. Where available, information is provided on etiologic agent, life cycle and transmission, signalment, risk factors, clinical signs, diagnosis, husbandry and medical management, and prevention.

Bacterial Diseases (General)

Overview

- Most bacterial diseases of fish are caused by opportunistic Gram-negative bacilli (rods).
- Some significant Gram-positive bacterial infections are reported (e.g. *Streptococcus* and *Renibacterium* spp.; *Mycobacterium* spp. may also take up Gram stain).
- Morbidity and mortality are often secondary to stressors.
- Systemic infections are most common, although local infections may be seen.
- Clinical signs are often nonspecific and definitive diagnosis requires ancillary testing.
- Antibiotic treatment should be based on culture and sensitivity results.

Etiology

- The bacteria that cause disease in fish can usually be grouped based on Gram stain and morphology, although some staining variability exists.
- Gram-negative bacilli are commonly reported to cause disease in fish.
  - *Acinetobacter* spp.
  - *Aeromonas* spp.
  - Chlamydia-like organisms.
  - *Chryseobacterium* spp.
  - *Citrobacter* spp.
  - *Edwardsiella* spp.
  - *Flavobacterium* spp.
  - *Francisella* spp.
  - *Moritella* spp.
  - *Pasteurella* spp.
  - *Photobacterium* spp.
  - *Piscirickettsia* spp. and piscirickettsial-like organisms.
  - *Pseudomonas* spp.
  - *Shewanella* spp.
  - *Tenacibaculum* spp.
  - *Vibrio* spp.
  - *Yersinia* spp.
- Gram-positive bacilli are less commonly reported.
  - *Erysipelothrix piscisicarius* sp. nov. (although Gram stain uptake is variable).
  - *Mycobacterium* spp. (although Gram stain uptake is variable).
  - *Nocardia* spp. (although Gram stain uptake is variable)
  - *Renibacterium* spp.
- Gram-positive cocci are emerging fish pathogens.
  - *Enterococcus* spp.
  - *Lactococcus* spp.
  - *Streptococcus* spp.
  - *Vagococcus* spp.

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- Obligate anaerobic bacteria are rarely reported, although this may be because fewer anaerobic diagnostic procedures are used in fish.

Transmission

- Horizontal transmission is seen with all bacteria, typically through ingestion and contact.
- Vertical transmission is an important route in a few (e.g. *Renibacterium salmoninarum*, *Flavobacterium psychrophilum*).
- Many are ubiquitous in the environment and are normal components of the biofilm, which can provide protection from immersion treatments and disinfectants.
- Pathogenic bacteria can be carried by fomites such as containers and nets.
- Bacteria can also be transmitted by mechanical and biological vectors and asymptomatic carriers, including aquatic invertebrates, fish, amphibians, and piscivorous birds.

Geographic Distribution

- Most bacterial pathogens of fish are ubiquitous and probably found worldwide.

Signalment

- All individuals are susceptible.
- Juvenile and geriatric fish are often more likely to show clinical signs.
- Species that are common in aquaculture dominate the literature, but bacterial diseases are common in all fish.
- Disease and epizootics are reported in both wild and managed fish populations.

Risk Factors

- Most bacterial pathogens are opportunistic and morbidity and mortality are primarily seen in fish under additional stressors. Increased exposure, such as from poor biosecurity, increases the probability of transmission.
- General risk factors are described here; specific examples are provided for each bacterial group.
- Stressors:
  - High stocking density.
  - Inappropriate social structure.
  - Inappropriate environment.
  - Aggression or displacement.
  - Poor water quality, particularly low dissolved oxygen and high ammonia.
  - Contaminants.
  - Inappropriate water temperature or changes in water temperature.
  - Poor nutrition.
  - Recent handling.
  - Recent transport.
  - Recent spawning.
  - Other diseases, particularly where they damage physical or physiological defenses (e.g. *Ichthyophthirius multifiliis*).
  - Immune-suppressive therapy (e.g. copper sulfate immersion).
- Exposure factors:
  - Poor biosecurity (e.g. use of surface water, exposure to wild fish).
  - Permissive water temperature.
  - Permissive salinity.
  - High organic loads.
  - Low water flow rate or turnover.
  - Trauma to the mucus, skin, or gills.

Signs/Clinical Findings

- Clinical course may be peracute, acute, or chronic.
- Clinical signs are typically nonspecific but can help differentiate between acute and chronic presentations. This delineation affects differentials and prognosis.
- Lethargy, increased hiding, or abnormal behavior is common.
- Inappetence or reduced appetite is common.
- Acute infections often present with erythema, petechiae, skin ulcers, and fin erosions. Ulcerative keratitis or uveitis may be seen.
- Chronic infections may present with poor body condition and change in coloration.
- Coinfections (e.g. viral, fungal, parasitic) are common.
- Some bacterial diseases of fish are cyclical.

Differential Diagnoses

- The clinical signs associated with a systemic bacterial infection are hard to distinguish from other causes of systemic inflammation (e.g. viral, fungal, toxic, parasitic).
- Full differentials are described in Section B; common differentials are highlighted for each bacterial group.

Diagnosis

- Diagnosis must be based on multiple consistent results, including history, clinical signs, cytology, necropsy, histology, culture, and molecular diagnostics. Mixed infections, autolysis, and contaminants are common and can create false impressions of a bacterial infection.
- Cytology (e.g. effusions, blood smears, tissue aspirates, tissue impressions) may show bacteria. Motility, morphology, and staining characteristics can help narrow down the differentials.
- Necropsy or coeliotomy and histology may show organomegaly, congestion, or necrosis. Chronic inflammation may cause granulomas. Intralesional bacteria may be

seen. Morphology and staining characteristics can help narrow down the differentials.

- Culture has been the mainstay of diagnosis but is limited to bacteria that grow in culture. Routine media such as trypticase soy agar with blood, brain–heart infusion agar, or MacConkey agar are particularly useful for *Aeromonas*, *Pseudomonas*, *Vibrio*, *Edwardsiella*, *Streptococcus*, and *Yersinia* spp. Selective media are needed for a number of fish pathogens (e.g. *Flavobacterium*, *Renibacterium*, *Francisella* spp.). False positives are possible from postmortem overgrowth and contaminants. False negatives are common, due to insufficient sample size, inappropriate temperature or media, and antibiotic treatment. It is best to use laboratories that are familiar with fish pathogens and to discuss possible differentials prior to sample collection and submission. Laboratories in the United States with expertise in fish pathogens are listed in Appendix 3.
- Molecular testing and sequencing allow definitive species identification. Identification based on phenotype alone (appearance and biochemical tests) may not be reliable for bacteria in fish.

Husbandry Management

- General management is described here; specific examples are provided for each bacterial group.
- Husbandry management typically relies on reducing or resolving stressors.
  - Increase aeration; target dissolved oxygen may be 95–100%.
  - Resolve any water quality issues.
  - Resolve any temperature stressors.
  - Increase water flow rate or turnover.
  - Reduce social stressors (e.g. add visual barriers).
  - Review the diet and consider additional vitamin C supplementation.
  - Minimize any additional stressors (e.g. transport).
- Husbandry changes can also reduce exposure and transmission rates.
  - Remove dead or moribund fish as soon as possible with appropriate disposition.
  - For virulent infections, isolate affected systems (e.g. limit access, use dedicated equipment).
  - Increase cleaning and disinfection of water. Many bacteria are sensitive to UV disinfection. Recommended doses are typically ~3–10 mJ/cm^2 or mWs/cm^2, but higher doses are reported for some, such as *Flavobacterium* and *Renibacterium* spp. Many bacteria are sensitive to ozone disinfection, although results vary across the studies. Dose depends on the pathogen and organic load, but common doses for fish are often TRO 0.1–0.2 mg/L for several minutes contact time with a system ORP of 300–350 mV. Higher doses have greater disinfection power but increase the risk of damage to fish. UV and ozone disinfection are synergistic and lower applied doses can be used when combined. UV and ozone are most effective after mechanical and biological filtration.
  - Increase cleaning and disinfection of fomites. Most bacteria are susceptible to routine disinfectants such as sodium hypochlorite and other chlorine-based disinfectants, alcohols, iodophors, peroxygen compounds (e.g. Virkon® Aquatic), chlorhexidine gluconate, phenols (e.g. Lysol®), and quaternary ammonium compounds (e.g. benzalkonium chloride) (see Appendix 2). All disinfectants are concentration-, time-, and temperature-dependent. Fomite disinfection is most effective after mechanical cleaning and when followed by drying, particularly in sunlight. Some bacteria are particularly resistant (e.g. *Mycobacterium* spp. and spore-forming bacteria).

Medical Management

- Treatment of an individual may differ from treatment or management of a group.
- Common treatments (see Chapters A12 and A13 for more details):
  - Antibiotic therapy is usually indicated and should be based on culture and sensitivity results.
  - For an individual, injectable or targeted oral medications may be considered along with supportive care such as fluid and nutritional support.
  - For a group of fish, medicated feed or immersion treatments are often required.
  - The effectiveness of oral treatments can be limited by reduced appetites.
  - Some formulations of the antibiotics florfenicol, oxytetracycline, and sulfadimethoxine/ormetoprim are FDA-approved in the United States for specific conditions in some fish species. Dose instructions and withdrawal periods must be followed.
  - Enrofloxacin, ceftazidime, amikacin, and sulfamethoxazole/trimethoprim are commonly used in aquarium fish but are not FDA-approved for use in food fish.
  - Low dose hypersalinity can be used to reduce the osmotic stress in freshwater fish (e.g. 1–3 g/L).
- Other reported treatments (see Chapters A12 and A13 for more details):
  - Anti-inflammatory doses of steroids may limit overstimulation of the immune system and improve prognosis.
- All legislation regarding medication use and disposal must be followed.

Prevention

- Morbidity and mortality can be prevented by reducing stressors, exposure, and severity of disease. While many bacterial pathogens are ubiquitous opportunists, good biosecurity can reduce the risk of introducing virulent strains.
- The general preventative measures below relate to all bacteria. Specific examples are provided for each bacterial group.
- Reduce or resolve stressors, particularly when disease is likely (e.g. after transport or at permissive water temperatures).
  - Low stocking density.
  - Suitable social groups and habitats.
  - Excellent water quality and preferred water temperature.
  - Good nutrition.
  - Suitable handling and transport protocols.
  - No other disease issues.
- Reduce exposure and transmission.
  - Isolation with no contact with free-ranging animals, particularly fish and aquatic invertebrates.
  - Pathogen-free water source (e.g. municipal water or ground water that has gone through fine filtration then UV and/or ozone disinfection). Avoid using untreated surface water.
  - Disinfection of recirculating water with fine filtration then UV and/or ozone.
  - High water flow rate or turnover.
  - Removal of dead or moribund fish as soon as possible with appropriate disposition.
  - Good cleaning and disinfection protocols.
  - Limits on visitors and potential fomites.
  - All-in-all-out management.
  - Suitable quarantine with isolation, monitoring, and diagnostic testing. This should apply to new animals as well as animals returning from shows or loans.
  - Frozen-thawed or commercial feeds, with no live feeds.
  - Routine monitoring of diseases of concern.
  - Disinfection of discharge water to reduce the risk of spill-back to wild populations.
- Reduce severity of disease.
  - Vaccination, where available.
  - High vitamin C in the diet; 500–1000 mg/kg of ascorbic acid equivalent in the diet consistently improves response to disease. Vitamin C is easily degraded by food processing, storage, light, oxygen, and high temperatures.
  - Immune-stimulants can be used prior to a known stressor (e.g. transport) or following an acute stressor (e.g. temperature change) to provide additional protection against extracellular bacteria such as *Aeromonas*, *Vibrio*, *Yersinia*, *Edwardsiella*, and *Streptococcus* spp. The range of products and doses is wide; examples of doses that are commonly effective in teleosts include: beta glucans at 1 g/kg of diet (0.1%) PO for one to two weeks; allicin or crushed garlic at 5–10 g/kg of diet (0.5–1%) PO for four weeks; or propolis ethanol extract at 5–10 g/kg of diet (0.5–1%) PO for four weeks.
  - Selective breeding of more resistant fish strains.
  - A healthy and diverse microbiome should reduce the probability of colonization by virulent strains; this might be most easily achieved with native species, local water, natural substrates, and suitable and stable environmental conditions.
- Regularly review morbidity and mortality trends and response to treatments.

Zoonotic Reports

- Several fish bacterial pathogens have zoonotic potential, but transmission is much more common from ingestion of raw or inadequately cooked or processed fish than from contact with fish.
- The highest risk from contact with fish is direct wound inoculation (e.g. fish spine injury or bite wound).
- Most fish bacterial pathogens are also common in the environment and transmission is possible through water contact with mucosal surfaces, open wounds, or food, although this appears to be rare.
- Disease is more likely with immune suppression.

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Abbreviations/Acronyms

- FDA: Food and Drug Administration
- ORP: Oxidation–reduction potential
- PO: Orally
- TRO: Total residual oxidant
- UV: Ultraviolet

Aeromonas salmonicida

Overview

- *Aeromonas salmonicida* subsp. *salmonicida* causes furunculosis, a serious disease of salmonids in aquaculture.
- Other *Aeromonas salmonicida* subspecies can cause atypical furunculosis in a variety of fish species.
- Both cause septicemia; skin ulcers and hemorrhages are common clinical signs.

Etiology

- Family *Aeromonadaceae.*
- *Aeromonas salmonicida* has several subspecies:
  - Typical furunculosis: *A. salmonicida* subsp. *salmonicida*.
  - Atypical furunculosis: *A. salmonicida* subsp. *achromogenes* (previously *Haemophilus piscium*), *A. salmonicida* subsp. *masoucida*, *A. salmonicida* subsp. *smithia*.
- This division of typical and atypical furunculosis is likely oversimplified, but remains a common categorization.
- *A. salmonicida* can be associated with other diseases, e.g. in carp (*Cyprinus* spp.), *A. salmonicida* is commonly associated with rhabdovirus carpio as part of infectious dropsy of carp (known as carp erythrodermatitis in Europe).
- These are nonmotile, Gram-negative bacilli and facultative anaerobes.

Transmission

- Transmission is typically horizontal, through ingestion and contact.
- The bacteria can survive for months in freshwater and sediment and for days in salt water.

Geographic Distribution

- Freshwater and saltwater habitats.

Signalment

- Typical furunculosis infects wild and cultured freshwater salmonids such as brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), and Pacific salmon (*Oncorhynchus* spp.), although rainbow trout (*Oncorhynchus mykiss*) seem less susceptible.
- Atypical furunculosis can infect a variety of freshwater and saltwater teleosts including salmonids (*Salmonidae*), sand eels (*Ammodytes lancea*), flatfish such as turbot (*Scophthalmus maximus*), plaice (*Pleuronectes platessa*), and halibut (*Hippoglossus* spp.), cyprinids such as goldfish (*Carassius auratus*) and koi (*Cyprinus carpio koi*), and Atlantic cod (*Gadus morhua*, causing ulcer disease of cod).

Risk Factors

- General risk factors are described under C4: *Bacterial Diseases (General).*
- Permissive water temperature is often >22°C (>72°F).

Signs/Clinical Findings

- Multiple fish from one or more species are usually affected.
- Clinical course may be peracute, acute, or chronic.
- Peracute death is common in juvenile fish.
- Lethargy may be seen.
- Inappetence or reduced appetite may be seen.
- Erythema, ecchymoses (particularly at the base of fins), hemorrhages, skin ulcers, or fin erosions are common.
- Boils (furuncles) or nodules may be seen prior to skin ulcers, although furuncles are not common, despite the name of the disease.
- Skin darkening may be seen.
- Exophthalmos may be seen.
- Coelomic distension and cloacal/anal distension may be seen.

- Gill pallor or gill edema may be seen.
- Generalized edema may be seen.
- Mortality is often high, up to 90%.

Differential Diagnoses

- Full differentials are described in Section B.
- Common differentials for bacterial septicemia include vibriosis, *Aeromonas*, *Citrobacter*, *Pseudomonas*, *Edwardsiella*, *Flavobacterium*, *Yersinia*, *Streptococcus*, and *Francisella* spp.

Diagnosis

- Diagnosis is typically based on a combination of cytology, histology, and culture.
- Short Gram-negative bacilli may be seen on blood smears, effusions, tissue cytology, or histology. They are nonmotile on wet mounts.
- Necropsy or coeliotomy and histology typically show petechiae, congestion, serosanguinous coelomic effusion, tissue pallor, organomegaly, or focal necrosis of any tissues with intralesional Gram-negative bacilli.
- Culture of affected tissues or blood is often successful. Routine or selective media (e.g. TSA with Coomassie brilliant blue) can be used aerobically at ~18–25°C (64–77°F). However, bacteria are easily killed by high temperatures, antibiotic treatment, and faster growing aeromonads.
- Biochemical identification schemes are often used, but species identification is rarely definitive. Even molecular techniques have limited ability to differentiate between species and subspecies.
- PCR tests are available commercially.
- ELISAs can be used to assess antibody response.

Husbandry Management

- General husbandry management is described under C4: *Bacterial Diseases (General)*.

Medical Management

- Common treatments (see Chapters A12 and A13 for more details):
  - Antibiotic therapy is indicated. Treatment should be based on culture and sensitivity as antibiotic resistance is common and published reports show different antibiotic susceptibilities.
  - Oxytetracycline dihydrate (Terramycin® 200) orally is approved in the United States for salmonids with furunculosis at 55–83 mg/kg every 24 hours for 10 days, with a 21-day withdrawal, as of 2020.
  - Sulfadimethoxine and ormetoprim (Romet® 30) orally is approved in the United States for salmonids with furunculosis at 50 mg/kg every 24 hours for five days, with a 42-day withdrawal, as of 2020.
  - Florfenicol (Aquaflor®) orally is approved in the United States for freshwater-reared salmonids with furunculosis at 10–15 mg/kg every 24 hours for 10 days, with a 15-day withdrawal, as of 2020.
- Other reported treatments (see Chapters A12 and A13 for more details):
  - Potassium permanganate immersion if infection is limited to the skin.
- All legislation regarding medication use and disposal must be followed.

Prevention

- General preventative measures are described under C4: *Bacterial Diseases (General)*.
- Effective commercial vaccines are available for typical furunculosis in salmonids, usually by intraperitoneal injection. Vaccines with mineral oil adjuvants provide the greatest protection but often cause adverse reactions including coelomic pigmentation, adhesions, and granulomas.
- Autogenous vaccines may be considered for other *A. salmonicida* subspecies; commercial vaccines are unlikely because of antigenic diversity.
- Immune-stimulants such as allicin, glucans, chitosan, and probiotics (e.g. *Lactobacillus*, *Lactococcus*, *Carnobacterium* spp.) can increase resistance to *A. salmonicida*. They are most effective at specific doses, short courses, and when given prior to infection.

Zoonotic Reports

- This disease has no known zoonotic potential.

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Abbreviations/Acronyms
- ELISA: Enzyme linked immunosorbent assay
- PCR: Polymerase chain reaction
- TSA: Trypticase soy agar

Motile Aeromonad Septicemia

Overview
- Several *Aeromonas* species are opportunists that can cause motile aeromonad septicemia (MAS).
- MAS is the most commonly reported bacterial infection of fish.
- The bacteria grow readily in culture and may be secondary infections or contaminants. This can result in a misdiagnosis or masking of the primary disease.

Etiology
- Family *Aeromonadaceae.*
- *Aeromonas hydrophila* (*liquefaciens*), *Aeromonas sobria*, *Aeromonas caviae*, *Aeromonas veronii*, and *Aeromonas dhakensis* are all reported in fish, although a variety of strains exist and the taxonomy changes frequently.
- The bacteria are common in the environment and many aeromonad species are nonpathogenic.
- Some *Aeromonas* species are normal commensals on fish skin.
- These are motile, short, straight, Gram-negative bacilli and facultative anaerobes.

Transmission
- Transmission is typically horizontal, through ingestion and contact.
- Amphibians and reptiles may act as carriers or vectors.

Geographic Distribution
- Predominantly in freshwater and brackish water habitats, probably worldwide.

Signalment
- Predominantly found in cultured and wild freshwater fish, including carp (*Cyprinus carpio*), goldfish (*Carassius auratus*), channel catfish (*Ictalurus punctatus*), tilapia (*Oreochromis* spp.), striped bass (*Morone saxatilis*), largemouth bass (*Micropterus salmoides*), and European eel (*Anguilla anguilla*).
- Infections are less common in marine teleosts. Examples include cod (*Gadus morhua*) and marine elasmobranchs.

Risk Factors
- General risk factors are described under C4: *Bacterial Diseases (General).*
- Permissive water temperature is often ~10–22°C (50–72°F) or higher.

Signs/Clinical Findings
- Individual or multiple fish from one or more species may be affected.
- Inappetence or reduced appetite may be seen.
- Lethargy may be seen.
- Erythema, petechiae, skin ulcers and fin erosions, scale loss, cutaneous edema, or boils (furuncles) are common.
- Exophthalmos may be seen.
- Coelomic distension may be seen.
- Gill pallor or edema may be seen.
- Neurologic signs may be seen.
- Mortality is typically low but can reach 100% in fry or fingerlings.

Differential Diagnoses
- Full differentials are described in Section B.
- Common differentials for bacterial septicemia include vibriosis, *Aeromonas*, *Citrobacter*, *Pseudomonas*, *Edwardsiella*, *Flavobacterium*, *Yersinia*, *Streptococcus*, and *Francisella* spp.

Diagnosis
- Diagnosis should be based on multiple consistent findings (e.g. clinical signs, cytology, necropsy, histology, and culture) and not culture alone.
- Short (1–3 µm), straight, Gram-negative bacilli may be seen on blood smears, effusions, tissue cytology, or histology. They are motile on wet mounts.

- Necropsy or coeliotomy and histology typically show petechiae, congestion, serosanguinous coelomic effusion, tissue pallor, organomegaly, or focal necrosis of any tissues with intralesional Gram-negative bacilli.
- Culture of affected tissues or blood is often successful. Routine or selective media (e.g. Coomassie brilliant blue) can be used aerobically at ~18–25°C (64–77°F). Bacteria are easily killed by high temperatures and concurrent antibiotic treatments.
- Traditional biochemical identification tests are often used, but species identification is frequently debated; definitive identification requires sequencing.
- PCR and ELISA tests may be available.

Husbandry Management

- General husbandry management is described under C4: *Bacterial Diseases (General)*.
- Reduction of stressors can be sufficient to control signs.

Medical Management

- Common treatments (see Chapters A12 and A13 for more details):
  - Antibiotic therapy may be indicated. Treatment should be based on culture and sensitivity as antibiotic resistance is common and published reports show different antibiotic susceptibilities.
  - Oxytetracycline dihydrate (Terramycin® 200) orally is approved in the United States for catfish and salmonids with *A. hydrophila* at 55–83 mg/kg every 24 hours for 10 days, with a 21-day withdrawal, as of 2020.
  - Enrofloxacin, ceftazidime, amikacin, florfenicol, and trimethoprim-sulfamethoxazole are commonly used in aquarium fish.
  - Low-dose hypersalinity can be used to reduce the osmotic stress in freshwater fish (e.g. 1–3 g/L).
- Other reported treatments (see Chapters A12 and A13 for more details):
  - Surgical debridement of lesions.
  - Potassium permanganate immersion if the disease is limited to the skin.
- All legislation regarding medication use and disposal must be followed.

Prevention

- General preventative measures are described under C4: *Bacterial Diseases (General)*.
- Motile aeromonads are ubiquitous and cannot be eliminated. Disease is usually secondary to other issues and prevention should focus on reducing stressors and reducing severity of disease.
- Autogenous vaccines can help with recurrent outbreaks; commercial vaccines are unlikely because of antigenic diversity.
- Immune-stimulants, such as beta glucans, allicin, and propolis, can increase resistance to MAS. They are most effective at specific doses, short courses, and when given prior to infection.

Zoonotic Reports

- Some aeromonad species and strains are zoonotic.
- Transmission may be foodborne, waterborne, or by inoculation.
- *A. hydrophila*, *A. sobria*, and *A. caviae* are implicated in foodborne and waterborne diarrhea from consumption of raw or inadequately cooked or processed fish and shellfish or contaminated food or water.
- Infection by inoculation can lead to local infection or septicemia.
- With a healthy immune system, no damage to the skin barrier, and good food hygiene, risk of transmission to humans from contact with fish or aquarium water appears to be low.

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Abbreviations/Acronyms
- ELISA: Enzyme linked immunosorbent assay
- MAS: Motile aeromonad septicemia
- PCR: Polymerase chain reaction

Vibriosis

Overview
- Vibriosis is caused by a variety of bacteria within the genus *Vibrio* and bacteria that used to be within the genus (e.g. *Aliivibrio*, *Moritella*, and *Photobacterium*).
- Some can be primary pathogens, but most are ubiquitous in the environment and cause disease secondary to other stressors.
- They can cause bacterial septicemia in a wide range of fish species.
- *Aliivibrio salmonicida* is particularly common in salmonid and cod aquaculture in Canada, Norway, and the United Kingdom.

Etiology
- Family *Vibrionaceae.*
- More than 20 *Vibrio* spp. may cause disease. Two are reported most commonly:
  - *Vibrio* (*Listonella*, *Beneckea*) *anguillarum*, causing saltwater furunculosis.
  - *Vibrio ordalii* (previously atypical strain of *V. anguillarum* biotype 2).
- Other *Vibrio* spp. that can cause disease are listed in alphabetical order below.
  - *V. alginolyticus.*
  - *V. cholera.*
  - *V. fischeri.*
  - *V. harveyi* (including previous *V. carchariae* and *V. trachuri*).
  - *V. ichthyoenteri.*
  - *V. logei.*
  - *V. parahaemolyticus.*
  - *V. pelagius.*
  - *V. splendidus.*
  - *V. tapetis.*
  - *V. vulnificus.*
- Several *Vibrio* spp. have been renamed but are still considered to cause vibriosis.
  - *Aliivibrio salmonicida*, causing hitra or cold-water vibriosis.
  - *Moritella viscosa.*
  - *Moritella marina.*
  - *Photobacterium damselae* subsp. *damselae.*
  - *Photobacterium* (*Pasteurella*) *damselae* subsp. *piscicida* (pseudotuberculosis, pasteurellosis, photobacteriosis).
- They are part of normal fish intestinal flora. Intestinal *Vibrio* flora closely resembles the populations in the water.
- These are common commensals (autochthonous bacteria) in elasmobranch tissues and blood, particularly *V. alginolyticus*, *V. harveyi*, and *P. damselae*.
- *Vibrio* spp. are present in light-emitting organs of marine fish and cephalopods.
- These are curved to spiral-shaped, Gram-negative bacilli that are usually motile and are facultative anaerobes.

Transmission
- Horizontal transmission is common; vertical transmission seems possible.
- Aquatic invertebrates (e.g. bivalves, penaeid shrimp) may act as carriers or vectors.

Geographic Distribution
- Predominantly in saltwater and brackish water habitats, probably worldwide.

Signalment
- Disease is likely possible in any marine or brackish fish, including elasmobranchs.
- Vibriosis is commonly reported in some groups:
  - Salmon and trout (*Salmonidae*).
  - Striped bass (*Morone saxatilis*), European bass (*Dicentrarchus labrax*).
  - Cod (*Gadus morhua*).
  - Yellowtail (*Seriola quinqueradiata*).
  - Seahorses (*Syngnathidae*).
  - Requiem sharks (*Carcharhinidae*).
- Disease is occasionally reported in freshwater fish (e.g. guppies, *Poecilia reticulata*).

Risk Factors
- General risk factors are described under C4: *Bacterial Diseases (General).*
- Permissive water temperature is high for most *Vibrio* spp. and low for *Aliivibrio salmonicida*, e.g. 15°C (59°F).

Signs/Clinical Findings
- Individual or multiple fish from one or more species may be affected.
- Clinical course may be peracute, acute, or chronic.
- Lethargy may be seen.

- Inappetence or reduced appetite may be seen.
- Skin darkening or pallor, erythema, petechiae, ecchymoses, skin ulcers, or fin erosions may be seen.
- Coelomic distension due to ascites or organomegaly or a pinched coelom due to loss of body condition may be seen.
- Corneal edema, keratitis, or exophthalmos may be seen.
- Gill pallor due to anemia may be seen.
- Neurologic or respiratory signs may be seen.
- Mortality can be >50%, up to 90% in rare cases.

Differential Diagnoses

- Full differentials are described in Section B.
- Common differentials for bacterial septicemia include vibriosis, *Aeromonas*, *Citrobacter*, *Pseudomonas*, *Edwardsiella*, *Flavobacterium*, *Yersinia*, *Streptococcus*, and *Francisella* spp.

Diagnosis

- Diagnosis should be based on multiple consistent findings (e.g. clinical signs, cytology, necropsy, histology, and culture) and not culture alone.
- Short (1–3 µm), curved to spiral-shaped, Gram-negative bacilli may be seen on blood smears, effusions, tissue cytology, or histology (Figure C4.1). They may be motile on wet mounts.
- Necropsy or coeliotomy and histology typically show petechiae, congestion, serosanguinous coelomic effusion, tissue pallor, organomegaly, or focal necrosis of any tissues (particularly kidney and spleen) with intralesional Gram-negative bacilli. In more chronic cases, inflammation may be granulomatous, particularly with *P. damselae*.
- Culture of affected tissues (especially the kidney and spleen) or blood is often successful. Routine or selective media (e.g. thiosulfate citrate bile salts) can be used. Culture success may be improved in marine species with 10 g/L sodium chloride. Incubation temperature needs to be low for *Aliivibrio salmonicida*. Autochthonous bacteria are present in elasmobranchs and blood cultures may be positive in healthy animals.
- Species identification using biochemical profiles can be difficult and PCR or sequencing is recommended.
- Serology is not routinely available.

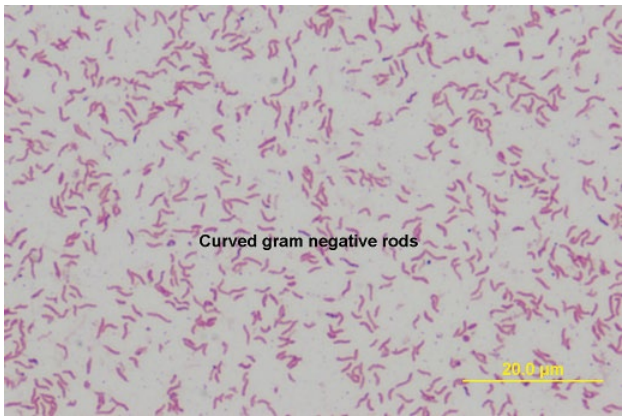

Figure C4.1 Cytologic appearance of curved Gram-negative bacilli, consistent with *Vibrio* spp. *Source*: Image courtesy of Jill Arnold, National Aquarium.

Husbandry Management

- General husbandry management is described under C4: *Bacterial Diseases (General)*.
- Reduction of stressors or other disease issues is sometimes enough to control signs.
- In aquaculture, regulations may require movement restrictions, depopulation, and disinfection.

Medical Management

- Common treatments (see Chapters A12 and A13 for more details):
  - Antibiotic therapy is usually indicated and should be based on culture and sensitivity results.
- All legislation regarding medication use and disposal must be followed.

Prevention

- General preventative measures are described under C4: *Bacterial Diseases (General)*.
- A healthy, diverse microbiome may reduce exposure to more pathogenic *Vibrio* spp. (e.g. survival of *V. ordalii* is reduced by the presence of other microbiota).
- Commercial immersion vaccines are available for some species (e.g. *V. anguillarum* and *V. ordalii* in salmonids).
- Autogenous vaccines may be considered.
- Immune-stimulants, such as beta glucans, can increase resistance to vibriosis. They are most effective at specific doses, short courses, and when given prior to infection.

Zoonotic Reports

- Vibriosis is predominantly a foodborne zoonosis through ingestion of raw or inadequately cooked shellfish (particularly oysters) or contaminated food and water. Species implicated in human disease include *V. parahaemolyticus*, *V. vulnificus*, *V. harveyi*, *V. alginolyticus*, and *P. damselae*. Signs may include nausea, vomiting, abdominal cramps, and pyrexia.
- Transmission is possible though inoculation into wounds, either from water or fish. This can cause local infections or septicemia, particularly if associated with immune suppression.
- The CDC estimates that vibriosis causes 80 000 human illnesses in the United States each year, of which two thirds are due to eating contaminated food.
- The species that causes cholera (*Vibrio cholerae*) is not found in fish.
- With a healthy immune system, no damage to the skin barrier, and good food hygiene, risk of transmission to humans from contact with fish or aquarium water appears to be low.

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Abbreviations/Acronyms

- CDC: Centers for Disease Control and Prevention
- PCR: Polymerase chain reaction

Enteric Septicemia of Catfish

Overview

- *Edwardsiella ictaluri* is the causative agent of enteric septicemia of catfish.
- It is a primary pathogen that causes significant economic losses in aquaculture of channel catfish (*Ictalurus punctatus*).
- It causes enteric, neurologic, and septicemic disease.

Etiology

- Family *Enterobacteriaceae*.
- The causative agent is *Edwardsiella ictaluri*. Several strains exist.
- *E. ictaluri* is a primary, obligate pathogen, unlike *Edwardsiella tarda* and *Edwardsiella piscicida*, which are opportunists.
- These are motile, short, pleomorphic, Gram-negative bacilli and facultative anaerobes.

Transmission

- Transmission is primarily by ingestion, often feco-oral or through scavenging. Transmission can also be waterborne through the gills and nares.
- *E. ictaluri* does not survive long in water but can survive in pond mud for >3 mo at 25°C (77°F).
- Fish that resolve clinical signs can become carriers.
- Piscivorous birds may act as vectors.

Geographic Distribution

- *E. ictaluri* has been reported in all areas of the United States where catfish aquaculture is common, including Alabama, Arkansas, Mississippi, Louisiana, and California.
- Other reports have included Japan, southeast Asia, Australia, and Europe.

Signalment

- Epizootic disease is seen primarily in channel catfish in aquaculture.
- Disease is also seen in blue catfish (*Ictalurus furcatus*), brown bullhead (*Ameiurus nebulosus*), and white catfish (*Ameiurus catus*), but signs are typically less severe.
- Hybrid catfish are typically more resistant, although reports of disease may be increasing.
- More recently, natural infection and clinical signs have been reported in a wider range of species, including wild ayu (*Plecoglossus altivelis*), cultured zebrafish (*Danio rerio*), and cultured Nile tilapia (*Oreochromis niloticus*), although these appear to be different strains.
- *E. ictaluri* has been isolated from a wider variety of freshwater fish without morbidity or mortality.

Risk Factors

- General risk factors are described under C4: *Bacterial Diseases (General)*.
- Permissive water temperature is often 22–28°C (72–82°F).

Signs/Clinical Findings

- Multiple conspecifics are usually affected.
- Most presentations are neurologic or enteric/septicemic, although the signs can overlap.
- The neurologic form is typically more chronic.
  - Spiral swimming, circling, and inappetence are common.
  - Lethargy or hanging in the water with a low tail position is also seen.
  - Fingerlings may show skin ulcers on the head (known as hole in the head).
- The enteric/septicemic form is typically more acute.
  - Erythema, petechiae, ecchymoses, hemorrhages, and skin ulcers are common.
  - Exophthalmos may be seen.

- Coelomic distension may be seen.
- Gill pallor due to anemia may be seen.

- A biphasic pattern is common with disease typically seen in the spring and fall.
- Mortality varies but can reach >50%, particularly with acute disease. Mortalities can increase rapidly if additional stressors are introduced, such as handling.

Differential Diagnoses

- Full differentials are described in Section B.
- Major differentials for septicemic disease in young channel catfish include *E. ictaluri*, *E. piscicida*, and channel catfish virus.
- Common differentials for bacterial granulomatous lesions in teleosts include *Mycobacterium*, *Nocardia*, *Edwardsiella*, *Francisella*, *Streptococcus*, *Photobacterium* spp., and piscirickettsial-like organisms.

Diagnosis

- Diagnosis should be based on multiple consistent findings (e.g. clinical signs, cytology, necropsy, histology, and culture) and not culture alone.
- Short (~1.25 µm) Gram-negative bacilli may be seen on blood smears, effusions, tissue cytology, or histology. They are motile on wet mounts.
- Necropsy or coeliotomy may show petechiae, congestion, serosanguinous coelomic effusion, tissue pallor, splenomegaly and renomegaly with nodules.
- Histology typically shows hemorrhagic enteritis and granulomatous splenitis, nephritis, and encephalitis with intralesional Gram-negative bacilli.
- Culture of affected tissues (particularly spleen, anterior kidney, liver, and brain) or blood is often successful. Routine media works well, usually at ~25–30°C (77–86°F).
- Biochemical tests (e.g. API 20E) can be used for tentative identification.
- PCR tests are available commercially and offer higher sensitivity; sequencing is required for confirmation.
- FA and ELISA may be available.

Husbandry Management

- General husbandry management is described under C4: *Bacterial Diseases (General)*.
- Consider slowly taking the water temperature out of the permissive range of 22–28°C (72–82°F), if possible.
- In catfish aquaculture, food is often reduced or withheld for 3–28 days to reduce fecal shedding of the bacteria. This is typically started immediately after identification of infection or when water temperature reaches >22°C (72°F). Withholding food can reduce mortality and antibiotic use, but does reduce growth rates, increase the risk of columnaris, and has welfare implications.

Medical Management

- Common treatments (see Chapters A12 and A13 for more details):
  - Antibiotic therapy is indicated if water temperature is likely to remain in the permissive range for weeks. Antibiotic resistance is common and choice should be based on culture and sensitivity results.
  - Sulfadimethoxine and ormetoprim (Romet® TC) orally is approved in the United States for catfish with *E. ictaluri* at 50 mg/kg every 24 hours for five days, with a three-day withdrawal, as of 2020.
  - Florfenicol (Aquaflor®) orally is approved in the United States for catfish with *E. ictaluri* at 10–15 mg/kg every 24 hours for 10 days, with a 15-day withdrawal, as of 2020.
- All legislation regarding medication use and disposal must be followed.

Prevention

- General preventative measures are described under C4: *Bacterial Diseases (General)*.
- Effective commercial immersion vaccines are available.
- Immune-stimulants, such as beta glucans, can increase resistance to *E. ictaluri*. They are most effective at specific doses, short courses, and when given prior to infection.

Zoonotic Reports

- This disease has no known zoonotic potential.
- A different *Edwardsiella* sp. (*E. hoshinae*) is reported in mammals.

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Abbreviations/Acronyms

- API: Analytical profile index
- ELISA: Enzyme linked immunosorbent assay
- FA: Fluorescent antibody
- PCR: Polymerase chain reaction

Edwardsiellosis

Overview

- *Edwardsiella tarda*, *Edwardsiella piscicida*, and *Edwardsiella anguillarum* are enteric bacteria that can cause bacterial septicemia known as edwardsiellosis.
- All bony fish seem susceptible.
- The response may be suppurative and in catfish, the disease is also known as emphysematous putrefactive disease.
- Clinical management is similar for all three species.

Etiology

- Family *Enterobacteriaceae.*
- *Edwardsiella tarda* was the original causative agent of edwardsiellosis.
- *Edwardsiella piscicida* and *E. piscicida*-like bacteria have been identified since 2013. Molecular diagnostics have shown that some infections previously reported as *E. tarda* were more likely *E. piscicida*.
- *Edwardsiella anguillarum* was first identified in 2015.
- Differentiation between the species requires molecular diagnostics.
- These are small, straight, Gram-negative bacilli that may be motile and are facultative anaerobes.

Transmission

- Transmission is typically oral.
- The bacteria are also found in invertebrates, amphibians, reptiles, and mammals, including humans; their role in transmission is unknown.

Geographic Distribution

- Ubiquitous in freshwater and saltwater habitats, probably worldwide.

Signalment

- Disease is likely possible in many marine and freshwater fish.
- Epizootics have been reported in several species in aquaculture, including eels (*Anguilla* spp.), tilapia (*Oreochromis* spp.), red seabream (*Pagrus major*), striped bass (*Morone saxatilis*), largemouth bass (*Micropterus salmoides*), channel catfish (*Ictalurus punctatus*), Amur catfish (*Silurus asotus*), olive flounder (*Paralichthys olivaceus*), and turbot (*Scophthalmus maximus*).
- Infection is common in marine aquarium teleosts but there are fewer reports in the literature.
- There is one report of disease in an elasmobranch: a round ribbontail ray (*Taeniura meyeni*).

Risk Factors

- General risk factors are described under C4: *Bacterial Diseases (General)*.

Signs/Clinical Findings

- Individual or multiple fish from one or more species may be affected.
- Clinical course may be acute or chronic; chronic is more common.
- Lethargy, spiral swimming, or positive buoyancy may be seen.
- Inappetence or reduced feeding may be seen.
- Erythema, petechiae, ecchymoses, skin ulcers, fin erosions, or loss of color are common. Some species, particularly catfish, may show skin abscesses or fistulas.
- Gill pallor due to anemia may be seen.
- Coelomic distension and cloacal/anal distension may be seen.
- Keratitis, uveitis, hypopyon, or exophthalmos may be seen.
- Mortality is usually low and chronic.

Differential Diagnoses

- Full differentials are described in Section B.
- Common differentials for bacterial septicemia include vibriosis, *Aeromonas*, *Citrobacter*, *Pseudomonas*,

Edwardsiella, *Flavobacterium*, *Yersinia*, *Streptococcus*, and *Francisella* spp.

Diagnosis

- Diagnosis to genus is typically based on signs, cytology, histology, and culture.
- Small, straight, Gram-negative bacilli may be seen on blood smears, effusions, tissue cytology, or histology. They may be motile on wet mounts.
- Necropsy or coeliotomy may show petechiae, congestion, serosanguinous coelomic effusion, tissue pallor, organomegaly, or nodules in any tissues. Malodorous abscesses may be found in the viscera or skeletal muscle, particularly in catfish.
- Histology typically shows suppurative inflammation, but it may be granulomatous. Intralesional Gram-negative bacilli are common.
- Culture of affected tissues (particularly kidney and spleen) or blood is often successful. Routine media works well, typically at ~30–35°C (86–95°F).
- FA can be used to highlight *Edwardsiella* sp. on histology or impression smears.
- PCR, qPCR, ELISA, LAMP assays, and sequencing may be available, but should be used in conjunction with other diagnostics.

Husbandry Management

- General husbandry management is described under C4: *Bacterial Diseases (General)*.

Medical Management

- Common treatments (see Chapters A12 and A13 for more details):
  - Antibiotics are usually indicated and should be based on culture and sensitivity.
  - In vitro testing usually shows a wide range of sensitivities, including tetracyclines, aminoglycosides, and fluoroquinolones. Resistance to macrolides and lincosamides is common.
- All legislation regarding medication use and disposal must be followed.

Prevention

- General preventative measures are described under C4: *Bacterial Diseases (General)*.
- Autogenous vaccines can help with recurrent outbreaks; commercial vaccines are unlikely because of antigenic diversity.
- Immune-stimulants, such as beta glucans, allicin, and propolis, can increase resistance to edwardsiellosis. They are most effective at specific doses, short courses, and when given prior to infection.

Zoonotic Reports

- *E. tarda* is zoonotic, although human disease is rare.
- Transmission is typically oral through ingestion of raw or inadequately cooked or processed fish. Gastroenteritis is the most common sequela, with several days of nausea, vomiting, diarrhea, and pyrexia.
- Inoculation into wounds is possible and can result in local wound infection with abscessation and necrosis.
- Septicemia and meningitis are rare sequelae.
- Humans can be asymptomatic carriers.

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Abbreviations/Acronyms
- ELISA: Enzyme linked immunosorbent assay
- FA: Fluorescent antibody
- LAMP: Loop-mediated isothermal amplification
- PCR: Polymerase chain reaction
- qPCR: Quantitative polymerase chain reaction

Columnaris and Flexibacteriosis

Overview
- *Flavobacterium columnare* is the causative agent of columnaris disease, sometimes known as saddle-back or peduncle disease.
- Columnaris is particularly common in freshwater ponds.
- Lesions are typically on the skin, fins, and gills, but the infection can become systemic.
- The marine counterpart is *Tenacibaculum maritimum*. Lesions are similar but systemic spread is less common.

Etiology
- Family *Flavobacteriaceae.*
- *Flavobacterium* (*Flexibacter*, *Bacillus*, *Cytophaga*) *columnare* is the cause of columnaris in freshwater fish.
- *Tenacibaculum* (*Flexibacter*, *Cytophaga*) *maritimum* (*marina*, *marinus*, *marimus*) is the cause of marine flexibacteriosis.
- These are motile, long, thin Gram-negative bacilli.

Transmission
- Horizontal transmission is common, through ingestion, contact, or inoculation.
- The bacteria are highly contagious.
- Data on survival in the environment varies from days to months depending on the conditions.
  - Survival of columnaris is increased in warm, hard, alkaline freshwater, and high organic loads. Water temperature of <5°C (<41°F) reduces survival.
  - Columnaris may be a component of the biofilm, which can provide protection for the bacteria.

Geographic Distribution
- *F. columnare* is ubiquitous in freshwater habitats worldwide.
- *T. maritimum* is ubiquitous in salt water habitats worldwide.

Signalment
- Columnaris is likely possible in all freshwater bony fish.
  - In aquariums and ornamental aquaculture, it is particularly common in goldfish (*Carassius auratus*), common carp and koi (*Cyprinus carpio*), mollies and guppies (*Poecilia* spp.), swordtails and platies (*Xiphophorus* spp.).
  - In food fish aquaculture, it is particularly common in channel catfish (*Ictalurus punctatus*), tilapia (*Oreochromis* spp.), sockeye salmon (*Oncorhynchus nerka*), and rainbow trout (*Oncorhynchus mykiss*).
- Marine flexibacteriosis is likely possible in all marine teleosts.
  - It is particularly common in several cultured food fish, including marine-reared Atlantic salmon (*Salmo salar*), barramundi (*Lates calcifer*), gilthead seabream (*Sparus auratus*), European bass (*Dicentrarchus labrax*), yellowtail amberjack (*Seriola lalandi*), olive flounder (*Paralichthys olivaceus*), and turbot (*Scophthalmus maximus*).
- Marine flexibacteriosis has also been identified in elasmobranchs under human care, including skates (*Rajidae*) and a sand tiger shark (*Carcharias taurus*).

Risk Factors
- General risk factors are described under C4: *Bacterial Diseases (General)*.
- Permissive water temperature is usually high (e.g. >15°C or 59°F, with morbidity and mortality highest at 24–32°C or 75–90°F), but is low for salmonid winter ulcer disease (e.g. <8°C or 46°F).
- High nitrite, alkalinity, hardness, and calcium are known risk factors.

Signs/Clinical Findings
- Individual or multiple fish from one or more species may be affected.
- An acute presentation may be seen, particularly in young fish.
  - Mortalities may be seen with no other signs.
  - Yellow-white or dark brown discoloration of the gills, particularly at the distal aspect of the primary lamellae, may be seen.
  - Dyspnea and tachypnea often develop.
  - Pruritus may be seen.

- A chronic presentation is more common.
  - Inappetence or reduced feeding is common.
  - Lesions on the skin, fins, and oral cavity are common. Multifocal skin pallor develops into ulcers covered with white or yellow cotton-like mucus plaques. Lesions often have a hyperemic, yellow, or white rim (Figure C4.2a). Skeletal muscle may be exposed.
  - Fin lesions often start at the base of the fin and progress distally, unlike typical bacterial fin rot.
  - Pruritus may be seen.
  - Edema, diffuse pallor, or darkening of the gills may be seen.
- Mortality is variable, but morbidity can be high.

Differential Diagnoses

- Full differentials are described in Section B.
- Common differentials for ulcers and mucus plaques on the skin of bony fish include oomycetes and scuticociliates, although a variety of skin pathogens and irritants can cause similar lesions.

Diagnosis

- Diagnosis is typically based on direct microscopy of skin scrapes or gill biopsies.
- Long (2–10 µm), thin, Gram-negative bacterial bacilli are seen, often grouped together in "haystacks" (Figure C4.2b and c). These show a slow, gliding and flexing motion on wet mounts.
- Wet mounts also often show increased mucus, opportunist pathogens (e.g. scuticociliates), gill hyperplasia and fusion of secondary filaments.
- Histology typically shows necrotizing, ulcerative dermatitis or branchitis with intralesional long, thin Gram-negative bacilli. Columnaris can become systemic with bacteremia and renomegaly. Marine flexibacteriosis rarely becomes systemic.
- Culture is possible, often collected from the edge of active lesions. However, contaminants are common with external cultures and the organisms are fastidious and inhibited by the high-nutrient content of routine media. Columnaris grows best with low-nutrient media (e.g. Shieh, Cytophaga, Hsu–Shotts), often at 25–30°C (77–86°F). Marine flexibacteriosis benefits from additional sodium chloride. Sensitivities can be problematic as the organisms do not grow well in Mueller–Hinton agar and other methodologies are needed (e.g. Gieseker et al. 2012).
- PCR and qPCR tests on gill, skin, or cranial kidney are available and provide specific, sensitive, and faster results than culture.
- LAMP from gills, skin, or cranial kidneys is used commonly in channel catfish.

Husbandry Management

- General husbandry management is described under C4: *Bacterial Diseases (General)*.
- Resolving environmental stressors can result in significant improvement.
- Slowly reduce the water temperature to <24°C (<75°F) and ideally <15°C (<59°F), if possible.
- In freshwater, consider low-dose hypersalinity treatment to reduce physiologic stress (e.g. 2–3 g/L).

Medical Management

- Common treatments (see Chapters A12 and A13 for more details):
  - Antibiotic therapy can improve morbidity and mortality but elimination is unlikely. Drug choice should be based on culture and sensitivity, but difficulties getting sensitivities mean that empirical therapy is common.
  - Florfenicol (Aquaflor®) orally is approved in the United States for freshwater-reared finfish with columnaris at 10–15 mg/kg every 24 hours for 10 days, with a 15-day withdrawal, as of 2020.
  - Oxytetracycline dihydrate (Terramycin® 200) orally is approved in the United States for freshwater-reared rainbow trout with columnaris at 83 mg/kg every 24 hours for 10 days, with a 21-day withdrawal, as of 2020.
  - Other antibiotics used in aquarium fish include enrofloxacin and trimethoprim sulfamethoxazole immersion.
  - Chloramine-T (HALAMID Aqua®) immersion is approved in the United States for walleye (*Sander vitreus*) and freshwater-reared salmonids and warm-water finfish with external columnaris under various treatment protocols, with a 0-day withdrawal, as of 2020.
  - Hydrogen peroxide (35% Perox-Aid®) immersion is approved in the United States for use in freshwater-reared cool-water and warm-water finfish with external columnaris at 50–75 mg/L for one hour every 48 hours for three treatments, with a 0-day withdrawal, as of 2020.
  - Potassium permanganate ($KMnO_4$) immersion treatment at 2 mg/L above the permanganate demand has been effective.
- Other reported treatments (see Chapters A12 and A13 for more details):
  - Oxolinic acid immersion.
  - Copper sulfate immersion.
- All legislation regarding medication use and disposal must be followed.

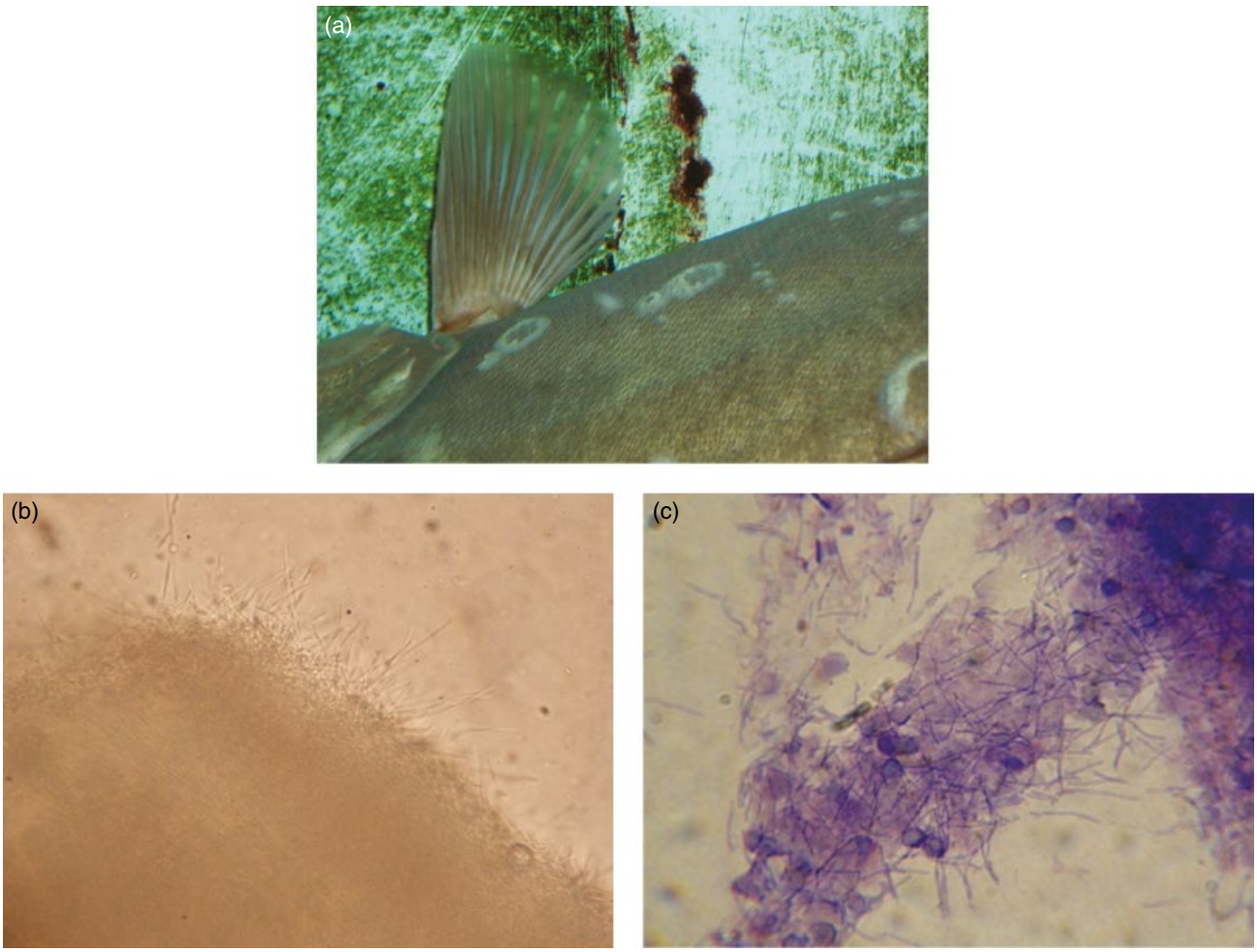

Figure C4.2 Gross appearance of columnaris lesions on a grouper (*Epinephelus* sp.) (a) and typical haystack structures on skin scrapes examined as a wet mount (b) and on Dif-Quik stain (c).

Prevention

- General preventative measures are described under C4: *Bacterial Diseases (General)*.
- High salinity (>5 g/L) and low hardness (<70 mg/L) reduce columnaris survival.
- Vaccination studies have shown variable success. A USDA-approved attenuated immersion vaccine is available for channel catfish.

Zoonotic Reports

- This disease has no known zoonotic potential.

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Abbreviations/Acronyms
- IM: Intramuscular
- LAMP: Loop-mediated isothermal amplification
- PCR: Polymerase chain reaction
- qPCR: Quantitative polymerase chain reaction
- PO: Orally
- USDA: United States Department of Agriculture

Flavobacterium psychrophilum

Overview
- *Flavobacterium psychrophilum* causes two serious diseases of salmonids.
- Bacterial cold-water disease (BCWD) is also known as peduncle or saddle-back disease and is typically seen in salmonid adults and fingerlings.
- Rainbow trout fry syndrome (RTFS) is also known as fry anemia syndrome and is typically seen in salmonid fry.
- Disease is seen when water temperature falls below 15°C (59°F).
- The diseases continue to be a significant problem in salmonid aquaculture and restocking programs.

Etiology
- Family *Flavobacteriaceae*.
- The causative agent is *Flavobacterium* (*Cytophaga*, *Flexibacter*) *psychrophilum*.
- These are motile, long, thin Gram-negative bacilli.

Transmission
- Horizontal and vertical transmission are reported.
- The bacteria are found in water and soil, as well as within the biofilm, which can provide protection for the bacteria.

Geographic Distribution
- Cold freshwater habitats.
- BCWD was first reported in the United States and RTFS was first reported in Europe, but they are now widespread.

Signalment
- Freshwater teleosts in cold-water only.
- This is a common problem in freshwater salmonids, particularly rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*).
  - BCWD is more common in adults and fingerlings.
  - RTFS is more common in fry.
- The disease has also been reported from other teleosts, including European eels (*Anguilla anguilla*), tench (*Tinca tinca*), common carp (*Cyprinus carpio*), Crucian carp (*Carassius carassius*), and ayu (*Plecoglossus altivelis*).

Risk Factors
- General risk factors are described under C4: *Bacterial Diseases (General)*.
- Permissive water temperature is usually low, e.g. 3–15°C (37–59°F).

Signs/Clinical Findings
- Multiple conspecifics are usually affected.
- In both BCWD and RTFS, lethargy, inappetence, reduced feeding, or gradual weight loss may be seen. Coelomic distension and cloacal/anal distension may be seen. Bilateral exophthalmos or gill pallor may be seen.
- BCWD:
  - Skin darkening and large cutaneous ulcers and erosions, particularly around the dorsal fins, are common. Erosions can be deep enough to expose vertebrae.
  - Mortality can reach 70–90%.
- RTFS:
  - Skin darkening or asymmetrical coloration may be seen, with the right and left side varying in color.
  - Neurologic signs are possible, particularly abnormal position in the water and spiral swimming.
  - Mortality is usually low.
- Chronic spinal deformities may be seen in survivors.

Differential Diagnoses
- Full differentials are described in Section B.
- Common differentials for skin ulcers in salmonids with long thin bacterial rods include *Flavobacterium columnare* and *F. psychrophilum*.

Diagnosis

- A tentative diagnosis is often possible from direct microscopy of skin scrapes or gill biopsies.
- Long (~2–6 µm), thin, Gram-negative bacilli may be seen on skin or gill samples; the bacilli are shorter than *F. columnare*. They are motile on wet mounts.
- Necropsy or coeliotomy may show splenomegaly, serosanguinous coelomic effusion, and tissue pallor.
- Histology typically shows pyogranulomatous inflammation and necrosis extending into muscle, bone, and cartilage with intralesional Gram-negative bacilli, particularly in the spleen, heart, gills, muscle, bone, and cartilage.
- Diagnosis is usually confirmed by PCR.
- Culture from blood or tissues (e.g. spleen, kidney) is possible, but the organisms are fastidious and inhibited by the high-nutrient content of routine media. Growth usually requires low-nutrient media (e.g. tryptone yeast extract salts), often at ~15–16°C (59–61°F).
- ELISA and FA tests have been developed for screening salmonid broodstock using kidney and ovarian tissues.

Husbandry Management

- General husbandry management is described under C4: *Bacterial Diseases (General)*.
- Environmental management alone can resolve morbidity and mortality.
- Slowly increase the water temperature, if possible. Clinical signs will typically resolve as temperature increases.
- Consider transferring fish to systems that have been cleaned and disinfected with sodium hypochlorite (e.g. 100 mg/L chlorine for one hour followed by sodium thiosulfate).
- Increase cleaning and disinfection; while *F. psychrophilum* is susceptible to most routine disinfectants, it is slightly less sensitive than *F. columnare*.

Medical Management

- Medical management has poor success due to reduced appetites limiting intake of oral medications and immune suppression at lower water temperatures.
- Common treatments (see Chapters A12 and A13 for more details):
  - Antibiotic therapy may be indicated and should be based on culture and sensitivity results.
  - Florfenicol (Aquaflor®) orally is approved in the United States for freshwater-reared salmonids with BCWD at 10–15 mg/kg every 24 hours for 10 days, with a 15-day withdrawal, as of 2020.
  - Oxytetracycline dihydrate (Terramycin® 200) orally is approved in the United States for freshwater-reared salmonids with BCWD at 83 mg/kg every 24 hours for 10 days, with a 21-day withdrawal, as of 2020.
- Other reported treatments (see Chapters A12 and A13 for more details):
  - Oxytetracycline immersion.
  - Potassium permanganate immersion.
  - Copper sulfate immersion.
  - Chloramine-T immersion.
- All legislation regarding medication use and disposal must be followed.

Prevention

- General preventative measures are described under C4: *Bacterial Diseases (General)*.
- Broodstock should be screened using diagnostic assays (e.g. ELISA).
- Disinfection of eggs (e.g. with iodophors) can reduce vertical transmission, but high doses may be needed.
- Experimental vaccines are reported but success has been inconsistent.

Zoonotic Reports

- This disease has no known zoonotic potential.

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Abbreviations/Acronyms
- BCWD: Bacterial cold water disease
- ELISA: Enzyme linked immunosorbent assay
- FA: Fluorescent antibody
- PCR: Polymerase chain reaction
- RTFS: Rainbow trout fry syndrome

Yersiniosis

Overview
- *Yersinia ruckeri* can cause hemorrhagic septicemia known as yersiniosis or enteric red mouth.
- It is an obligate pathogen of freshwater salmonids and has caused significant losses in aquaculture.

Etiology
- Family *Enterobacteriaceae*.
- The causative agent is *Yersinia ruckeri*.
- It was originally identified as a *Salmonella* sp.
- It is genetically similar to *Salmonella* spp., *Yersinia enterocolitis*, and *Yersinia pseudotuberculosis*.
- Several serotypes and biotypes exist. Serotypes are more relevant to virulence than biotypes. Serotype O1a is the cause of most epizootics.
- These are short, straight, Gram-negative bacilli that are usually motile.

Transmission
- Horizontal transmission is reported, predominantly feco-oral but waterborne transmission via the gills may also occur.
- There is some evidence of vertical transmission.
- The bacteria can survive for at least 4 mo outside the host, particularly within the biofilm, which can provide protection for the bacteria.
- Carriers include fathead minnows (*Pimephales promelas*). Fish that resolve clinical signs continue to shed the bacteria for up to 2 mo.
- *Y. ruckeri* is also found in aquatic invertebrates, reptiles, birds, and mammals, and these may be vectors.

Geographic Distribution
- Predominantly in freshwater habitats, probably worldwide.

Signalment
- Freshwater teleosts are most susceptible, particularly salmonids in aquaculture such as rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*).
- *Y. ruckeri* has been isolated from other freshwater fish, including goldfish (*Carassius auratus*), common carp and koi (*Cyprinus carpio*), eels (*Anguilla* spp.), channel catfish (*Ictalurus punctatus*), and turbot (*Scophthalmus maximus*).

Risk Factors
- General risk factors are described under C4: *Bacterial Diseases (General)*.
- Permissive water temperature is often 20–28°C (68–82°F).

Signs/Clinical Findings
- Multiple conspecifics are usually affected.
- Clinical course is usually acute presentation, particularly in juveniles. A more chronic course is seen in adults.
- Lethargy or swimming near the surface may be seen.
- Inappetence or reduced feeding may be seen.
- Petechiae, erythema, or hemorrhages are common, particularly in and around the oral cavity and eyes. This often progresses to severe oral ulceration.
- Skin darkening may be seen.
- Hyphema, keratitis, and exophthalmos (unilateral or bilateral) may be seen; fish may appear to be blind.
- Gill edema or pallor may be seen.
- Coelomic distension and cloacal/anal distension may be seen.
- Mortality rate is usually low, but cumulative losses can be high.

Differential Diagnoses
- Full differentials are described in Section B.
- Common differentials for bacterial septicemia include vibriosis, *Aeromonas*, *Citrobacter*, *Pseudomonas*, *Edwardsiella*, *Flavobacterium*, *Yersinia*, *Streptococcus*, and *Francisella* spp.

Diagnosis
- Diagnosis is typically based on clinical signs in susceptible species and culture results.

- Short (1–3 µm), straight, Gram-negative bacilli may be seen on blood smears, effusions, tissue cytology, or histology. They are typically motile on wet mounts.
- Necropsy or coeliotomy and histology typically show petechiae, congestion, yellow to serosanguinous coelomic effusion, tissue pallor, splenomegaly, with intralesional Gram-negative bacilli. Enteritis with intestines filled with thick, yellow fluid may be seen.
- Culture of affected tissues (particularly kidney in acutely sick fish and intestines in carriers) is often successful. Routine or selective media (e.g. Shotts–Waltman) can be used, often at ~20–28°C (68–82°F).
- PCR, RFLP, ELISA, FA, and LAMP tests may be available on blood, fecal, or tissue samples.

Husbandry Management

- General husbandry management is described under C4: *Bacterial Diseases (General).*

Medical Management

- Common treatments (see Chapters A12 and A13 for more details):
  - Antibiotic therapy is usually indicated and should be based on culture and sensitivity results.
  - Commonly used antibiotics include oxytetracycline, potentiated sulfonamides, and florfenicol.
- All legislation regarding medication use and disposal must be followed.

Prevention

- General preventative measures are described under C4: *Bacterial Diseases (General).*
- Restrict access by birds and mammals as they may act as vectors.
- A USDA-approved, monovalent vaccine is available for biotype 1 in rainbow trout and usually provides good protection, but outbreaks of biotype 2 remain possible. Bivalent vaccines are less commonly available.
- Autogenous vaccines may be considered.

Zoonotic Reports

- This disease has no known zoonotic potential.
- The *Yersinia* sp. that causes plague (*Y. pestis*) has not been identified in fish.
- The *Yersinia* spp. that cause yersiniosis in humans (*Y. enterocolitica* and *Y. pseudotuberculosis*) have not been identified in fish.

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Abbreviations/Acronyms

- ELISA: Enzyme linked immunosorbent assay
- FA: Fluorescent antibody
- LAMP: Loop-mediated isothermal amplification
- PCR: Polymerase chain reaction
- RFLP: Restriction fragment length polymorphism
- USDA: United States Department of Agriculture

Streptococcosis

Overview

- *Streptococcus* spp. are emerging pathogens of bony fish, often causing meningoencephalitis, panophthalmitis, and septicemia.
- They are one of the few Gram-positive bacterial infections of fish.
- They are a common cause of epizootics in wild and cultured fish.

Etiology

- Family *Streptococcaceae*.
- Two streptococcal species are most commonly reported to cause disease in fish:
  - *S. agalactiae* (also known as group B *Streptococcus*, GBS, and very similar to *Streptococcus difficilis*); there are several serotypes and biotypes.
  - *S. iniae.*
- Several other streptococcal species have been reported to cause disease in fish and may be increasing in importance.
  - *S. dysgalactiae.*

- *S. ictaluri.*
- *S. milleri.*
- *S. parauberis.*

- These are aerobic Gram-positive cocci.

Transmission

- Transmission is typically horizontal through ingestion and contact.
- The bacteria survive well in organics and suspended solids.
- Feed can remain a source of infection after >6 mo of freezing.

Geographic Distribution

- Predominantly in saltwater and brackish water habitats, probably worldwide.

Signalment

- Disease is reported from marine, brackish, and freshwater fish, particularly teleosts.
- The majority of reports are from cultured fish including tilapia (*Oreochromis* spp.), striped bass and hybrids (*Morone saxatilis*), gilthead seabream (*Sparus auratus*), golden shiners (*Notemigonus crysoleucas*), coho salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*), sea trout (*Cynoscion nebulosus*), mullet (*Mugilidae*), sturgeon (*Acipenseridae*), yellowtail (*Seriola quinqueradiata*), pinfish (*Lagodon rhomboides*), rabbitfish (*Siganidae*), bluefish (*Pomatomus saltatrix*), zebrafish (*Danio rerio*), olive flounder (*Paralichthys olivaceus*), barramundi (*Lates calcarifer*), golden pompano (*Trachinotus ovatus*), and channel catfish (*Ictalurus punctatus*, although some reports suggest this species can be resistant).
- Reports from aquarium fish are less common. They include red-tail black sharks (*Epalzeorhynchos bicolor*), rainbow sharks (*Epalzeorhynchos erythrurus*), rosy barbs (*Pethia conchonius*), tetras (*Characidae*), and cichlids (*Cichlidae*).
- Reports from elasmobranchs are rare. They include stingrays (*Hypanus* spp.), mangrove whiprays (*Himantura granulata*), and shovelnose rays (*Glaucostegus* and *Aptychotrema* spp.).
- Epizootics have been reported in wild teleosts, including Queensland grouper (*Epinephelus lanceolatus*), striped bass (*Morone saxatilis*), and Caribbean reef fish.
- Common carp and koi (*Cyprinus carpio*) and goldfish (*Carassius auratus*) may be resistant.

Risk Factors

- General risk factors are described under C4: *Bacterial Diseases (General)*.

Signs/Clinical Findings

- Individual or multiple fish from one or more species may be affected.
- Clinical course may be acute or chronic.
- Spiraling, circling, or erratic swimming, and loss of buoyancy are common signs as the bacteria tend to localize in the central nervous system. Fish may lose their flight response.
- Reduced appetite or weight loss may be seen.
- Darkening of the skin, petechiae, erythema (particularly on the ventrum and fin bases), skin ulcers, or fin erosions may be seen. There may be abscess-like swellings, particularly along the peduncle.
- Exophthalmos (unilateral or bilateral), periocular and periorbital hemorrhage, corneal hypopyon, or corneal opacity may be seen.
- Coelomic distension due to ascites may be seen.
- Spinal deformities may be seen.
- Poor reproductive success may be seen.
- Acute mortalities are common, usually in a few days. Mortality is usually low (<10%), but may reach 50–70%. Large-scale epizootics have been reported for *S. iniae*.
- Signs can be cyclic.

Differential Diagnoses

- Full differentials are described in Section B.
- Differentials for Gram-positive bacterial cocci in fish include *Streptococcus*, *Vagococcus*, *Enterococcus*, and *Lactococcus* spp.

Diagnosis

- Diagnosis is typically based on a combination of cytology, histology, and culture.
- Gram-positive cocci that form chains may be seen on blood smears, effusions, cerebrospinal fluid, tissue cytology, or histology (Figure C4.3).
- Necropsy or coeliotomy may show petechiae, congestion, serosanguinous coelomic effusion, and organomegaly.
- Histology typically shows granulocytic, pyogranulomatous, or granulomatous inflammation in the central nervous system, vertebrae, and eyes. Meningoencephalitis, panophthalmitis, and septicemia are common. There are usually abundant intralesional Gram-positive cocci that form long chains. *S. ictaluri* in channel catfish is unusual in that it does not show tropism for the central nervous system and causes epidermal necrosis, myositis, and arthritis.
- Culture of affected tissue (particularly brain or kidney) or blood can be done on routine or selective media (e.g. Todd Hewitt or thallium acetate).
- Sequencing is required for definitive identification.

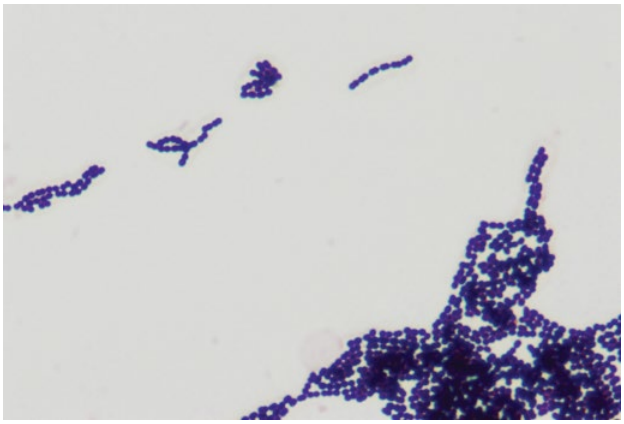

Figure C4.3 Typical appearance of *Streptococcus* spp. on Gram stain showing chains of Gram-positive cocci. *Source*: Image courtesy of Jill Arnold, National Aquarium.

Husbandry Management

- General husbandry management is described under C4: *Bacterial Diseases (General)*.

Medical Management

- Common treatments (see Chapters A12 and A13 for more details):
  - Antibiotic therapy is usually indicated and should be based on culture and sensitivity results.
  - Florfenicol (Aquaflor®) orally is approved in the United States for freshwater-reared warm-water finfish with *S. iniae* at 10–15 mg/kg every 24 hours for 10 days, with a 15-day withdrawal, as of 2020.
  - Other commonly used antibiotics include erythromycin, enrofloxacin, oxytetracycline, ampicillin, and amoxicillin.
- All legislation regarding medication use and disposal must be followed.

Prevention

- General preventative measures are described under C4: *Bacterial Diseases (General)*.
- Experimental vaccines have been challenging because of the rapid emergence of new strains. Autogenous vaccines show the most promise.

Zoonotic Reports

- Many *S. agalactiae* and *S. iniae* strains are zoonotic and can be associated with neonatal septicemia, pneumonia, and meningitis in humans. However, fish strains of these bacteria are different from the typical human isolates.
- Zoonotic transmission from fish has been reported rarely. Cases have been associated with processing infected tilapia, likely via inoculation through a wound. Cellulitis, endocarditis, and arthritis developed. All reported cases resolved.
- The *Streptococcus* spp. that cause strep throat, scarlet fever, impetigo, and pneumococcal disease have not been identified in fish.

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Renibacterium salmoninarum

Overview

- *Renibacterium salmoninarum* is the cause of bacterial kidney disease (BKD) in salmonids.
- BKD is a slowly progressive, systemic disease that has been reported from wild and cultured salmonids.

- Typical pathology consists of granulomatous renomegaly with Gram-positive diplobacilli.
- It is a significant concern for salmonid culture and restoration projects.
- Medical treatment is rarely effective.

Etiology
- Family *Micrococcaceae*.
- The causative agent is *Renibacterium salmoninarum*, an obligate pathogen.
- These are nonmotile, small, Gram-positive diplobacilli that are facultatively intracellular.

Transmission
- Horizontal and vertical transmission are reported.
- The bacteria survive for up to a few weeks in the environment.
- Asymptomatic carriers are common.
- Transmission to and from wild and cultured fish is likely.

Geographic Distribution
- Freshwater and saltwater salmonid habitats, probably worldwide.

Signalment
- BKD infects freshwater and marine salmonids.
- Pacific salmonid species are the most susceptible, such as coho salmon (*Oncorhynchus kisutch*), Chinook salmon (*Oncorhynchus tshawytscha*), sockeye salmon (*Oncorhynchus nerka*), brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), and Arctic char (*Salvelinus alpinus*).
- Disease is most likely in fish <6–12 months of age.

Risk Factors
- General risk factors are described under C4: *Bacterial Diseases (General)*.
- Permissive water temperature is usually low, with signs seen in fall and winter.
- Recent transfer to salt water and spawning are known risk factors.

Signs/Clinical Findings
- BKD is usually a chronic disease that is slowly progressive.
- Multiple salmonids are usually affected.
- Lethargy is common.
- Poor growth rates are common.
- Coelomic distension is common.
- Darkening of the skin, petechiae, erythema, ulcers, or pustules along the lateral line, often known as spawning rash, may be seen.
- Exophthalmos may be seen.
- Mortality rate is typically low but chronic, sporadic mortalities tend to continue.

Differential Diagnoses
- Full differentials are described in Section B.
- Common differentials for bacterial granulomatous inflammation in salmonids include *Renibacterium salmoninarum*, *Flavobacterium psychrophilum*, *Mycobacterium*, *Nocardia*, *Streptococcus*, *Francisella*, *Photobacterium* spp., and piscirickettsial-like organisms.
- Differentials for Gram-positive bacterial rods in fish include *Mycobacterium* and *Lactobacillus* spp.

Diagnosis
- Diagnosis is typically based on gross necropsy in susceptible species and molecular diagnostics.
- Small (1–2 µm), Gram-positive diplobacilli may be seen on blood smears, effusions, tissue cytology, or histology (Figure C4.4). They are nonmotile on wet mounts. The bacteria stain positively with periodic acid–Schiff.
- Necropsy findings usually include renomegaly with granulomas (Figure C4.4). Granulomas may also be present in the spleen, liver, and heart. Serosanguinous ascites and pseudodiphtheritic serosal membranes may be present. Cavernous lesions in the skeletal muscle adjacent to the kidney may be seen.
- Culture from tissues (particularly kidney) is possible, but the organisms are fastidious, requiring kidney disease media with cysteine and growth is slow, taking several weeks. Media may need antibiotics and antifungals to prevent overgrowth.
- Nested PCR has a high sensitivity and is a common test outside of hatcheries.
- Standard ELISAs are used commonly to screen broodstock in hatcheries, although quantitative ELISAs have a higher sensitivity and specificity. Samples are usually serum, ovarian fluid, or kidney supernatant.
- FA tests can show organisms on kidney slides.
- LAMP may provide a faster, simpler, cheap test with a similar sensitivity to nested PCR.
- The disease may be reportable to national, regional, or local agencies (as of 2020, it is not notifiable to the OIE).

Husbandry Management
- Control measures may be dictated by national, regional, or local agencies to prevent spread.
- Consider culling of fish with clinical signs to reduce further transmission, as medical management often shows limited success.

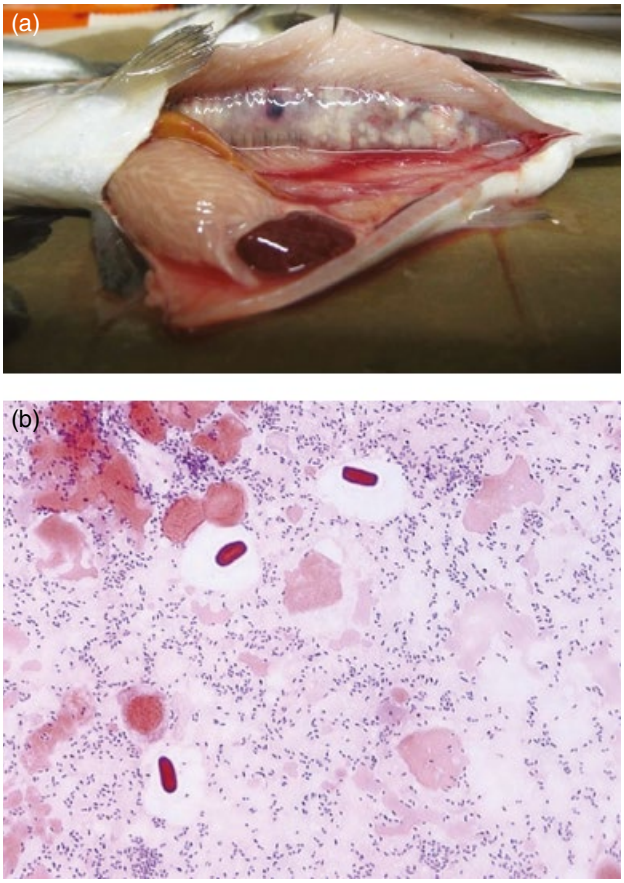

Figure C4.4 Severe granulomatous renomegaly due to bacterial kidney disease (a) and Gram stain of a kidney impression smear showing abundant Gram-positive bacilli at x1000 (b). *Source*: Images courtesy of Aimee Reed, Oregon Department of Fish and Wildlife, Fish Health Services.

Medical Management

- Common treatments (see Chapters A12 and A13 for more details):
  - Antibiotic therapy may reduce morbidity and mortality if early in the disease process, but signs often recur after treatment. Choice should be based on culture and sensitivity results.
  - Macrolide antibiotics are commonly used, particularly erythromycin.
- All legislation regarding medication use and disposal must be followed.

Prevention

- General preventative measures are described under C4: *Bacterial Diseases (General)*.
- Good biosecurity has been shown to reduce prevalence in aquaculture, including isolation from other farms, all-in-all-out management, and separation of generations.
- Regular examination of broodstock is helpful, so that fish with clinical signs can be excluded from breeding.
- ELISAs on ovarian fluid in broodstock prior to breeding allows affected fish to be excluded.
- Erythromycin injection of females pre-spawning can reduce vertical transmission.
- Erythromycin immersion treatment of eggs can reduce transmission.
- Experimental live vaccines have shown some promise.

Zoonotic Reports

- This disease has no known zoonotic potential.

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Abbreviations/Acronyms

- BKD: Bacterial kidney disease
- ELISA: Enzyme linked immunosorbent assay
- FA: Fluorescent antibody
- LAMP: Loop-mediated isothermal amplification
- OIE: World Organisation for Animal Health
- PCR: Polymerase chain reaction

Mycobacteriosis

Overview

- Mycobacteriosis is a common chronic disease of teleosts.
- It is caused by non-tuberculous mycobacteria (NTM), particularly *Mycobacterium fortuitum*, *Mycobacterium marinum*, and *Mycobacterium chelonae*. These are ubiquitous bacteria, common in water and biofilms.
- Almost all fish are susceptible, particularly *Cichlidae*, *Syngnathidae*, and *Cyprinidae*.
- Lesions are usually granulomatous. The acid-fast bacteria are readily identified on cytology and histology.
- Identification to species level is not essential, but can be helpful if control options, antigenic variation, or zoonotic potential need to be assessed.
- Medical treatment is rarely effective.

Etiology

- Family *Mycobacteriaceae*.
- These are NTM, also known as atypical mycobacteria. This is an important differentiation from the tuberculous mycobacteria such as *Mycobacterium tuberculosis* and *Mycobacterium bovis*.
- Species classification changes rapidly, and new genera are being proposed. The three predominant *Mycobacterium* spp. remain:
  - *M. fortuitum*, typically in freshwater.
  - *M. marinum*, typically in salt water but also in freshwater; this may include isolates previously reported as *M. piscium*.
  - *M. chelonae*, particularly in zebrafish (*Danio rerio*).
- Less commonly reported mycobacterial species:
  - *M. abscessus* (previously *M. chelonae* subsp. *abscessus*).
  - *M. avium*; identified on rare occasions in fish.
  - *M. gordonae* (previously *M. aquae*).
  - *M. haemophilum*, particularly in zebrafish.
  - *M. hippocampi* novel sp., in seahorses (*Hippocampus* spp.).
  - *M. montefiorense*, particularly in moray eels (*Gymnothorax* spp.) and rockfish (*Sebastes* spp.).
  - *M. neoaurum*.
  - *M. peregrinum*.
  - *M. poriferae*.
  - *M. pseudoshottsii*, particularly in striped bass (*Morone saxatilis*).
  - *M. salmoniphilum*, particularly in marine salmonids (*Salmonidae*).
  - *M. shottsii*, particularly in striped bass.
  - *M. simiae*.
  - *M. triplex*.
- These are nonmotile, acid-fast positive, non-spore forming, pleomorphic and often beaded bacilli. They may take up Gram stain.
- They are aerobic, facultatively intracellular, and slow-growing.

Transmission

- Transmission is typically horizontal, often through ingestion (e.g. from live prey such as rotifers, unpasteurized dead prey, scavenging, picking at the biofilm). Other routes include inoculation and immersion.
- Vertical transmission can occur but does not seem to be common.
- NTM are frequently found in water, sediment, and biofilms.
- NTM are common in amphibians and reptiles and these may act as carriers or vectors.
- NTM are occasionally reported from invertebrates.
- Fomites may carry NTM, including sterilized medical equipment.

Geographic Distribution

- Ubiquitous in freshwater and saltwater habitats, probably worldwide.

Signalment

- Mycobacteriosis is reported from a wide range of fish (>180 spp.).
- It is common in teleosts, with some species and groups appearing to be particularly susceptible.
  - Bettas and gouramis (*Anabantidae*).
  - Sunfish (*Centrarchidae*).
  - Tetras (*Characidae*).
  - Cichlids (*Cichlidae*).
  - Zebrafish (*Danio rerio*), goldfish (*Carassius auratus*), and other cyprinids (*Cyprinidae*).
  - Cod (*Gadus morhua*).
  - Striped bass (*Morone saxatilis*).
  - Flatfish (*Pleuronectiformes*).
  - Yellowtail (*Seriola quinqueradiata*).
  - Rabbitfish (*Siganus rivulatus*).
  - Seahorses, pipefish, and seadragons (*Syngnathidae*).
- Some teleosts seem relatively resistant to clinical disease, such as catfish (*Siluriformes*).
- Mycobacteriosis is rare in elasmobranchs. It has been limited to individual animals under long-term human care, including an Atlantic guitarfish (*Rhinobatos lentiginosus*), an epaulette shark (*Hemiscyllium ocellatum*), and a yellow stingray (*Urobatis jamaicensis*).
- Disease is more common in adult and geriatric fish.

Risk Factors

- General risk factors are described under C4: *Bacterial Diseases (General)*.
- Permissive water temperature is often high (e.g. 28–30°C (82–86°F) for *M. marinum*).
- Low pH, high humic and fulvic acids, and contaminants (e.g. zinc, iron) are known risk factors.

Signs/Clinical Findings

- Fish are often asymptomatic.
- Where disease is seen, it is usually chronic (weeks, months, or years following infection) and progressive, although an acute presentation is possible.
- Inappetence, reduced appetite, weight loss, or poor growth rates are common.
- Lethargy, abnormal swimming, or abnormal buoyancy may be seen.
- Pigment changes (hyper- and hypopigmentation), petechiae, skin ulcers, scale loss, cutaneous nodules, or firm or soft cutaneous masses with possible exudate may be seen (Figure C4.5a).
- Coelomic distension may be seen.
- Exophthalmos, or less commonly enophthalmos, may be seen.
- Spinal deformities may be seen.
- Chronically low fecundity or poor breeding success may be seen.
- Mortalities are typically sporadic, but epizootics are possible.

Differential Diagnoses

- Full differentials are described in Section B.
- Common differentials from granulomatous dermatitis include *Mycobacterium* spp., *Nocardia* spp., *Renibacterium salmoninarum*, *Exophiala* spp., and *Mesomycetozoea*.
- Common differentials for granulomatous inflammation associated with acid-fast positive structures include *Mycobacterium* spp., *Nocardia* spp., myxozoans, and *Cryptosporidium* spp., although stain artifacts should also be considered.

Diagnosis

- Presumptive diagnosis is usually based on granulomas with acid-fast beaded bacilli on cytology or histology. Definitive diagnosis requires culture and PCR.
- Variable length (1–10 µm), beaded bacilli may be seen on blood smears, effusions, scrapes from lesions, tissue cytology, or histology, but staining depends on species and growth phase. They are Gram-positive but do not stain reliably. On Romanowsky (e.g. Diff-Quik) and Gram stains, they often take up no stain ("ghosting"). Acid-fast stains (e.g. Ziehl–Neelsen, Kinyoun, Fite) show pink to red beaded rods. Sensitivity of cytology is often higher than culture.
- Necropsy or coeliotomy and histology typically show organomegaly, granulomas, congestion, and necrosis (particularly the liver, spleen, kidney, and gonads) with intralesional acid-fast bacilli (Figure C4.5b–d). If bacilli are only found within the gastrointestinal tract, they may be transient. In rare cases, granulomas are not seen and a more acute inflammatory response is noted. 10% formalin, Bouin's, and Dietrich's fixatives do not affect cytology outcome, but Cal-Ex decalcifier may cause false negatives.
- Diagnostic imaging may show organomegaly particularly splenomegaly, hepatomegaly, renomegaly, or large gonads. Mineralization is rare.
- Culture is possible using some routine media, but selective media is often preferred (e.g. Lowenstein–Jensen, Middlebrook 7H10, Mitchison 7H11). Homogenization of tissues facilitates recovery. The growth rate and ease of culture vary significantly by species. Some (e.g. *M. chelonae*, *M. fortuitum*, *M. abscessus*) have a short generation time, while others require months to grow in culture (e.g. *M. marinum*, *M. haemophilum*, *M. pseudoshottsii*).
- PCR or nested PCR is recommended for slow-growing species and should be followed by sequencing for species identification.
- Mycolic acid or fatty acid profiles can be used for species identification.
- RFLP shows inconsistent results. PCR-RFLP (also known as terminal-RFLP) can provide more reliable identification.

Husbandry Management

- The best way to reduce morbidity and mortality is to minimize exposure and stressors.
- Minimize environmental stressors.
- Consider reducing the water temperature to reduce morbidity and mortality from *M. marinum*.
- Consider culling of fish with clinical signs to reduce further transmission.
- With virulent strains or to protect valuable collections, isolate affected systems. Consider depopulation and disinfection. If done, all substrate and filter media must be disposed of and the biofilm must be removed. Biosecurity must be strict to prevent reintroduction of virulent strains. Reintroduction of some strains via fish or water will happen.
- Increase cleaning and disinfection, with thorough mechanical cleaning to remove any organic material. In vitro testing has shown some effect from:
  - Heat (e.g. reports of >60–121°C or 140–250°F).
  - Sunlight.
  - Chlorine at >500 mg/L for 30 minutes.

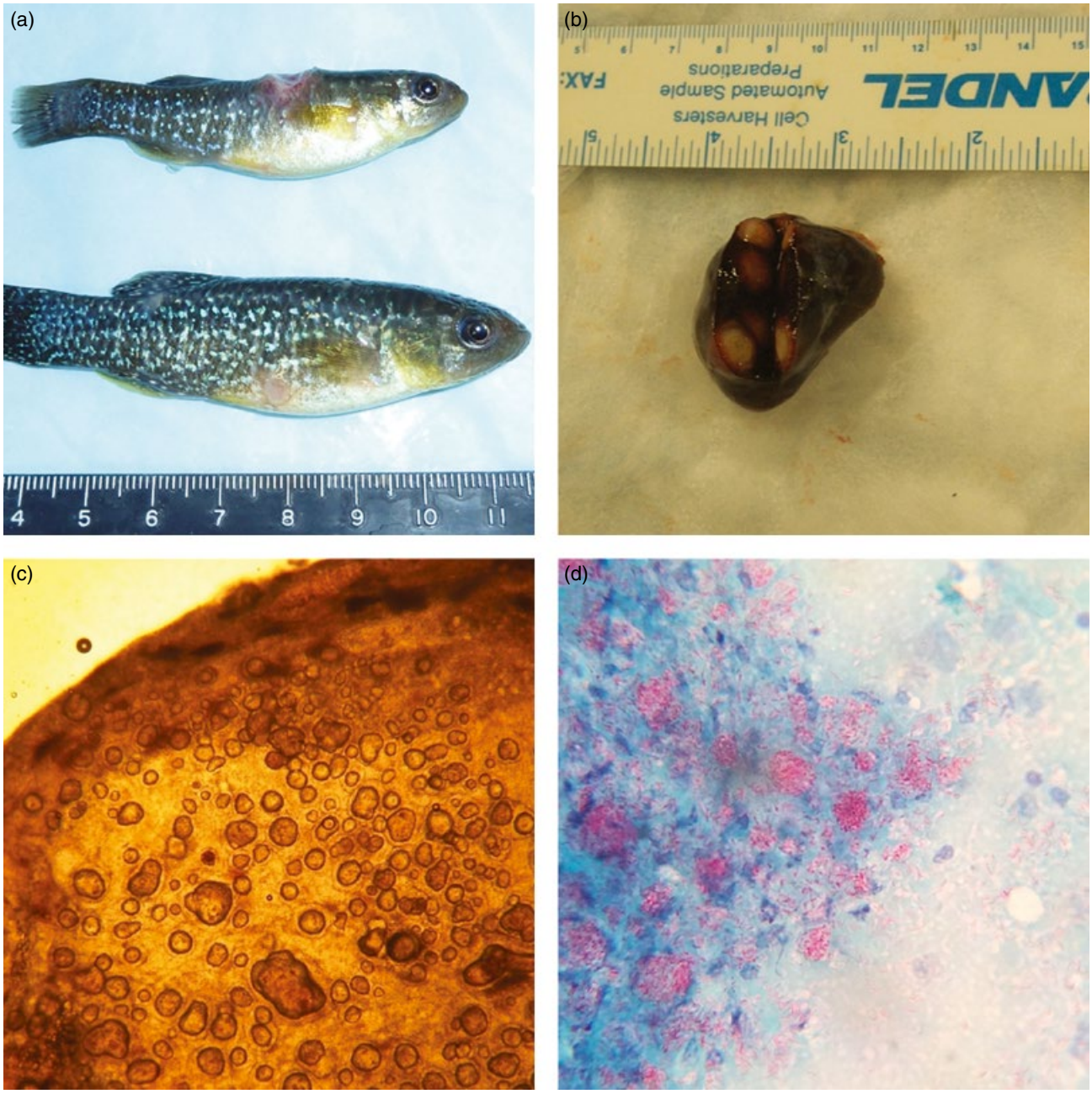

Figure C4.5 Mycobacterial lesions: cutaneous ulceration in mummichogs (*Fundulus* sp.) (a), granulomas visible grossly on a spleen (b), granulomas on a liver wet mount (c), and acid-fast positive beaded bacilli on a tissue impression smear at x400 (d).

- Phenols (e.g. benzyl-4-chlorophenol/phenylphenol (Lysol) 1% for one minute).
- Ethyl alcohol 70% for one minute.
- UV disinfection with high applied doses (e.g. >90 mJ/cm^2).

- No effective disinfection has been seen from ammonium chloride, peroxymonosulfate, or freezing.

Medical Management

- Reported treatments (see Chapters A12 and A13 for more details):
  - Medical treatment is rarely recommended due to the poor response and risk of resistance.
  - If antibiotic therapy is pursued (e.g. for valuable genetic stock), it should be based on culture and sensitivity results.

- Reported antibiotics include azithromycin, kanamycin, tobramycin, and amikacin.
- Medications routinely used for human treatment of mycobacterial diseases should be avoided, including isoniazid, rifampin, and clarithromycin.
- Multidrug therapy is more likely to be helpful than single-drug therapy.

- All legislation regarding medication use and disposal must be followed.

Prevention

- General preventative measures are described under C4: *Bacterial Diseases (General)*.
- Routine monitoring should include acid-fast staining at necropsy and histology.
- Pasteurization of fish feeds can reduce transmission.
- Elderly fish should not be used as broodstock.
- Sentinel programs are used in zebrafish colonies to monitor for virulent strains.
- Routine disinfection of eggs and larvae is used in zebrafish colonies.
- No commercial vaccines are available. Experimental live attenuated vaccines (including BCG) and DNA vaccines given by ICe or IM injection can provide some protection. Killed vaccines have shown no effect.

Zoonotic Reports

- About one-third of NTM are zoonotic.
- The fish NTM that is most commonly zoonotic is probably *M. marinum*. It is usually transmitted by inoculation into wounds. The typical presentation is a nonhealing skin lesion on an extremity. The lesion may include papules, nodules, plaques, or exudate. Lesions may progress slowly over months.
- Systemic infections are possible with NTM species that can tolerate higher temperatures (e.g. *M. fortuitum*, *M. gordonae*), usually in immune-suppressed individuals. These cases may be fatal.
- Gloves should be worn when working with high-risk fish or systems. It is essential to avoid contamination of wounds.
- Immune-suppressed individuals should not work with high-risk fish or systems.
- There are extremely rare reports of fish infection with *M. avium* complex and *M. ulcerans*. Human infections with these bacteria are possible, but are much more likely from other sources.
- *Mycobacterium* spp. within the tuberculous complex (e.g. *M. tuberculosis* and *M. leprae*) have not been identified in fish.

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Abbreviations/Acronyms
- BCG: Bacillus Calmette and Guérin
- ICe: Intracoelomic
- IM: Intramuscular
- NTM: Non-tuberculous mycobacteria
- PCR: Polymerase chain reaction
- RFLP: Restriction fragment length polymorphism
- UV: Ultraviolet

Nocardiosis

Overview
- Nocardiosis is a poorly understood disease in fish.
- It is similar to mycobacteriosis, with a chronic presentation, systemic granulomatous inflammation, and acid-fast positive rods. However, *Nocardia* spp. are long, branching rods that look like fungal hyphae.
- Medical treatment is rarely effective.

Etiology
- Order *Actinomycetales*.
- Family *Nocardiaceace*.
- Several *Nocardia* species cause disease in fish:
  - *N. seriolae* (previously *N. kampachi*) in marine fish.
  - *N. asteroides* in freshwater fish.
  - *N. salmonicida* (previously *Streptomyces salmonicida*).
  - *N. crassostreae* in fish and oysters.
- These are nonmotile, Gram-positive and acid-fast positive, slender, filamentous, branching bacterial rods that are facultatively intracellular.

Transmission
- Transmission is typically horizontal through contact, although ingestion has been reported.
- These are often found in invertebrates, which may act as carriers or vectors. Fish pathogens can cause severe disease in some species such as oysters.

Geographic Distribution
- Ubiquitous in freshwater and saltwater habitats, probably worldwide.

Signalment
- Disease is likely possible in many freshwater and saltwater fish.
- Susceptible aquarium fish include neon tetras (*Paracheirodon innesi*) and seahorses (*Hippocampus* spp.).
- Susceptible cultured fish include salmonids (*Oncorhynchus*, *Salmo*, *Salvelinus* spp.), largemouth bass (*Micropterus salmoides*), Japanese seabass (*Lateolabrax japonicus*), three-striped tiger fish (*Terapon jarbua*), yellow croaker (*Larimichthys crocea*), yellowtail (*Seriola quinqueradiata*), and greater amberjacks (*Seriola dumerili*).

Risk Factors
- General risk factors are described under C4: *Bacterial Diseases (General)*.

Signs/Clinical Findings
- Individual fish are usually affected at one time.
- Lethargy may be seen.
- Inappetence, reduced feeding, or weight loss may be seen.
- Later signs include skin ulcers, nodules, or boils (furuncles).
- Coelomic distension may be seen.
- Mortalities are seen intermittently, but because of the chronic nature, cumulative mortality may reach 30%.

Differential Diagnoses
- Full differentials are described in Section B.
- Common differentials for granulomatous inflammation with hyphae or rod-like structures include mycobacteriosis and systemic fungal disease, although stain artifacts should be considered.

Diagnosis
- Presumptive diagnosis is typically based on cytology and histology, although additional testing is required for species identification.
- Long, thin branching rods that resemble fungal hyphae may be seen on blood smears, scrapes from lesions, or tissue cytology or histology (Figure C4.6). The rods are Gram-positive, but do not take up stain well. The rods are acid-fast positive, with better stain uptake using Fite's stains than Ziehl–Neelsen stains.
- Necropsy or coeliotomy and histology typically show multiple white to yellow nodules due to granulomatous inflammation within the viscera, especially the gills, heart, spleen, liver, kidney, and swim bladder.
- Culture from affected tissues can be successful. *Nocardia* spp. will grow on blood agar, as well as mycobacterial and fungal media (e.g. Sabouraud's).

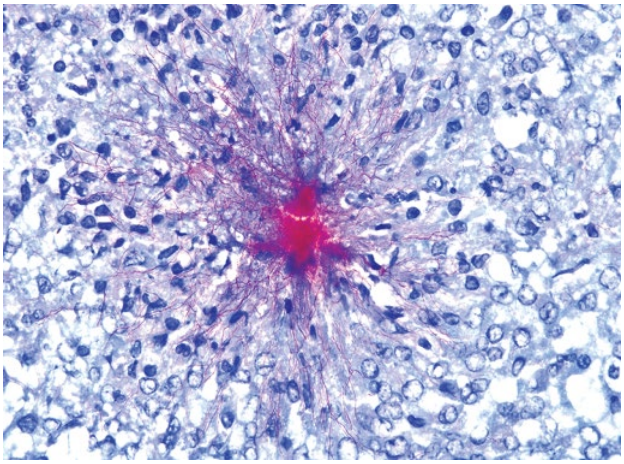

Figure C4.6 Long, branching, acid-fast rods consistent with *Nocardia* sp. on histology of the skeletal muscle of an Atlantic salmon (*Salmo salar*); acid-fast x1000. *Source*: Image courtesy of David Groman, University of Prince Edward Island.

- PCR testing may be available.
- Serological tests are not routinely available.

Husbandry Management

- General husbandry management is described under C4: *Bacterial Diseases (General)*.
- *Nocardia* spp. are relatively resistant to disinfection; benzalkonium chloride is considered most effective.

Medical Management

- Common treatments (see Chapters A12 and A13 for more details):
  - Medical treatment is rarely recommended due to the poor response and risk of resistance.
  - If antibiotic therapy is pursued (e.g. for valuable genetic stock), it should be based on culture and sensitivity results.
  - In vitro, trimethoprim sulfamethoxazole is often effective.
  - Long-term therapy is recommended, ideally for several months.
- All legislation regarding medication use and disposal must be followed.

Prevention

- General preventative measures are described under C4: *Bacterial Diseases (General)*.

Zoonotic Reports

- *N. asteroides* is zoonotic and systemic disease has been reported in humans with immune suppression.
- In humans, nocardiosis is typically transmitted by aerosolization of bacteria in soil, standing water, or decaying plants. It usually presents as a slowly progressive pneumonia with chronic cough. In some cases, endocarditis or encephalitis can develop.
- Inoculation into a wound or surgical site can result in cellulitis or abscessation.
- The CDC estimates that there are 500–1000 new cases in the United States each year.
- The author can find no reports of disease transmission from fish.

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Abbreviations/Acronyms

- CDC: Centers for Disease Control and Prevention
- PCR: Polymerase chain reaction

Epitheliocystis

Overview

- Epitheliocystis is an emerging bacterial disease that often infects fish gills.
- The Gram-negative bacilli have been hard to classify. They are considered chlamydia-like, but they may be *Proteobacteria*.
- Pathology is usually identified on histology; lesions are characterized by large hypertrophied cells that look like spherical cysts.
- Morbidity and mortality are rare but have been reported in cultured freshwater and marine fish.

Etiology
- The taxonomy is complicated.
- They were historically grouped within the phylum *Chlamydiae* and reported as chlamydia-like organisms (CLOs).
- Most of the species found in fish are described under the term *Candidatus*, e.g. *Candidatus* Piscichlamydia salmonis, *Candidatus* Clavichlamydia salmonicola, *Candidatus* Renichlamydia lutjani.
- More recent analyses suggest these may instead belong within the phylum *Proteobacteria*. Isolates from elasmobranchs appear to cluster within *Gammaproteobacteria*.
- These are obligate intracellular, Gram-negative bacteria.

Transmission
- Fish CLOs appear to show the typical developmental cycle of *Chlamydiales* with three forms: elemental, reticular, and intermediate bodies.
- Horizontal transmission following rupture of the infected cells seems likely.
- Amoeba and other protists can harbor environmental chlamydiae and it is suspected that fish CLOs may use amoeba as intermediate hosts.

Geographic Distribution
- Their distribution has not been determined.

Signalment
- CLOs have been reported from >90 species of freshwater and marine teleosts, including several cultured species such as Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), Arctic charr (*Salvelinus alpinus*), lake trout (*Salvelinus namaycush*), white sturgeon (*Acipenser transmontanus*), barramundi (*Lates calcarifer*), European bass (*Dicentrarchus labrax*), largemouth bass (*Micropterus salmoides*), yellowtail (*Seriola quinqueradiata*), greater amberjack (*Seriola dumerili*), gilthead seabream (*Sparus aurata*), carp (*Cyprinus carpio*), and pacu (*Piaractus mesopotamicus*).
- CLOs have been reported from aquarium species including leafy sea dragons (*Phycodurus eques*).
- In Atlantic salmon, a CLO is considered a possible etiological agent of proliferative gill inflammation, seen around 3–5 mo after transfer to salt water.
- There have been increasing reports in elasmobranchs including spiny dogfish (*Squalus acanthias*), smooth dogfish (*Mustelus canis*), leopard sharks (*Triakis semifasciata*), spotted eagle rays (*Aetobatus narinari*), and bonnethead sharks (*Sphyrna tiburo*).

Risk Factors
- General risk factors are described under C4: *Bacterial Diseases (General)*.

Signs/Clinical Findings
- Fish are often asymptomatic.
- Individual fish are usually affected.
- Small, white, spherical nodules up to 0.5 mm diameter may be seen on the gills, oral cavity, and skin, often with no other signs.
- Lethargy may be seen.
- Reduced appetite or poor growth rates may be seen.
- Exophthalmos may be seen.
- Abnormal swimming may be seen.
- Skin ulcers may be seen.
- Dyspnea, tachypnea, or bradypnea may be seen.
- Morbidity and mortality are highest in young fish in culture.
- Disease may be cyclic.

Differential Diagnoses
- Full differentials are described in Section B.
- Common differentials for nodules on the gills include herpesviruses, papillomas, granulomatous inflammation, epitheliocystis or other CLOs, *Mesomycetozoea*, microsporidial xenomas (e.g. *Loma* spp.), *Ichthyophthirius multifiliis*, and *Cryptocaryon irritans*.

Diagnosis
- Tentative diagnosis may be based on cytology and histology of the gills or skin (Figure C4.7). Definitive diagnosis requires PCR and sequencing.
- Small bacteria may be seen within cells on cytology or histology; they may be bacilli or cocci and typically stain Gram-negative and Giemsa-positive.
- Histology of epithelial tissue shows large hypertrophied cells that resemble spherical cysts with a single intracytoplasmic, basophilic inclusion or vacuole (10–30 µm) that contains the bacteria. There is usually minimal inflammatory response, but there may be a mild granulocytic or lymphocytic response and local tissue necrosis.
- Culture has been unsuccessful to date. This means that Koch's postulates have not been met and little is known about pathogenesis.
- TEM shows homogeneous bacteria and typical elementary, reticular, and intermediate bodies.
- PCR and sequencing are commonly used.
- ISH and IHC may be available.

Husbandry Management
- General husbandry management is described under C4: *Bacterial Diseases (General)*.

Medical Management
- This may be an incidental finding with no treatment needed.
- Reported treatments (see Chapters A12 and A13 for more details):

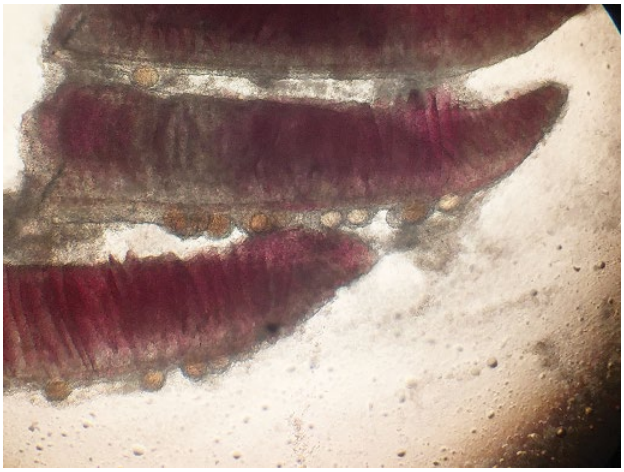

Figure C4.7 Nodules due to epitheliocystis on direct microscopy of a gill biopsy in a yelloweye rockfish (*Sebastes ruberrimus*).

- CLOs are often susceptible to macrolides (e.g. azithromycin) and tetracyclines (e.g. doxycycline, oxytetracycline).
- Oxytetracycline immersion at 25 mg/L every 12 hours for three days in largemouth bass in freshwater raceways resolved clinical signs. This is likely to be less effective in salt water.

- Enrofloxacin has been largely ineffective.
- All legislation regarding medication use and disposal must be followed.

Prevention

- General preventative measures are described under C4: *Bacterial Diseases (General)*.

Zoonotic Reports

- There are no reports of zoonotic transmission from fish, but this is an emerging disease and its true status is not known.
- The *Chlamydiales* that commonly infect humans (*Chlamydia trachomatis*, *Chlamydophila pneumoniae*, and *Chlamydia psittaci*) are phylogenetically distinct from the species in fish.

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Abbreviations/Acronyms

- CLO: Chlamydia-like organism
- IHC: Immunohistochemistry
- ISH: In situ hybridization
- PCR: Polymerase chain reaction
- TEM: Transmission electron microscopy

Francisellosis

Overview

- *Francisella* species are emerging, global pathogens that can cause septicemia and granulomatous inflammation in multiple fish species.
- *Francisella noatunensis* subsp. *noatunensis* and *Francisella noatunensis* subsp. *chilensis* are reported from cold-water marine teleosts and are serious pathogens of cultured Atlantic cod (*Gadus morhua*).
- *Francisella orientalis* is reported from warm-water teleosts and is a serious pathogen of cultured tilapia (*Oreochromis* spp.).

Etiology

- Family *Francisellaceae*.
- These are piscirickettsial-like organisms (PLOs).
- Taxonomy is debated, but the proposed taxonomy is:

- *Francisella noatunensis* subsp. *noatunensis* (previously *F. philomiragia* subsp. *noatunensis* and *F. piscicida*).
- *Francisella noatunensis* subsp. *chilensis* (previously within *F. noatunensis* subsp. *noatunensis*).
- *Francisella orientalis* (previously *F. noatunensis* subsp. *orientalis*, *F. asiatica*, and *F. victoria*).

- These are nonmotile, small, pleomorphic, Gram-negative and acid-fast negative coccobacilli that are facultatively intracellular and strictly aerobic.

Transmission

- Transmission is typically horizontal, predominantly feco-oral; vertical transmission has not been reported.
- Survival in the environment is reported as days to months. Survival rates seem highest in mud and brackish water.
- Invertebrates such as mussels and crabs may act as vectors.

Geographic Distribution

- *F. noatunensis* in saltwater habitats in Europe and Chile.
- *F. orientalis* in freshwater and saltwater habitats including South, Central, and North America, Southeast Asia, and the United Kingdom.

Signalment

- *F. noatunensis*: Marine teleosts in cold water, particularly Atlantic cod and Atlantic salmon (*Salmo salar*).
- *F. orientalis*: Freshwater, brackish, and marine teleosts in warm water. This is a particular problem for tilapia in aquaculture. It is also reported from ornamental cichlids (*Cichlidae*), striped bass and hybrids (*Morone* spp.), chicken grunts (*Parapristipoma trilineatum*), French and Caesar grunts (*Haemulon* spp.), fairy wrasses (*Cirrhilabrus* spp.), and green chromis (*Chromis viridis*).

Risk Factors

- General risk factors are described under C4: *Bacterial Diseases (General)*.
- Mortalities from *F. orientalis* are higher at 20–28°C (68–82°F).

Signs/Clinical Findings

- Individual or multiple fish from one or more species may be affected.
- Clinical course may be acute or chronic.
- Lethargy may be seen.
- Inappetence, reduced feeding, or poor growth rates may be seen.
- Abnormal swimming (e.g. spiraling or circling) may be seen.
- Erythema, petechiae, or raised hemorrhagic nodules are common. Skin ulcers are less common but may be seen. Dark coloration may be seen.
- Exophthalmos may be seen.
- White gill nodules, hyperplasia, or pallor may be seen.
- The acute form may have a mortality of up to 90% with few clinical signs.
- The chronic form may have a mortality of up to 20%.

Differential Diagnoses

- Full differentials are described in Section B.
- Common differentials for bacterial granulomatous lesions in teleosts include *Mycobacterium*, *Nocardia*, *Edwardsiella*, *Francisella*, *Streptococcus*, *Photobacterium* spp., and other piscirickettsial-like organisms.

Diagnosis

- Diagnosis is difficult and usually relies on consistent signs and molecular testing.
- Small (1.5 µm), Gram-negative coccobacilli may be seen. The bacteria are acid-fast negative and Giemsa-positive. They are nonmotile on wet mounts.
- Necropsy and histology usually show severe splenomegaly and renomegaly with white-tan nodules that consist of granulomas and intracellular bacteria. Adjacent muscle and skin are often affected. Granulomas are rare in the brain. Generalized pallor may be seen. More acute systemic signs may be seen.
- Culture is limited as the organisms are fastidious and need additional cysteine and iron to grow. Specialized media include cysteine heart or Thayer–Martin agar. They can also be grown in cell lines. False negatives are still common due to antibiotic therapy and low bacterial loads. Homogenization of tissues in saline or phosphate buffered saline can improve recovery. A recommended protocol for sensitivity testing is provided by Soto et al. (2016).
- PCR, qPCR, IHC, and ISH tests on fresh spleen or kidney are available experimentally. Sequencing is required to confirm identification.

Husbandry Management

- General husbandry management is described under C4: *Bacterial Diseases (General)*.
- For *F. orientalis*: Slowly increase water temperature if possible; mortalities stop rapidly at >28°C (>82°F).

Medical Management

- Common treatments (see Chapters A12 and A13 for more details):
  - Antibiotic therapy is usually indicated and should be based on culture and sensitivity results, but results are variable.
  - In vitro antibiotic sensitivity has been high for florfenicol and oxolinic acid, variable for oxytetracycline and enrofloxacin, and low for potentiated sulfonamides and erythromycin.
  - Florfenicol orally at 15–20 mg/kg every 24 hours for 10 days reduced mortalities from *F. orientalis* in Nile

tilapia at 25°C (77°F); 10 mg/kg every 24 hours was less effective.
- All legislation regarding medication use and disposal must be followed.

Prevention
- General preventative measures are described under C4: *Bacterial Diseases (General)*.

Zoonotic Reports
- These bacteria have no known zoonotic potential, but other *Francisella* spp. do.
- The *Francisella* sp. that causes tularemia (*F. tularensis*) is genetically similar, but has never been identified in fish.

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Abbreviations/Acronyms
- IHC: Immunohistochemistry
- ISH: In situ hybridization
- PCR: Polymerase chain reaction
- qPCR: Quantitative polymerase chain reaction

Piscirickettsiosis

Overview
- *Piscirickettsia salmonis* causes rickettsial septicemia, also known as piscirickettsiosis and salmon rickettsia syndrome.
- Pacific salmonids in seawater are the most susceptible.
- It is a major concern for salmonid culture and restoration projects in some areas.

Etiology
- Family *Piscirickettsiaceae*.
- These are piscirickettsial-like organisms (PLOs).
- The first species identified in fish was *Piscirickettsia salmonis*. The taxonomy for other species is unclear and this section will focus on *P. salmonis*.
- These are nonmotile, Gram-negative and acid-fast negative pleomorphic coccobacilli that are facultatively intracellular.

Transmission
- Transmission is typically horizontal, through contact with fish or water. Vertical transmission seems uncommon.
- Crustaceans may act as vectors, as with other rickettsials.

Geographic Distribution
- Freshwater and saltwater habitats along the west coast of North America, South America, and Europe. Epidemics have been common in Chile.

Signalment
- *P. salmonis* is reported from coho salmon (*Oncorhynchus kisutch*), pink salmon (*Oncorhynchus gorbuscha*), Chinook salmon (*Oncorhynchus tshawytscha*), rainbow

trout (*Oncorhynchus mykiss*), and Atlantic salmon (*Salmo salar*).
- Other PLOs are reported from a variety of freshwater and marine bony fish.

Risk Factors
- General risk factors are described under C4: *Bacterial Diseases (General)*.
- Specific examples for *P. salmonis* include:
  - Transfer to salt water within the previous 6 mo.
  - Season: fall or spring.
  - High prevalence of infected stock within a 7–10 km radius.

Signs/Clinical Findings
- The incubation period is thought to be weeks to months following natural infection.
- Several conspecifics are usually affected.
- Lethargy is common, with fish often seen swimming at the surface or edges of enclosures.
- Inappetence or reduced feeding is common.
- Dark coloration is common.
- Gill pallor due to anemia is common.
- Erythema, petechiae, skin ulcers, and fin erosions are common. Nodules may be seen.
- Coelomic distension may be seen.
- Mortality rate can reach 90% but is typically lower.

Differential Diagnoses
- Full differentials are described in Section B.
- Common differentials for bacterial septicemia include vibriosis, *Aeromonas*, *Citrobacter*, *Pseudomonas*, *Edwardsiella*, *Flavobacterium*, *Yersinia*, *Streptococcus*, and *Francisella* spp.

Diagnosis
- Diagnosis is typically made based on a combination of clinical signs, cytology, necropsy, histology, and serologic or molecular testing.
- Gram-negative and acid-fast negative pleomorphic coccobacilli that are often found in pairs or ring-shaped structures up to 1.5 µm in diameter may be seen in cells on blood smears, effusions, tissue cytology, or histology. The bacteria stain positively on Giemsa, methylene blue, and acridine orange staining. They are nonmotile on wet mounts.
- Necropsy or coeliotomy may show serosanguinous coelomic effusion, tissue pallor, hepatomegaly, splenomegaly, renomegaly, along with pathognomonic gray to yellow ring-shaped foci on the liver. There may be nodules in the viscera.
- Histology of the liver, spleen, kidney, and intestines often show the intracellular bacteria within macrophages. The bacteria may be associated with necrosis and granulomatous inflammation.
- Culture is limited as the organisms are fastidious and need additional cysteine or specific eukaryotic cell lines at low temperatures, typically ~15–18°C (59–64°F). Highest culture rates are from liver, spleen, or kidney tissue during acute infection. The growth rate is slow.
- FA, ELISA, IHC, or PCR tests may be available.

Husbandry Management
- General husbandry management is described under C4: *Bacterial Diseases (General)*.

Medical Management
- Common treatments (see Chapters A12 and A13 for more details):
  - Clinical response to medical treatment is often limited, likely due to the intracellular nature of the bacteria, antibiotic resistance, and reduced feeding responses. Treatment success is highest if started early in the disease process.
  - In vitro sensitivity is typically highest for aminoglycosides, tetracyclines, macrolides, and quinolones.
- All legislation regarding medication use and disposal must be followed.

Prevention
- General preventative measures are described under C4: *Bacterial Diseases (General)*.
- A variety of commercial vaccines are in use, but efficacy is debated.
- Early harvesting of cultured salmonids, less than 6 mo after transfer to salt water or prior to high water temperatures in summer, may reduce the risk.

Zoonotic Reports
- These bacteria have no known zoonotic potential.

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Abbreviations/Acronyms

- ELISA: Enzyme linked immunosorbent assay
- FA: Fluorescent antibody
- IHC: Immunohistochemistry
- PCR: Polymerase chain reaction
- PLO: Piscirickettsial-like organism