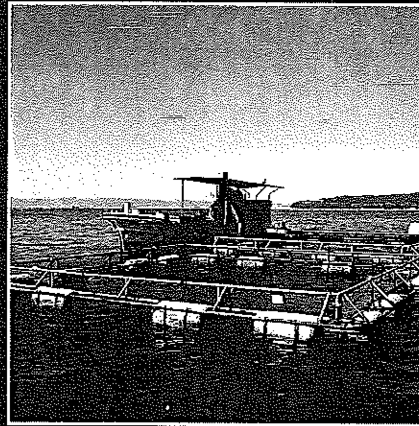
ENCYCLOPEDIA OF AQUACULTURE



EDITED BY

ROBERT R. STICKNEY

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BACTERIAL DISEASE AGENTS

JOHN P. HAWKE
Louisiana State University
Baton Rouge, Louisiana

OUTLINE

Gram-Negative Agents
Gram-Positive Agents
Bibliography

Bacterial diseases have historically been among the most significant problems facing aquaculturists worldwide. The oldest record of bacterial disease in aquaculture names "redpest" in eels in the Mediterranean as early as 1718 (1), and the causative agent *Vibrio anguillarum* was first cultured in 1883 (2). It has been estimated that 10% of fish loss in aquaculture is due to disease and that, of this portion, more than half is due to bacterial disease (3,4). Economic losses resulting from bacterial disease are difficult to assess, because many cases go unreported; however, a single bacterial disease, Enteric Septicemia of Catfish (ESC), is believed to cost the U.S. catfish industry US$19 million in direct fish losses annually (5,6). Bacterial pathogens are, in some cases, highly host-specific, infecting and causing disease in only one genus or even one species of fish. Others have a broad host range. Some are obligate pathogens, not normal free-living components of the aquatic ecosystem but ones requiring a fish host to survive. Others are facultative pathogens that are normal inhabitants of the aquatic environment and require stress to assist in initiating disease in a fish population. Despite our current knowledge of approximately 70 species of bacterial agents having the capability of causing disease in aquaculture (7), new diseases caused by previously undocumented bacterial fish pathogens continue to surface almost yearly. The aquaculture industry has experienced tremendous growth in recent years, and, as culture practices have become more intensive in order to increase profitability and as new species of fish are cultured, new, highly virulent bacterial pathogens have emerged. Currently, bacterial taxonomy is in transition, due to the utilization of more precise molecular methods of classification, and the names of many previously recognized pathogens have changed. This entry addresses only those bacteria that are established as pathogens in aquaculture. The most currently approved names, as well as the older names with which readers may be more familiar, are included. Older names or taxonomically invalid names are highlighted by quotation marks, (i.e., *"Haemophilus piscium"*). Despite the importance of bacterial disease in aquaculture, only one book is available that covers management techniques (7), and only two books are available that deal specifically with description of bacterial pathogens and of the diseases that they cause (8,9). Review articles that deal with various mechanisms of bacterial pathogenesis, have been written and several books are available that contain chapters devoted to methods of diagnosis of bacterial diseases and of identification of bacterial pathogens (10–13).

GRAM-NEGATIVE AGENTS

A. ***Enterobacteriaceae.*** Facultatively anaerobic, oxidase negative, gram-negative rods.

*1. **Edwardsiella tarda.*** Causative agent of *Edwardsiella* Septicemia. Synonyms: Emphysematous-putrefactive disease of catfish; red disease of eels; edwardsiellosis of salmon, of tilapia, and of striped bass.

a. History—The bacterium was originally described in 1962, from 256 isolates obtained from various sources in Japan; however most of the isolates were from snakes. Five isolates from human gastrointestinal infections were referred to as the "Asakusa group" (14). In the same year, a new disease of eels, "red disease," was described, and the causative agent was named *"Paracolobactrum anguillimortiferum"* by Hoshina (15). A new genus, *Edwardsiella*, including a new species, *E. tarda*, was described from 37 human isolates in the U.S. by Ewing, in 1965 (16). In 1973, *E. tarda* was almost simultaneously recognized as the cause of "red disease" of eels in Japan and Taiwan (17) and of "emphysematous putrefactive disease of catfish" in the U.S. (18). Cultures of *"P. anguillimortiferum"* were no longer available for comparison, so *E. tarda* became the valid scientific name.

b. Culture—Primary isolation of the bacterium from diseased fish is achieved on standard media, such as brain heart infusion agar (BHIA) or tryptic soy agar (TSA) with 5% sheep blood and incubation at 25–37 °C. Growth is rapid, with 1–2 mm colonies present in 24 hours. On EIM-selective medium (19), *E. tarda* forms a small green colony with a black center.

c. Description—Short-gram negative rod, 0.6 × 2.0 μm, motile by peritrichous flagella at 25 and 37 °C, oxidase-negative, indole-positive, fermentative in semisolid glucose motility deeps (GMD), K/A with hydrogen sulfide production and gas in triple sugar iron (TSI) agar slants. The bacterium is differentiated from closely related organisms in Table 1.

d. Epizootiology—*Edwardsiella* septicemia appears to be favored by high water temperatures [28 °C (82 °F) and above] and by the presence of high levels of organic matter in catfish ponds. Infections in eels in Taiwan occur when water temperatures are fluctuating between 10 and 18 °C (50–64 °F). The incidence of *E. tarda* infections is relatively rare in channel catfish (*Ictalurus punctatus*) ponds and, mortality rates are usually low (~5%), but, when infected fish are moved into confined areas such as holding tanks, mortalities can reach levels as high as 50%.

Table 1. Biochemical and Biophysical Characteristics Important in Differentiating Three Fish Pathogens Belonging to the Family *Enterobacteriaceae*

Characteristic	*E. ictaluri*	*E. tarda*	*Y. ruckeri*
Motility at 25 °C	+	+	+
Motility at 35 °C	−	+	−
Indole	−	+	−
Citrate (simmons)	−	−	+
Trehalose	−	−	+
Gelatin (22 °C)	−	−	+
Gas from glucose (25 °C)	+	+	−
H_2S	−	+	−

The reservoir of infection is unclear, but the bacterium has been associated with a variety of aquatic invertebrates and of aquatic and terrestrial vertebrates. Fecal contamination of water from human or animal sources, may be a source of the bacterium. There is also some speculation that *E. tarda* may comprose a part of the normal microflora of the surfaces of certain fishes or snakes. In the U.S., *E. tarda* was isolated from as many as 88% of domestic dressed channel catfish (20) and was found in 30% of imported dressed fish. The bacterium was also found in 75% of catfish pond water samples, in 64% of pond mud samples, and in 100% of frogs, turtles, and crayfish from catfish ponds.

e. Geographic range and host susceptibility—*E. tarda* is found in both freshwater and brackish-water environments. It has been reported from 25 countries in North and Central America, Europe, Asia, Australia, Africa, and the Middle East. The bacterium has been isolated from over 20 species of freshwater and marine fish; it occurs most commonly in the following: the channel catfish, *I. punctatus*; the common carp, *Cyprinus carpio*; the largemouth bass, *Micropterus salmoides*; the striped bass, *Morone saxatilis*; the red sea bream, *Chrysophrys major*; the Japanese flounder, *Paralichthys olivaceus*; tilapia, *Oreochromis* sp.; yellowtail, *Seriola quinqueradiata*; and rarely in salmonids. The bacterium also causes disease in snakes, alligators, and sea lions and in various birds, cattle, and swine (8).

f. Clinical signs of disease and treatment—*Edwardsiella* septicemia in eels is characterized by hemorrhagic fins, by petechiae on the belly, by a swollen protruding anus, and by necrotic foci in the internal organs. Gas filled pockets may form between the skin and muscle. In channel catfish with emphesematous putrefactive disease, 3–5 mm (0.12–0.20 in.) cutaneous ulcers form in the skin. These lesions progress into larger abcesses deep in the tissues and emit a foul odor when ruptured. Eye disease is common in tilapia and striped bass, and infected fish exhibit exophthalmia and corneal opacity. Treatment in catfish is best achieved with medicated feeds: Romet 50 mg/kg/day (23.0 mg/lb/day) for 5 days and Terramycin 23.0–34.0 mg/lb/day for 10–14 days, both of which are approved for use in catfish by the United States Food and Drug Administration (USFDA). Japanese strains of *E. tarda* are resistant to many antibiotics; however, nalidixic acid has been used with success (7). *E. tarda* is serologically heterogeneous, with 49 O antigens and 37 H antigens; therefore, production of effective vaccines has met with difficulty (55).

II. Edwardsiella ictaluri. Causative agent of Enteric Septicemia of Catfish

a. History—The disease was first documented from cases involving diseased channel catfish submitted to the Southeastern Cooperative Fish Disease Laboratory at Auburn University in 1976. The laboratory, under the direction of Drs. W.A. Rogers and J.A. Plumb, recorded 26 cases of this new disease syndrome, from ponds primarily in Alabama and Georgia, between January 1976 and October 1979. The disease was described by Hawke in a published account in 1979 (21), and the causative organism was described as a new species, *E. ictaluri*, in 1981 (22). Records from fish disease diagnostic laboratories indicate that the disease was not prevalent in the industry immediately following its discovery. Enteric septicemia of catfish (ESC) occurred in only 8% of the total cases reported by the Mississippi Cooperative Extension Service in 1980 and 1981. Between 1982 and 1986, however, the increase in ESC incidence was explosive, and the economic impact on the catfish industry was significant (3). Enteric septicemia of catfish is believed to have been present in cultured catfish prior to its description, because archived tissues from the Fish Farming Experiment Station at Stuttgart, Arkansas reacted with a monoclonal antibody specific for *E. ictaluri* (23). Currently, ESC is the most important disease of farm raised catfish, accounting for approximately 30% of all disease cases submitted to fish diagnostic laboratories in the Southeastern United States. In Mississippi, where catfish make up the majority of case submissions, it has been reported at frequencies as high as 47% of the yearly total (3). It is estimated that ESC costs the catfish industry $19 million yearly in direct fish losses (6) and ESC is considered a disease of current or potential international significance by the OIE (65).

b. Culture—Primary isolation from the kidney, liver, spleen, or brain of diseased fish is achieved on standard media such as BHIA or TSA with 5% sheep blood. The bacterium has a narrow temperature range for optimum growth (25–28 °C), and primary isolation plates should be incubated within this range. Growth is slow even at these temperatures, with 1–2 mm colonies present in 48 hours. *E. ictaluri* forms a small pale green colony on EIM-selective medium (19).

c. Description—The bacterium is a short Gram-negative rod, 0.75 × 1.25 μm, motile by peritrichous flagella at 25–30 °C but not at 35 °C, oxidase-negative, fermentative in GMD, indole-negative, K/A with H_2S negative in TSI slant, and citrate-negative. The bacterium is differentiated from closely related fish pathogens in Table 1. The species is antigenically and physiologically homogeneous, regardless of the geographic source of the isolate. Confirmatory identification can be made with serological tests, among them the slide agglutination test, the indirect florescent antibody test (IFAT), the enzyme immunoassay (EIA), and the enzyme linked immunosorbent assay (ELISA). *E. ictaluri* may be identified with the API 20E

system (BioMerieux Vitek Inc.) by generation of the code number 4004000.

d. Epizootiology — Outbreaks of ESC are strongly correlated with pond water temperature, with high stocking densities, and with stress resulting from poor water quality. The so-called "ESC window" occurs in the spring and in the fall, when water temperatures are in the range 22–28 °C (72–82 °F). When initially described, *E. ictaluri* was thought to be an obligate pathogen, because its survival was limited to approximately 8 days in sterile pond water (21); however, it was later found to survive for 95 days in pond mud at 25 °C (77 °F) (24). *E. ictaluri* infections are very common in commercial catfish ponds in the southeastern U.S. but are rarely found as the cause of natural fish kills. Mortality rates in cultured fish may vary from less than 10% to 50% or more of the population. The disease is most common, and causes the highest mortalities, in fingerling channel catfish, but production-size catfish with no previous exposure are also highly susceptible. Survivors of outbreaks are believed to become asymptomatic carriers that can transmit the disease if stocked into a pond containing a population of naive fish.

e. Geographic range and host susceptibility — *E. ictaluri* is known to occur primarily in the southeastern U.S. Isolated instances of disease have been reported from Thailand and Australia, in atypical species. The bacterium is specific for channel catfish, with other species of catfish apparently being less susceptible. Rare outbreaks have been documented in the blue catfish (*Ictalurus furcatus*), in the white catfish (*Ictalurus melas*), and in the brown bullhead (*Ictalurus nebulosus*). Natural infections have been found in the walking catfish (*Clarias batrachus*) in Thailand, and in two aquarium species, the Bengal danio (*Danio devario*) and the glass knife fish (*Eigemannia virescens*). Experimental infection and disease have been induced in chinook salmon (*Oncorhynchus tshawytscha*) and in rainbow trout (*Oncorhynchus mykiss*), but many species of warm and coldwater fish are refractory (7,25).

f. Clinical signs of disease and treatment — ESC occurs in acute, subacute, and chronic forms in channel catfish. The acute form is characterized by only a few clinical signs; clear or straw colored fluid in the body cavity, exophthalmia, petechial hemorrhage around the head and operculum, and enlargement of the kidney and spleen. The subacute form is characterized by more obvious external signs; small 2–3 mm (0.2–0.25 in.) ulcerative lesions (red or white spots) in the skin, hemorrhage and necrotic foci in the liver, hemorrhage in the intestine, and bloody ascites. The chronic form is characterized by an ulcer in the top of the head in the area of the sutura fontanelle between the two frontal bones of the skull (a phenomenon resulting in the name "hole in the head disease"). This lesion develops from inflammatory accumulations in the brain cavity, as a result of meningoencephalitis. The meningoencephalitis results in such behavioral signs as spinning or spiraling, prior to death. Treatment of channel catfish with ESC is best achieved orally with medicated feeds (Romet for 5 days with 3 days of withdrawal or Terramycin for 10–14 days with 21 days of withdrawal). The emergence of resistant strains in the late 1980s prompted investigations into alternative antimicrobials (sarafloxacin, amoxicillin, florfenicol); however, none has achieved USFDA clearance. Currently, experimental live attenuated vaccines show promise, but an effective commercial vaccine is not available.

III. Yersinia ruckeri. Causative agent of Enteric Redmouth Disease. Synonyms: "Hagerman redmouth," "salmonid blood spot."

a. History — The disease was first observed in rainbow trout aquaculture in the Hagerman Valley, Idaho, in the 1950s and was described by Rucker in 1966 (26). A description of the causative bacterium was first published by Ross et al. in 1966 (27), and the pathogen was named *Y. ruckeri* by Ewing et al., in 1978 (28). The common name, enteric redmouth disease (ERM), was adopted by the Fish Health Section of the American Fisheries Society in 1975 (29). Enteric redmouth disease has historically been one of the most significant diseases in salmonid aquaculture, with the potential for cumulative losses of 70% of some populations. The economic impact on the trout industry in the late 1970s was significant, with over US$2 million in losses annually; however, the current practice of vaccinating trout fingerlings with commercially available vaccines has reduced the impact on the industry.

b. Culture — Primary isolation of *Y. ruckeri* is achieved on general purpose bacteriological media (BHI or TSA w/5% sheep blood) and incubation in a normal atmosphere at 22–25 °C. Colonies (1–2 mm) are visible in 24–48 hours. The bacterium is capable of growth over a wide temperature range (9–37 °C); however, the strains that grow at 37 °C are avirulent for trout. *Y. ruckeri* forms a green colony surrounded by a zone of hydrolysis on Shotts and Waltman (SW) selective medium (30), and it produces a yellow colony on the selective medium of Rodgers (31). These media, although useful in selective isolation of the organism from exposed surfaces or intestinal cultures, are not specific enough for confirmatory identification.

c. Description — The bacterium is gram-negative and oxidase-negative. Cells of *Y. ruckeri* are rod shaped (1.0 × 1.0–3.0 μm), becoming filamentous in older cultures. The bacterium is motile by peritrichous flagella in the optimum temperature range, but non-motile strains occasionally occur (27). With the exception of sorbitol fermentation (approximately 32% of strains are positive), the strains are fairly homogeneous in biochemical phenotype. Most strains are positive in the following reactions: glucose fermentation in GMD; ONPG; lysine and ornithine decarboxylase; citrate utilization; Jordans tartrate; and gelatin liquefaction. The bacterium gives a negative reaction for H_2S, indole, and Voges–Proskauer. Features useful in differentiating *Y. ruckeri* from related fish pathogens are given in Table 1. Genetically, *Y. ruckeri* is distantly related to other members of the genus, being only 38% homologous by DNA–DNA hybridization (28). Five different serovars of *Y. ruckeri* are recognized, on the basis of formalin-killed whole-cell serology, and their occurrence seems to be correlated with the geographic region from which they are isolated. The serovars are described as follows: Serovar I (Idaho strains), Serovar II (Oregon

strains), Serovar III (Australian strains), Serovar IV (excluded), Serovar V (Colorado strains), and Serovar VI (Ontario strains).

d. Epizootiology—Although *Y. ruckeri* is thought to be an obligate pathogen of salmonids, the role of stress is very important in the initiation of outbreaks (27). The bacterium has also been shown to survive up to two months in mud. Experimentally, the efficiency of transmission of ERM from carriers to susceptible fish and subsequent disease is greatly enhanced by the addition of stress. Reservoirs, other than survivors of outbreaks, have not been identified. Survivors carry the bacterium in their intestine and shed organisms into the water on a cyclical basis every 35–40 days; shedding precedes recurrent outbreaks of the disease by several days. Vertical transmission of *Y. ruckeri* has not been demonstrated. Fingerlings are the most susceptible to infection, and mortality in acute outbreaks may range from 30–70%. Enteric redmouth commonly occurs as a chronic disease, particularly in older fish > 12 cm (4.75 in.), with cumulative mortality being approximately 30%. Most outbreaks occur when water temperatures are in the range 11–18 °C (52–64 °F), with the greatest severity occurring between 15 and 18 °C (59–64 °F).

e. Geographic range and host-susceptibility—Since its initial isolation in the Hagerman Valley of Idaho, the disease has been found throughout U.S. trout-growing areas. Fish disease caused by *Y. ruckeri* has been confirmed in many regions throughout the world, including North America, Europe, Australia, Finland, Norway, and South Africa. The rainbow trout is the species most susceptible; however, all salmonids can be infected. Some nonsalmonid species, such as the fathead minnow, *Pimephales promelas*, the cisco, *Coregonus artedii*, the whitefish, *Coregonus clupesformis*, the sturgeon, *Acipenser* spp., and the turbot, *Scophthalmus maximus*, are susceptible. The bacterium has also been isolated from invertebrates, sea gulls, and muskrats, from sewage, and from river water. One human clinical isolate was reported (7,8).

f. Clinical signs of disease and treatment—As the common name of the disease suggests, reddening around the mouth and operculum (due to subcutaneous hemorrhage) is the most obvious of the gross external clinical signs. Other external signs that are commonly reported are exophthalmia and a general darkening of body pigmentation. Internal clinical signs are indistinguishable from other gram-negative bacterial septicemias. Treatment of ERM is best accomplished by feeding Romet or Terramycin medicated feeds at 50 mg/kg (23 mg/lb/day) body weight/day for 5 or 14 days respectively. ERM is successfully managed by vaccination of fingerlings with commercial bath vaccines.

IV. Other Enterobacteriaceae. Other members of the *Enterobacteriaceae* have, rarely, been implicated as the cause of disease in fish. Bacteria of the genera *Proteus*, *Serratia*, *Citrobacter*, *Enterobacter*, and *Hafnia* have been implicated as causative agents of fish disease; however, these diseases have not appeared with any consistency in aquaculture settings (9).

B. *Aeromonadaceae.* Facultatively anaerobic, oxidase-positive, 0/129-resistant, Gram-negative rods.

I. Aeromonas hydrophila ("A. punctata, A. liquefaciens"), A. sobria, A. caviae. Causative agents of Motile Aeromonad Septicemia. Synonyms: hemorrhagic septicemia, infectious dropsy, infectious abdominal dropsy, red sore. The motile aeromonads are treated here as a group, because the number of species involved is in question and because the taxonomy is in a state of transition.

a. History—Although reference is made as early as 1891 to fish with hemorrhagic septicemia (32), the description of a bacterial disease in cultured carp with infectious dropsy caused by *"Pseudomonas punctata"* (*A. punctata*), by Schaperclaus in 1930, is believed to be the first documented case of motile aeromonad septicemia (MAS) (33). Ewing et al., in 1961 (34), proposed that *A. punctata*, *A. hydrophila*, and *A. liquefaciens* were all variants of the same species and included them all in a single species, *A. hydrophila*. The name of the disease syndrome was changed to "motile aeromonad septicemia" in 1975, to reflect the multispecies etiology of the disease. Seven species of motile aeromonads were recognized by Carnahan et al., in 1991; however, only *A. hydrophila*, *A. sobria*, and *A. caviae* are currently recognized as representative of the motile aeromonad fish pathogens.

b. Culture—Primary isolation is achieved by streaking on standard media such as BHIA or TSA and incubation at 30–37 °C. Growth is rapid at these temperatures, with 2–3 mm, cream-colored colonies visible in 18–24 hrs. Most strains are beta-hemolytic on blood agar. Motile *Aeromonas* spp., form yellow–orange colonies on Rimler–Shotts selective medium when incubated at 35 °C (36).

c. Description—Short, gram-negative rods, 0.8 × 1.0 μm, motile by a single polar flagellum, oxidase-positive, fermentative in glucose motility deeps. Aeromonads also uniformly reduce nitrate to nitrite. Motile aeromonads are easily differentiated from the motile vibrios and *Photobacterium* by resistance to vibriostatic agent, 0/129 (2,4-diaminiop-6,7-diisopropyl pteridine phosphate). The motile aeromonad fish pathogens are differentiated from related fish pathogens in Table 2.

d. Epizootiology—Motile aeromonad septicemia is considered one of the most common diseases of cultured warm-water fish in freshwater environments. The bacterium is also known to cause disease in brackish-water fish culture at salinities up to 15 ppt. Aeromonads are common members of the microflora of natural waters, are ubiquitous in a variety of environments, and are commonly found as part of the normal microflora of the intestine and skin of fish. Motile aeromonad septicemia occurs most frequently in the spring of the year but can occur year round (7). Natural fish kills on lakes and reservoirs in the spring are often a result of MAS epizootics acting either alone or in concert with parasitic infestation (37). The motile aeromonads are considered opportunistic pathogens, and MAS is usually considered a stress related disease. Such stress factors as rising water temperatures, handling, transport, poor water quality, and parasitic load may contribute to outbreaks. Those outbreaks are chronic

Table 2. Biochemical and Biophysical Characteristics Important in Differentiating Some Fish Pathogens in the Family *Aeromonadaceae*

Characteristic	*A. hydrophila*	*A. sobria*	*A. caviae*	*A. salmonicida* subsp. *salmonicida*	*A. salmonicida* subsp. acromogenes
Motility at 25 °C	+	+	+	−	−
Gas in GMD[a]	+	+	−	+	−
Esculin hydrolysis	+	−	+	+	−
Brown pigment	−	−	−	+	−
Voges–Proskauer	+	+	−	−	−
Growth at 37 °C	+	+	+	−	−
Acid from arabinose	+	−	+	+	−

[a] GMD = glucose motility deep, incubated at optimum growth temperature of bacterium tested.

in nature, with low mortality rates. "Poststocking syndrome" is a disease that is characterized by a peak in mortality approximately three days following the transport and stocking of fish in a new pond or unit. Infection, disease, and mortality may continue for a few days; then the losses diminish and stop. Motile aeromonads are often a major component in "winter mortality syndrome" or "winterkill," a disease in catfish ponds related to rapid temperature drops in the winter months, to fungal disease, and to secondary bacterial infection. The exception to this may be certain strains of *A. sobria* and *A. hydrophila* that possess a surface protein layer (S-layer) that serves as a virulence factor (38). Highly virulent strains may cause a typical hemorrhagic septicemia, with rapid progress and high mortality rates.

e. Geographic range and species susceptibility—Motile aeromonads are found in warm-water freshwater and brackish-water environments throughout the world. This group of bacteria shows little or no host specificity; it infects a variety of freshwater and brackish-water fish hosts. Aquatic animals other than fish are susceptible to motile aeromonad infections; Compare redleg disease of frogs and fatal disease of reptiles (39). Although not considered a serious health concern, *A. hydrophila* has occasionally been responsible for wound infections and even fatal septicemia in humans (7).

f. Clinical signs of disease and treatment—MAS occurs in acute and chronic forms. The acute form is a typical bacterial septicemia characterized by exophthalmia, abdominal distension due to ascites, petechial hemorrhage in the skin, and diffuse necrosis in the internal organs. The progress of infection may be so rapid that few clinical signs are evident (38). The chronic form is characterized by the formation of deep muscular ulcerations, with associated hemorrhage and inflammation. Treatment for acute disease is with medicated feeds (Romet® or Terramycin®) at 50 mg/kg/day. The chronic form of the disease usually responds to improvement of water quality and to removal of stress. Vaccination is not considered feasible, because of the multiplicity of strains and serotypes encountered.

II. Aeromonas salmonicida. Nonmotile Aeromonads.

Three subspecies or groups of *A. salmonicida* have been proposed by McCarthy and Roberts (40), and supported by various taxonomists (41) on the basis of epizootiology and phenotype, even though DNA homology studies do not support the necessity for subspecies.

Group 1. *A. salmonicida* subsp. *salmonicida*
Causative agent of furunculosis in salmonids.

Group 2. *A. salmonicida* subsp. acromogenes ("*Haemophilus piscium*")
Causative agent of ulcer disease in salmonids and bacterial septicemia of salmonids (phenotypically atypical strains).

Group 3. *A. salmonicida* subsp. *nova*
Causative agent of carp erythrodermatitis and goldfish ulcer disease (phenotypically atypical strains).

a. History—The first report of furunculosis is from 1894, by Emmerich and Weibel, from a trout hatchery in Germany (42). The bacterium was referred to as *Bacillus der Forellenseuche* in Germany and *Bacillus salmonicida* in England in the early 1900s and was reclassified as *A. salmonicida* by Griffin in 1953 (43). The name furunculosis is somewhat of a misnomer and was given to describe the "boil-like" lesions of the original form of the disease in trout; however, from a pathologic standpoint, there is little similarity to the pus-filled boils that occur in humans (44). Judging from clinical signs, one can speculate that ulcer disease of trout was probably first seen in 1899 by Calkins (45). Ulcer disease of trout (UD) was described by Snieszko (46), and the causative bacterium was named *H. piscium* (from diseased brook trout) in 1980 (47). The relationship between *A. salmonicida* and *H. piscium* was investigated by Paterson (48) and by McCarthy and Roberts (40), and it was concluded that *H. piscium* was simply an atypical, achromogenic strain of *A. salmonicida*. Carp erythrodermatitis (CE) was described in 1972 by Fijan (49) from common carp, and goldfish ulcer disease (GUD) was described in 1980 by Elliott and Shotts (50).

b. Culture—Primary isolation of typical strains is achieved on BHI agar, TSA agar, or TSA agar with 5% sheep blood and incubation at 20–25 °C. Growth is fairly rapid even at these temperatures, with 2-mm cream-colored colonies visible in 48 hours. Some atypical strains from ulcer disease may be somewhat fastidious, and growth may be enhanced by the addition of fish peptone to the primary isolation medium (45).

c. Description—*A. salmonicida* is a gram-negative, facultatively anaerobic, nonmotile rod, 0.8–1.3 ×

1.3–2.0 μm. Typical strains, when grown on media containing tyrosine, produce a brown, water-soluble pigment. Atypical strains do not produce this pigment; they also vary on a few tests involving enzyme production and carbohydrate fermentation. Fresh isolates from fish are virulent, produce "rough" colonies (because of the presence of a surface protein "A-layer"), and autoagglutinate in broth culture. Colonies that have been subcultured, particularly at higher temperatures 25 °C (77 °F), are nonvirulent, become "smooth," are A-layer negative, and do not autoagglutinate (51). Once the bacterium has lost the A-layer via high-temperature incubation, it cannot be induced to revert to being A-layer positive. The nonmotile subspecies of *A. salmonicida* are differentiated from related fish pathogens in Table 2.

d. Epizootiology—*A. salmonicida* is considered an obligate pathogen, even though recent studies have shown it can survive for months in fresh water and in muds. The current belief is that furunculosis and other related diseases caused by the subspecies of *A. salmonicida* are transmitted horizontally from fish to fish by close contact through the skin or by ingestion of water containing high numbers of the organism (40). Skin damage due to abrasion or to external parasites (such as salmon lice) may open portals of entry for the bacterium. Asymptomatic infected carriers are vehicles for persistence within a population. Vertical transmission is not believed to be important in the spread of furunculosis. The disease has always been an important economic problem in salmonid aquaculture, and it has recently been cited as a problem in such new aquaculture venues as the sea-ranching of salmon in Scotland and Norway, where losses as high as 20% have been reported (8).

e. Geographic range and host susceptibility—Furunculosis of salmonids is found in almost every region of the world in which trout are grown, most notably in North America, Great Britain, Europe, Asia, and South Africa (7). All salmonids are susceptible to the disease. Atypical strains produce carp erythrodermatitis in common carp (*C. carpio*) in Europe and Great Britain but not in North America or Asia. Goldfish ulcer disease has been diagnosed from the goldfish, (*Carassius auratus*), in the U.S., Italy, Great Britain, Japan, and Australia (7).

f. Clinical signs of disease and treatment—Gross external clinical signs in salmonids with furunculosis vary depending on the time course of the disease process. In acute disease, fish show very few clinical signs, and mortality is high. In chronic disease, the following signs become apparent: darkening, lethargy, anorexia, petechial hemorrhage, swellings in the skin/muscle containing necrotic debris (furuncles), and hemorrhage in the gill filaments. In nonsalmonids infected with atypical strains, the disease begins with small localized skin infections that progress into larger ulcerations, which may be secondarily infected by motile aeromonads or pseudomonads. The disease ultimately becomes a septicemia, if the fish does not die first from a secondary infection. Medicated feeds with Terramycin® or Romet® administered at 50 mg/kg/day (23 mg/lb/day) have traditionally been the treatment of choice in the U.S. During the 1980s, drug-resistant strains of *A. salmonicida* began to appear in Europe and Norway, and additional antimicrobials had to be explored. In Scotland, there are currently four antibacterial agents licenced for control of furunculosis: oxytetracycline, oxolinic acid, trimethoprim-sulfadiazine, and amoxicillin. Multiple resistance to these drugs is common now, with the exception of amoxicillin (52); however, some isolates of *A. salmonicida* subsp. acromogenes have recently been reported to be resistant to amoxicillin (53). Florphenicol has been reported to be a very effective chemotherapeutic in experimental trials (54). *A. salmonicida* is an antigenically homogeneous species, with variability only in the presence or absence of A-layer; however, there have over the last 50 years been many failed attempts at producing successful commercial vaccines (55). The use of oil-adjuvanted vaccines has given the first encouraging results (56).

C. Vibrionaceae. Facultatively anaerobic, oxidase positive, 0/129 sensitive, gram-negative curved rods. All but two species are halophilic and thus are known primarily as pathogens of marine fish.

I. Vibrio anguillarum, Listonella anguillarum and Vibrio ordalii. Causative agents of vibriosis. Synonyms: salt water furunculosis, boil disease, ulcer disease, red pest of eels.

a. History—As stated in the introduction to this chapter, vibriosis may well be the longest known bacterial disease in aquaculture. The clinical signs of a disease in eels cultured in the Mediterreanean as early as 1718 are consistent with vibriosis (1). The causative agent was first cultured from diseased eels with "red pest" in 1883 (2) and named *V. anguillarum* in 1909 by Bergeman (57). Two distinct biochemical phenotypes of *V. anguillarum* were recognized by Nybelin in 1935; that work led to the designation of *V. anguillarum biovar I* and biovar II (9). The more fastidious and nonreactive strains composing biotype II received their own species status in 1981, after the work of Schiewe (58). Today, the taxonomy of this group of organisms is in a confused state, after the suggestion of MacDonell and Colwell in 1985 that *V. anguillarum* and *V. ordalii* be reclassified in the genus *Listonella* (59). Currently references to both names are found in the literature.

b. Culture—*V. anguillarum* may be cultured from diseased fish tissues on standard culture media such as TSA with or without 5% sheep blood or BHIA and incubation at 25–30 °C. The organism grows rapidly and 2-mm colonies are visible within 24 hours. *V. anguillarum* and *V. ordalii* are halophilic and grow on culture media with a final salt concentration of 1–3%. Primary isolation of *V. ordalii* is improved by using seawater agar with 3% salt and incubation at 15–25 °C. Growth is slow, and up to seven days may be required for typical colonies to develop (7). *V. anguillarum*, but not *V. ordalii*, can be isolated on thiosulfate citrate bile sucrose (TCBS) selective medium, on which it produces a yellow colony. This medium is not differential, so additional testing is required to speciate the organisms forming yellow colonies. A new selective and differential medium, *V. anguillarum* medium (VAM), used alone or in combination with dot-blot hybridization,

Table 3. Biochemical and Biophysical Characteristics Important in Differentiating Some Fish Pathogens of the Family Vibrionaceae

Character	*V. anguillarum*	*V. ordalii*	*V. salmonicida*	*V. vulnificus* 2	*P. damselae* subsp. *damselae*	*P. damselae* subsp. *piscicida.*	*P. shigelloides*
Motility	+	+	−	+	+	−	+
Sensitive to 0/129	+	+	+	+	+	+	+
Production of:							
Arginine dihydrolase	+	−	−	−	+	+	+
Lysine decarboxylase	−	−	nr	+	−	−	+
Ornithine decarboxylase	−	−	nr	−	−	−	+
β-galactosidase	+	−	−	+	−	−	+
Indole	+	−	−	−	−	−	+
Degredation of:							
Gelatin	+	+	−	+	−	V	−
Starch	+	−	−	+	+	+	nr
Urea	−	−	−	−	+	−	−
Lipids	+	−	−	+	−	−	nr
Nitrate reduction	+	V	−	+	+	−	+
Voges–Proskauer	+	−	−	−	+	+	−
Growth at 37 °C	+	−	−	+	nr	−	+

Symbols: V = variable results, nr = no record.

has been employed for environmental enumeration of *V. anguillarum* (60).

c. Description — Short, gram-negative, slightly curved rods, 0.5 × 1.4–2.6 μm, motile by monotrichous or multitrichous sheathed polar flagella at 25–30 °C, oxidase-positive, fermentative in glucose deeps, and sensitive to vibriostat 0/129. *V. anguillarum* is more reactive in biochemical tests than *V. ordalii*, being positive for arginine dihydrolase, Voges–Proskauer, beta–galactosidase, indole, citrate, arabinose, and sorbitol. The vibrionaceae pathogenic to fish are differentiated in Table 3.

d. Epizootiology — *V. anguillarum* is regarded as the marine counterpart to *A. hydrophila*, being a part of the normal microflora of the marine aquatic environment and marine fish (61). The bacterium exhibits long-term survival in aquatic environments, and virulent and nonvirulent strains may occur. Transmission is horizontal, via the water column, and infection is a result either of direct invasion of the skin or intestine by virulent strains, or of colonization of skin abrasions by less virulent strains, or of both. Perhaps the best-characterized virulence mechanism in a fish pathogen is the siderophore-mediated, plasmid-encoded, high-affinity iron-transport system in *V. anguillarum* strain 775 (62). The expression of this system requires a stretch of about 25 kilobases (kb) of a 65-kb plasmid, pJM1, and curing *V. anguillarum* strain 775 of pJM1 results in loss of virulence (63). *V. anguillarum* is a serious problem in marine aquaculture, particularly in stressed populations, and it is therefore considered an opportunistic pathogen. Temperature also plays a role, with mortalities in salmonids being highest (60%) at 18–20 °C (64–68 °F) and lowest (4%) at 6 °C (43 °F) (64).

e. Geographic range and host susceptibility — *V. anguillarum* is found worldwide in a variety of marine fish species, and on rare occasions in freshwater species. It is a significant fish pathogen in all coastal areas of North America, the North Sea, the Atlantic and Mediterreanean coasts of Europe and North Africa, and Asia. *V. ordalii* outbreaks are confined to the Northwest Pacific Coast of North America and Japan (7). Approximately 50 species of marine fish have been listed as being susceptible to vibriosis, but salmonids, eels (*Anguilla* spp.), hybrid striped bass (*Morone* sp.), milkfish (*Chanos chanos*), and ayu (*Plecoglossus altivelis*) are the most important aquaculture species. *V. ordalii* occurs most commonly in salmon and trout.

f. Clinical signs of disease and treatment — Clinical signs of vibriosis are similar to motile aeromonad septicemia. Acute disease is accompanied by few clinical signs other than erratic swimming behavior and lethargy. The spleen may be enlarged, and the liver, kidney, and spleen may contain necrotic foci. In chronically infected fish, skin/muscle ulcerations become prominent, as does anemia. In *V. ordalii*-infected fish, the bacteria are less dispersed in the tissue, and microcolonies can be observed in sections of heart and skeletal muscle. Control of vibriosis is best achieved by maintaining good husbandry practices and by using commercially available multivalent vaccines. Treatment of the disease in salmonids is by medicated feed (Romet® 50 mg/kg/day (23 mg/lb/day) for 5 days or Terramycin® 50–75 mg/kg/day (23–34 mg/lb/day) for 10–14 days). Clearance for Romet® in salmonids is 42 days that for Terramycin® is 21 days.

II. Vibrio salmonicida. Causative agent of coldwater vibriosis. Synonyms: Hitra disease, hemorrhagic syndrome.

a. History — Coldwater vibriosis (CV) is a disease that affects primarily the Atlantic-salmon industry in Norway. The disease was first described as a multifactorial malady by Poppe in 1977 (66), from Atlantic salmon cultured on the island of Hitra off the coast of Norway. Egidius (67)

suggested a bacterial etiology for the condition and, along with work by Holm (68), provided a description of the etiologic agent of CV. The bacterium was fully described, and named *V. salmonicida*, by Egidius et al., in 1986 (69). Monoclonal antibodies to a major surface antigen, VS-P1, were used to confirm the identity of *V. salmonicida* (by immunohistochemistry) in archived tissues from original outbreaks of "Hitra disease" in 1977 (70).

b. Culture — Primary isolation of *V. salmonicida* is difficult, but it can be accomplished on TSA with 5% sheep blood or BHIA, provided 1.5–2% salt is added to the medium. Optimum temperature for incubation of primary isolation plates is 15–17 °C, and growth is slow, requiring 72 hrs to form 1–2 mm, smooth, grey colonies.

c. Description — The bacterium is a gram-negative, slightly curved or pleomorphic, motile rod 0.5–2.0 μm long. *V. salmonicida* is psychrophilic, and fails to grow above 25 °C. It is oxidase-positive, motile, and sensitive to vibriostat 0/129. The bacterium is generally non-reactive on many biochemical tests; it is differentiated in Table 3 from related organisms. Strains of *V. salmonicida* are serologically and biochemically homogeneous (68) and are distinct from *V. anguillarum*, *V. ordalii*, and other vibrios. Rare isolates from Atlantic cod belong to a different serotype.

d. Epizootiology — Coldwater vibriosis is transmitted via the water from carrier fish (71), and infection is primarily through the gills. The organism is capable of survival in seawater and sediments for over one year. Mortalities in natural CV outbreaks are high, losses of 5% per day being common. Mortality in experimentally infected Atlantic salmon was 90% at 45 days postinfection. Most natural infections on fish farms occur in the autumn and winter, when water temperatures are between 4 and 9 °C. All ages and size classes of salmonids are susceptible.

e. Geographic range and host susceptibility — CV has been reported from the coast of Norway, the Shetland Islands of northern Scotland, the Faroe Islands, eastern Canada, and the northeastern U.S. (7). Atlantic salmon grown in salt water or brackish-water net-pens are most susceptible. An outbreak of CV has been reported from a highly stressed, net-pen cultured population of juvenile Atlantic cod (*Gadus morhua*) (72).

f. Clinical signs of disease and treatment — Acutely infected fish come to the surface of net-pens, swim erratically, and exhibit few (if any) external clinical signs. In subacute to chronic stages of the disease, pale gills, hemorrhage at the base of the fins and in the muscle, reddish and prolapsed anus, bloody ascetic fluid in the peritoneal cavity, and hemorrhage and watery contents in the posterior intestine are all notable clinical signs. Histopathologically, CV is similar to vibriosis caused by *V. anguillarum*, except with more pronounced heart and muscle damage (13). CV is believed to be a multifactorial disease, so reducing stress on the population is a primary management strategy. Effective commercial vaccines have had a positive impact on the Norwegian Atlantic salmon aquaculture industry. In one field trial, mortalities were reduced from 24.9% to 1.87% (73). Feeds medicated with Tribrissen, Terramycin®, or furazolidone were used, initially at 75–100 mg/kg (34–45 mg/lb/day) body weight/day for 10 days, with positive results. Recent development of resistant strains, however, has led to the evaluation of new drugs, such as Florfenicol (54).

III. Vibrio vulnificus Biogroup 2. Causative agent of *V. vulnificus* infection of eel.

a. History — *V. vulnificus* biogroup 2 was originally documented as the cause of serious outbreaks of bacterial disease in eel populations in Japan between 1975 and 1977 (74). The disease is now known from Europe, where it has caused similar outbreaks in Spain (75), the Netherlands, and England (9). The bacterium was properly classified as *V. vulnificus*, and a new biotype (biogroup 2) was established by Tison et al. in 1982 (76).

b. Culture — Primary isolation is accomplished on seawater agar or TSA with an additional 2% NaCl. Growth is slow, requiring 4–7 days at 20–25 °C.

c. Description — Short gram-negative rods 0.5–2.0 μm long, oxidase-positive, sensitive to vibriostat 0/129, motile by a single polar flagellum, with growth between 20 and 37 °C. *V. vulnificus* biogroup 2 differs from typical *V. vulnificus* by negative results in tests for indole, for ornithine decarboxylase, and for acid from mannitol or sorbitol and by growth at 42 °C (9). Characteristics that differentiate *V. vulnificus* are found in Table 3.

d. Epizootiology — Little has been reported on the epizootiology of this disease, but it is believed to be an opportunistic infection, because the organism has low virulence in experimental infections and is ubiquitous in the marine environment.

e. Geographic range and host susceptibility — The disease is currently known to be a problem in eel mariculture in Japan, England, the Netherlands, and Spain. Thus far, only Japanese eels (*Anguilla japonica*) and European eels (*Anguilla anguilla*) are known to be susceptible. All strains pathogenic to fish form a homogeneous group in terms of serology and of biochemical phenotype.

f. Clinical signs of disease and treatment — The disease is characterized by hemorrhage in the gills and along the flank or tail. Internally necrotic lesions may be found in the liver, the spleen, the heart, and the intestine; they bear close resemblance to lesions seen with classical vibriosis in eel.

IV. Other Vibrio spp. Several additional species of vibrios have been found on various occasions to be pathogenic for fish and shellfish (7,9,13). Uncertainty about their overall impact on aquaculture has led to listing these additional species here as potential pathogens, without going into detail. With time, some of these bacteria may emerge as significant pathogens in aquaculture:

V. alginolyticus — gilthead seabream, mullet, penaed shrimp.

V. vulnificus biogroup 1 or typical strains — hybrid striped bass, red drum, tilapia, prawns.

V. carchariae — sharks.

V. cholerae, *V. mimicus* — ayu, channel catfish, red drum, crayfish.

V. fischeri—turbot, penaed shrimp.

V. harveyi—snook.

***V. Photobacterium damselae* subsp. *damselae* ("*Vibrio damsela*," "*Listonella damsela*").** Causative agent of ulcerative disease of damselfish and of hemorrhagic septicemia of various other species. Synonym: Vibriosis

a. History—*P. damselae* subsp. *damselae* ("*Vibrio damsela*") was initially discovered as the causative agent of an ulcerative disease in damselfish (*Chromis punctipinnis*) inhabiting the coastal waters of southern California (77). Additional work by Grimes in 1984, on isolates from captive sharks, confirmed the new species (78). The organism has also been isolated from human wound infections (77). The taxonomy of the organism has changed since its initial description, having been moved to *Listonella* in 1985 (59) and later to *Photobacterium* in 1991 (79).

b. Culture—Primary isolation is accomplished on BHIA or TSA with 5% sheep blood and incubation at 25 °C for 48 hours. Addition of 2% NaCl to primary isolation medium is suggested. Growth is rapid, with typical 2-mm colonies visible after 48 hours incubation at 25 °C. TCBS agar may be used for selective isolation; however, additional testing is required for speciation.

c. Description—Short (0.5–2.0 μm), gram-negative, slightly curved or pleomorphic rods weakly motile by one or more sheathed, polar flagella. *P. damselae* subsp. *damselae* is positive for oxidase, urease, and arginine dihydrolase and negative for acid from sucrose. Additional characteristics that differentiate it from related organisms are found in Table 4.

d. Epizootiology—*P. damselae* subsp. *damselae* is a normal inhabitant of marine waters; it may invade highly susceptible fish directly, and less susceptible fish secondarily to injury. Host specificity was investigated in laboratory experiments by scarifying the dermis of a variety of fish species and swabbing the area with a solution containing 10^8 viable cells of *P. damselae*. Only damselfish succumbed to the disease by this method.

e. Geographic range and host susceptibility—*P. damselae* subsp. *damselae* has been documented as a pathogen of natural populations of damselfish in coastal southern California (77), in sharks and dolphins held in captivity (78), in cultured turbot and sea bream in Europe (80,81), in yellowtail (*S. quinqueradiata*) in Japan (82), in rainbow trout in Denmark (83), and in barramundi (*Lates calcarifer*) in Australia (84). Stress associated with temperatures higher than normal is a common theme in aquaculture outbreaks.

f. Clinical signs of disease and treatment—*P. damselae* subsp. *damselae* causes ulcerative lesions in the skin of affected damselfish, usually near the pectoral fin and caudal peduncle. Ulcers sometimes increase in size to ~20 mm (1.75 in.), prior to death of the fish. In turbot and seabream, ulcers are not noticed, but hemorrhages in the eyes, at the base of the fins, and around the anus, as well as abdominal distension are typical clinical signs. Treatment of *P. damselae* infections in Europe and Japan is done with oxytetracycline, oxolinic acid, and trimethoprim–sulfamethoxazole.

***VI. Photobacterium damselae* subsp. *piscicida* ("*Pasteurella piscicida*").** Causative agent of photobacteriosis. Synonyms: Pasteurellosis, pseudotuberculosis.

a. History—Photobacteriosis was first described by Snieszko et al., in 1964 (85), from a massive natural fish kill involving white perch (*Morone americana*) and striped bass (*M. saxatilis*) in the upper Chesapeake Bay, U.S. The authors placed the causative organism in the genus *Pasteurella*, on the basis of physiological, morphological, and staining characteristics. The bacterium was

Table 4. Biochemical and Biophysical Characteristics Important in Differentiating Among Gram-Positive Cocci and Cocco Bacilli which have been Isolated from Fish

	S. iniae	*S. difficilis*	*L. garvieae*	*L. piscium*	*V. salmoninarum*	*E. faecium*	*C. piscicola*
Shape	cocci	cocci	ovoid	cocci	ovoid	ovoid	cocco-bacilli
Hemolysis	β	γ	α	γ	α	α, β, or γ	γ
Esculin	+	−	+	+	+	+	+
Hippurate	−	+	−	−	d	−	−
ADH	+	+	+	−	−	+	+
PYR	+	−	+	−	+	+	+
β-Gur	+	−	ND	ND	−	−	ND
PAL	+	+	−	ND	+	−	ND
H_2S	−	−	−	−	+	−	−
Growth at/in:							
45 °C	−	−	+	−	−	+	−
10 °C	v	−	+	+	+	+	+
pH 9.6	v	+	+	−	+	+	+
40% bile	−	+	+	ND	+	+	−
L-Arabinose	−	−	−	+	−	−	−
Inulin	−	−	−	−	−	−	+
Lactose	−	−	v	+	−	−	v
Mannitol	+	−	+	+	−	+	+
Raffinose	−	−	−	+	−	−	v

studied morphologically, physiologically, and serologically in 1968 by Janssen and Surgalla (86), who concluded that it represented a new species and proposed the name "*P. piscicida*." Outbreaks of "pseudotuberculosis" in cultured yellowtail in the late 1960s in Japan were attributed to this bacterium (87). Photobacteriosis remains one of the most serious diseases affecting Japanese mariculture; losses of yellowtail in excess of 2000 tons were reported in 1989 (88). Prior to 1990, there were no reports of photobacteriosis from Europe. After an account by Toranzo et al., in 1991 (89) of the disease in gilthead seabream (*Sparus aurata*) cultured in Spain, the disease seemed to spread throughout the region. Currently, photobacteriosis represents a significant economic problem in European and Meditereanean mariculture. The first report of photobacteriosis from cultured fish in the U.S. was from striped bass cultured in brackish water ponds on the Alabama Gulf Coast by Hawke et al., in 1987 (90). In 1990, the disease recurred on the U.S. Gulf Coast, in Louisiana, and yearly outbreaks have severely damaged an emerging hybrid striped bass mariculture industry (91). "*P. piscicida*" was never accepted as a valid name by bacterial taxonomists, and ultimately the bacterium was renamed *P. damselae* subsp. *piscicida* in 1995, on the basis of 16 sRNA sequencing (92); the name was later corrected to *P. damselae* subsp. *piscicida*.

b. Culture—Primary isolation is accomplished on TSA with 5% sheep blood or BHIA with 1–2.5% NaCl and incubation at 25–28 °C. Typical 1–2 mm, grey, nonhemolytic colonies appear after 48 hours of incubation. A selective medium is not available.

c. Description—*P. damselae* subsp. *piscicida* is a gram-negative, slightly curved or pleomorphic, nonflagellated, nonmotile rod (0.7 × 0.7–2.6 μm), often exhibiting bipolar staining. Coccoid forms predominate in older cultures. It is positive in tests for oxidase, catalase, Voges–Proskauer, and arginine dihydrolase, and it ferments glucose, mannose, galactose, and fructose. It is halophilic, requiring 0.5% salt to grow, and it is sensitive to vibriostatic agent 0/129. Strains of *P. damselae* subsp. *damselae* are serologically and phenotypically homogeneous regardless of the geographic source (93) and are easily differentiated from *P. damselae* subsp. *damselae* and other Vibrionaceae (Table 3).

d. Epizootiology—*P. damselae* subsp. *piscicida* was initially considered an obligate pathogen, because it fails to survive in brackish water for more than five days (94); however, Magarinos et al., demonstrated long term survival in seawater and in sediment, in a viable but nonculturable form that retains virulence (95). A carrier or latent state has not been demonstrated in susceptible hosts, but it has been theorized that other species of fish in the vicinity of fish farms, or perhaps an invertebrate, may harbor the pathogen (96). Most susceptible hosts can be infected via the water, so horizontal transmission from fish to fish within a culture unit is the most likely method of spread during epizootics. Outbreaks on fish farms are explosive and are characterized by sudden reduction in feeding response and rapid onset of mortality. Cumulative mortalities in striped bass (80%), gilthead seabream (40%), and striped jack (34%) have all been documented as occurring over a four week period. Outbreaks of photobacteriosis are correlated with water temperatures of 18–25 °C (64–77 °F) and salinities of 5–25 ppt.

e. Geographic range and host susceptibility—Since its initial description from white perch in Chesapeake Bay, reports of new hosts and of an extented range have continued to increase. At present, the disease is known from Japan, the Atlantic and Gulf Coasts of the U.S., Europe, the Mediterranean, and Israel. The list of susceptible hosts of aquaculture importance includes the yellowtail, the striped jack (*Pseudocaranx dentex*), the ayu, the black seabream (*Mylio macrocephalus*), the red seabream (*Pagrus major*), striped bass (and hybrids), the gilthead seabream (*S. aurata*), and the sea bass (*Dicentrarchus labrax*) (7).

f. Clinical signs of disease and treatment—Photobacteriosis is an acute bacterial septicemia with a striking lack of gross clinical signs in most susceptible hosts. In hybrid striped bass and striped bass, redness in the operculum, enlargement of the spleen, and pallor of the gills are the only consistent signs. Necrotic foci are seen microscopically in the spleen and kidney, but an inflammatory response is lacking. In yellowtail, the disease progresses slightly more slowly, and white miliary lesions of about 1–2 mm (0.1–0.2 in.) become visible in the spleen and kidney. Necrotic foci containing bacterial colonies in the splenic parenchyma, and an associated chronic inflammatory response, has led to the use of the misleading pathological term "pseudotuberculosis" (13). Because of the rapid onset of disease, medicated feeds are usually not offered early enough in the infection to be effective. If timely application is achieved, the pathogen responds well to treatment with oxytetracycline, Romet, oxolinic acid, ampicillin, amoxicillin, and florfenicol medicated feeds. Currently, there are no drugs approved for treatment of photobacteriosis in hybrid striped bass in the U.S. With the widespread use of antibiotics on Japanese fish farms, resistant strains of the pathogen, carrying R-plasmids marking multiple drug resistance, have been isolated. Because of the general ineffectiveness of medicated feeds in combating the disease, vaccination is a logical approach for future management. Commercial vaccines are currently in the developmental stage.

VII. Plesiomonas shigelloides and

VIII. Shewanella putrefaciens (Pseudomonas putrefaciens, Alteromonas putrefaciens). *P. shigelloides* and *S. putrefaciens* are bacteria of only questionable importance as pathogens in aquaculture. Only one published report lists *P. shigelloides* as a fish pathogen. The bacterium was isolated from diseased, farmed rainbow trout in Portugal (98). *S. putrefaciens* has also been reported on only one occasion, from rabbitfish grown in sea cages in the Red Sea (99). There are other anecdotal accounts of *P. shigelloides* and *S. putrefaciens* as suspected pathogens in a variety of fresh and marine fish species (9). Both species are commonly isolated as post-mortem contaminants from decomposing fish tissue and from the intestines of healthy fish. With this in mind, further description of

the organisms is not given, other than to include data on identification of *P. shigelloides* to allow comparison with closely related organisms with which it might be confused (Table 3). The bacteria are presently classified in the Vibrionaceae but may be subject to a reclassification, one pending acceptance of the recommendations of MacDonell and Colwell (59).

D. *Pseudomonadaceae*

I. Pseudomonas anguilliseptica. Causative agent of red spot disease of eels, hemorrhagic septicemia of marine fish. Synonym for Japanese eel disease, Sekiten-byo.

a. History—*P. anguilliseptica* was first described in 1972, as the causative agent of red spot disease of cultured eels (*A. japonica*) in Japan, by Wakabayashi and Egusa (100). The disease has become one of the most significant problems in eel culture in Japan and recently has been reported from European eels (*A. anguilla*) cultured in Scotland (101). The disease seems to be increasing in importance in eel growing areas throughout many parts of the world (9). Recent outbreaks of disease on mariculture farms in Finland (102), in Malaysia (103), in Japan (104,105), and along the Mediterranean and Atlantic Coasts of France in a variety of fish species (106) are referred to as "hemorrhagic septicemia," and *P. anguilliseptica* is the causative agent. The lack of host specificity represents a potential hazard for many farmed species in the future.

b. Culture—Primary isolation can be made on nutrient agar with 10% horse blood or nutrient agar containing 0.5% (w/v) NaCl adjusted to pH 7.4. Incubation should be between 20–25 °C, and the resulting growth is slow, requiring 72 hours for small (~1 mm), shiny, pale-grey colonies to form.

c. Description—*P. anguilliseptica* is a Gram-negative rod (5.0–10.0 × 0.8 μm), motile by a single polar flagellum at 15 °C but not at 25 °C. The bacterium is oxidase and catalase-positive, does not produce acid from glucose or any of a number of other carbohydrates oxidatively or fermentatively, and is resistant to vibriostatic agent (0/129). Growth occurs between 5 and 30 °C at 0–4% NaCl. The occurrence of pleomorphic, filamentous rods 5–10 μm in length makes this bacterium unique in appearance.

d. Epizootiology—In Japan, the disease occurs in eels during April and May, when temperatures are between 15 and 20 °C (59–68 °F). Mortalities decline in the summer months, when water temperatures are between 20 and 25 °C (68–77 °F); however, outbreaks may recur in the fall, when temperatures decrease. Because of the salt tolerance of the organism, disease outbreaks are prevalent in brackish-water ponds (107). In experimental infections, Japanese eels are most susceptible to infection and disease at around 19–20 °C (66–68 °F), and very little mortality occurs above 25 °C (77 °F). Juvenile European eels (elvers) are much more susceptible to infection than adults (96% mortality vs 3.9% mortality) (101), and applications of copper sulfate at 25 to 100 μg/L increase the susceptibility of eels to infection and disease.

e. Geographic range and host susceptibility—Red spot disease primarily affects the Japanese eel in Japan and the European eel in Scotland (100,101). The Japanese eel has been shown to be the more susceptible of the two species. *P. anguilliseptica* has also been reported from eels in Taiwan (7). Recently, outbreaks of the disease have occurred in farmed black sea bream and ayu in Japan (104,105), in salmonids in Finland (102), and in sea bass, sea bream, and turbot cultured on the French Mediterreanean and Atlantic coasts (106). Heavy mortalities were reported in 1987 during infections of the giant sea perch (*L. calcarifer*) and in grouper cultured in offshore sea cages in Malaysia (103). *P. anguilliseptica* has also been isolated from wild Baltic herring on the southwest coast of Finland (108). Wild fish were suspected to serving as a vector to transmit the disease to farmed salmonids. Experimental infections have been achieved in ayu, bluegill, carp, goldfish, and loach (109).

f. Clinical signs of disease and treatment—Japanese eels exhibit extensive petechial hemorrhage in the subepidermal layer of the jaws, on the underside of the head, and along the ventral body surface. Internally, there are petechiae in the peritoneum, enlargement of the liver, and atrophy of the kidney and spleen. Pericarditis is also commonly observed. Clinical signs in other fish species are typical of hemorrhagic septicemia. Treatment of the disease in eels is achieved in a variety of ways, including the following: administering feed medicated with Romet® [50 mg/kg/day (23 mg/lb/day) for five days] or oxolinic acid [5–20 mg/kg/day (0.9–9.0 mg/lb) for three days]; antibiotic baths with oxolinic acid [2–10 mg/L (ppm)]; or raising the water temperature to >27 °C (80.6 °F). Formalin-killed bacterins are effective vaccines against red spot when administered by injection, but not when delivered by immersion (110).

II. Pseudomonas fluorescens. Causative agent of Pseudomoniasis. Synonyms: bacterial tail rot, fin rot, hemorrhagic septicemia.

a. History—*P. fluorescens* was described originally (as *Bacillus fluorescens*) by Trevisan in 1889 (111). The bacterium is a normal inhabitant of soil and water and has been associated with the spoilage of such foods as eggs, fish, and milk (111). Early accounts of hemorrhagic septicemia in cultured carp by Otte in 1963 included *P. fluorescens* along with the motile aeromonads as potential causative agents (9). The importance of *P. fluorescens* in aquaculture is not clear, and its occurrence over the years has been sporadic.

b. Culture—Primary isolation is achieved on nutrient agar, TSA with 5% sheep blood, or BHIA and incubation at 22–25 °C. Cetrimide agar (Difco) or Pseudosel (BBL) are selective media used to isolate pseudomonads from mixed populations of gram negative rods (112). Growth on standard isolation media is rapid, with 1–2 mm, cream-colored colonies visible in 24 hours at 25 °C.

c. Description—*P. fluorescens* is a gram-negative rod (0.5 × 1.5–4.0 μm) that is motile by 1–3 polar flagella and is positive in tests for oxidase, catalase and arginine dihydrolase. *P. fluorescens* produces acid from glucose

aerobically, but is not fermentative (giving a +/no change reaction in GMD). Colonies on TSA, Cetrimide agar, or Mueller Hinton agar plates produce a diffusible yellow–green pigment (pyoverdin) that is fluorescent under short wavelength (ca. 254 nm) ultraviolet light (112). Growth occurs in the range of 4–37 °C but not at 42 °C. Five biovars are reported in Bergey's Manual of Systematic Bacteriology (111), but strains pathogenic to fish have not been classified by this method.

d. Epizootiology—*P. fluorescens* is an opportunistic pathogen that usually invades the host secondarily to stress or injury. Ghittino, in 1966, stated that degraded environmental conditions were vital to initiation of outbreaks of hemorrhagic septicemia caused by *P. fluorescens* and that often the infection was secondary to viral infection (45). *P. fluorescens* is most often seen causing fin rot and skin lesions in a variety of fish species reared under stressful conditions (45). Pseudomonad infections display many similarities to motile aeromonad septicemia. One exception is that *P. fluorescens* infections can also occur at low temperatures. For instance, 100% mortality was observed in tench fry over a 10-day period at 10 °C (50 °F) (113). Winter mortality in silver and bighead carp at temperatures near freezing has also been reported by Csaba (114).

e. Geographic range and host susceptibility—*P. fluorescens* is ubiquitous in aquatic environments world-wide. Published accounts of pseudomoniasis have listed a wide range of susceptible fish species, including silver and bighead carp, goldfish, koi, tench, grass carp, black carp, white catfish, rainbow trout, and freshwater tropical aquarium species (12).

f. Clinical signs of disease and treatment—The most common clinical sign in pseudomoniasis is fin or tail rot, in which large portions of the fins are lost due to necrosis. In carp, the disease is usually indistinguishable from motile aeromonad septicemia, displaying hemorrhages in the skin and ascetic fluid accumulations in the body cavity. In rainbow trout and koi, the presence of skin lesions similar to those seen in ulcer disease are common. Treatment of pseudomonas septicemia is best achieved by improvement of water quality conditions and by elimination of environmental stress. On nonfood fish, bath treatments with benzalkonium chloride [1–2 mg/L (ppm) for 1 hour] and furnace [0.1 mg/L (ppm) for 24 hours] may be effective (9). Pseudomonads are notorious for multiple drug resistance; therefore, isolation of the pathogen and determination of antimicrobial susceptibility is essential before a recommendation can be made. Vaccines are currently not in use, presumably because of the relationship of stress to disease outbreaks.

***III. Other Pseudomonas* spp.** A variety of pseudomonads have been implicated as fish pathogens (9), but, because of a lack of consistency in their occurrence, they are simply listed here, along with their susceptible host(s) and geographic location:

P. chlororaphis—amago trout (*Oncorhynchus rhodurus*); Japan (9).

P. pseudoalcaligenes—rainbow trout; England (9).

P. sp. ("hemorrhagic ascites")—ayu; Japan (115).

GRAM-POSITIVE AGENTS

A. ***Streptococcaceae.*** Small cocci, usually in chains, facultatively anaerobic, catalase negative.

Recent changes in the classification of the streptococci, stemming from molecular taxonomic studies, from the taxonomic reassignment of previously known fish pathogens, and from the recent discovery of several new fish pathogens, have led to a great many changes in this group, and, hopefully, to elimination of some of the confusion. Streptococci have been recognized as fish pathogens since 1958 (116); they have traditionally been grouped on the basis of a few phenotypic characteristics (biochemical test reactions), of hemolytic reactions, and of Lancefield serology. This system breaks down because some streptococci do not possess a known Lancefield antigen, because members of different species may belong to the same Lancefield group, and because strains within a species may have heterogeneous Lancefield antigens. Also, hemolysis patterns may be interpreted differently in different laboratories. In spite of these problems, these methods are still useful as initial steps in the identification process (117). Because the older references use this system, many of the early descriptions of streptococci pathogenic to fish are incomplete. This defect has resulted in a multitude of published reports of streptococcal disease in a variety of fish species, giving the impression that many different species are the etiologic agents of disease. Some reviewers, out of frustration, simply lump them all together as causative agents of a single disease: "streptococcosis." In cases where strains were archived, these agents have been renamed and (often) reclassified; however, information on other unavailable strains is too sketchy for them to be classified properly. Discussed under this heading are diseases caused by members of the genera *Streptococcus*, *Enterococcus* (previously group D streptococci), *Lactococcus* (formerly group N streptococci), and *Vagococcus* (formerly motile group N streptococci).

I. Streptococcus iniae ("Streptococcus shiloi"). Causative agent of streptococcosis. Synonyms: -hemolytic streptococcal disease, bacterial meningoencephalitis, mad fish disease, golf ball disease of freshwater dolphin.

a. History—*S. iniae* was first described from diseased Amazon freshwater dolphin (*Inia geoffrensis*) housed at a public aquarium in San Francisco, California (118). The dolphin (an aquatic mammal) had numerous subcutaneous abcesses (a condition called "golf ball disease") from which β-hemolytic streptococci were isolated. The disease was reported from the same species at another aquarium in New York two years later (119). There were no further reports of this organism causing disease in the U.S. until 1994, when Perera et al. (120) described *S. iniae* as the causative agent of an epizootic in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) cultured in Texas. Eldar et al. (121), described a new species in 1994, *S. shiloi*, as the causative agent of "bacterial meningoencephalitis" in cultured tilapia and trout in

Israel; however, *S. shiloi* was later shown to be a junior synonym of *S. iniae* (122). Epizootics caused by β-hemolytic streptococci occurred on Japanese fish farms between 1979 and 1986 in a variety of species. Although the descriptions are difficult to compare, because of the different methods used, all appear consistent with *S. iniae*, and one archived strain has been identified by serology as *S. iniae* (8). The first report of *S. iniae* infection in commercially reared hybrid striped bass in the U.S. was by Stoffregen et al., in 1996, from fish cultured in closed recirculating systems (123). With the development of closed-recirculating-system technology, *S. iniae* has also emerged as the first significant disease of cultured tilapia in the U.S., having been identified in 14 states (124). In Israel and Japan, it has become an even more significant problem, causing losses in the millions of dollars yearly (125).

b. Culture—Primary isolation is on TSA with 5% sheep blood. Incubation should be between 30–35 °C in a normal atmosphere. Growth is slow, requiring 48 hours for typical opaque-white colonies to develop on blood agar. Selective isolation is best achieved on Columbia CNA agar with 5% sheep blood. The bacterium fails to grow on enterococcal-selective media containing sodium aside.

c. Description—*S. iniae* is a gram-positive coccus (0.6–0.8 μm) in pairs or chains. Typical colonies on TSA blood agar are surrounded by a very narrow zone of β-hemolysis and a broader zone of α-hemolysis, although more strongly β-hemolytic strains can occur. Hemolysis is best demonstrated by stabbing the blood agar with the inoculating loop. Growth is between 10, 40 and 50 °C, and optimum growth is at 37 °C. The bacterium is nonmotile, fermentative in GMD, negative for catalase and bile esculin, and positive for esculin, leucine arylamidase (LAP), pyrrolidonyl-arylamidase (PYR), and the CAMP test. *S. iniae* does not react with any of the available Lancefield typing antisera. Biochemical tests useful in differentiating *S. iniae* from other gram-positive cocci are found in Table 4.

d. Epizootiology—Opinions vary as to the source of *S. iniae* in the aquatic environment and its degree of pathogenicity. One school of thought is that the organism is widespread in the environment, possibly disseminated by homoiothermic animals, and that it causes disease in fish only under conditions of stress. Others feel that the bacterium is well adapted as a fish pathogen with limited host specificity and that it must be transmitted horizontally from sick or carrier fish to susceptible hosts. Currently this question is unresolved. What is known is that disease outbreaks are more common in high-density aquaculture environments and that epizootics are often preceded by degraded water quality conditions or by injury resulting from handling. Tilapia appear to become more susceptible when they are subjected to temperature extremes or rapid temperature shifts. Experimental infections were achieved in tilapia by injection, by oral intubation, and by immersion at 15 °C (59 °F) to 35 °C (95 °F), with the highest rate of mortality at 20 °C (68 °F) (126). The bacterium survives for only two days in sterile saline (0.85% NaCl), although it is able to survive in muds around sea farms in Japan from year to year (127). The zoonotic potential of *S. iniae* was realized in the winter of 1995–96 in Toronto, Canada, when eight cases of invasive disease were reported in people who sustained minor injuries (cuts or scratches) while preparing fresh whole tilapia purchased in local markets but originating from fish farms in the U.S. A single clone (determined by genetic analysis) is believed to be responsible for the human disease (128).

e. Geographic range and host susceptibility—*S. iniae* is currently known in the U.S. and Canada as a pathogen of tilapia and of hybrid striped bass, in Japan as a pathogen of yellowtail, ayu, tilapia, and flounder (8), and in Israel as a pathogen of tilapia and trout. The host specificity has not been well defined, but red drum (*Sciaenops ocellatus*), channel catfish, carp, and black seabream are refractory to experimental infection (126,129). The *Streptococcus* sp., described by Al-Harbi from hybrid tilapia in Saudi Arabia (130) and the *Streptococcus* sp., described from spinefoot (*Siganus canaliculatus*) cultured in Singapore (131) are believed to be biotypes of *S. iniae* (Al-Harbi, personal communication) (8).

f. Clinical signs of disease and treatment—Tilapia and hybrid striped bass infected with *S. iniae* circle listlessly at the surface, spiral, spin, or swim erratically. The disease is a typical bacterial septicemia in the acute phase. Infected fish often have petechiae around the anus and mouth, have congestion in the fins, and may exhibit bilateral or unilateral exophthalmia accompanied by corneal opacity. The liver, kidney, and spleen are pale and enlarged, and ascites can cause abdominal swelling. The chronic stages of the disease are characterized by infection of the brain, the optic nerve, and the eye, by subcutaneous abcesses, by fibrin deposits in the peritoneal cavity, and by pericarditis. Clinical signs in other species are very similar to those described above. Although the bacterium is sensitive to ampicillin, amoxicillin, oxytetracycline, and erythromycin, feeds medicated with these antimicrobic agents have met with only limited success, with recurrence of disease several weeks following treatment. Enrofloxacin in feed at 5 or 10 mg/kg/day (2.3–4.5 mg/lb) was an effective chemotherapeutic (123) when used to treat cultured hybrid striped bass. There are currently no USFDA-approved antimicrobic agents for treatment of streptoccocal disease in hybrid striped bass or tilapia in U.S. aquaculture. In Japan, erythromycin at 50 mg/kg/day (23 mg per lb) for four to seven days has been effective in cultured yellowtail. Formalin-killed injectable vaccines have been investigated by Eldar and have induced protection lasting four months in rainbow trout (132).

***II. Streptococcus difficilis ("Streptococcus difficile")—Group B, Type Ib Streptococcus* sp.** Causative agent of group B Streptococcosis. Synonyms: nonhemolytic streptococcal disease, bacterial meningoencephalitis.

a. History—Nonhemolytic group B, type Ib streptococci were first described as fish pathogens by Robinson and Meyer in 1966 (133) from diseased golden shiners (*Notemigonus crysoleucas*) cultured in farm ponds in

Arkansas, U.S. Koch's postulates were fulfilled in shiners, and several other species of fish were found to be susceptible to experimental infection with the streptococci. Unspeciated group B, type Ib nonhemolytic *Streptococcus* sp., were documented as causative agents of large fish kills in estuarine bays along the Florida and Alabama U.S. Gulf Coast in the fall of 1972 by Plumb et al., (134), in Lake Pontchartrain, Louisiana, U.S. in the fall of 1978 (135), and in the Chesapeake Bay, Maryland, U.S. in the fall of 1988 by Baya et al. (136). A variety of marine and estuarine species were affected during these outbreaks. A group B, type Ib *Streptococcus* has been responsible for mortality in bull minnows (*Fundulus grandis*) and in hybrid striped bass at mariculture facilities on the Alabama and Louisiana Gulf Coast from 1984 to the present (124,137). In 1994 Eldar et al., described a new species, *S. difficile*, as the causative agent of bacterial meningoencephalitis in cultured tilapia in Israel (121). *S. difficile* was later shown to be a group B, type Ib nonhemolytic *Streptococcus* that by whole cell protein electrophoretic analysis was identical to *S. agalactiae* (138). Biochemically, the bacterium was more similar to other group B, type Ib streptococci isolated from fish. In a study conducted in 1990, Elliott demonstrated the similarity in whole cell protein electrophoretic profiles of group B streptococci from humans, mice, cattle, frogs and fish (139). The group B streptococci isolated from shiners, tilapia, and estuarine fish are probably biotypes of the same species, although this has yet to be demonstrated by genetic analysis. The name was corrected to *S. difficilis* in 1998 (140).

b. Culture—Primary isolation is on TSA with 5% sheep blood or BHIA. Incubation is between 25–30 °C in a normal atmosphere; however, growth may be enhanced in an atmosphere of 5% O_2, 10% CO_2, and 85% N_2. Growth is slow, requiring 48 hours for typical opaque–white colonies to develop on blood agar. Selective isolation is best achieved on Columbia CNA agar with 5% sheep blood, and the bacterium fails to grow on enterococcal selective media containing sodium aside.

c. Description—*S. difficilis* is a gram-positive coccus (0.6–0.8 µm) in pairs or chains. The bacterium is nonmotile, and colonies on blood agar are (non) γ-hemolytic. Growth occurs between 20–30 °C, and the organism fails to grow above 35 °C or below 15 °C in a normal atmosphere. Growth occurs in BHI broth with 0.5–4.0% salt, but not in 6.5% salt. The bacterium is fermentative in GMD, negative for catalase and esculin hydrolysis, and positive for Voges–Proskauer, hippurate, alkaline phosphatase (PAL), leucine arylamidase (LAP), arginine dihydrolase (ADH), and acid from ribose. The CAMP reaction is variable. Biochemical tests useful in differentiating *S. difficilis* from other gram-positive cocci are found in Table 4.

d. Epizootiology—In natural fish kills caused by group B type Ib streptococci, high water temperatures, combined with poor tidal flushing in tributaries leading into estuaries, were speculated to be the stressors that initiated infections. In Alabama and Florida, the infections seemed to begin in menhaden (*Brevoortia* sp.), a schooling planktivorous fish, and spread to carnivores and scavengers that fed on the moribund and dead menhaden. Outbreaks on mariculture farms in Louisiana and Alabama were strongly correlated with high water temperatures in late summer and early fall and with bouts of low dissolved oxygen. Experimental infections have been conducted with tilapia (125) and bullminnows (137). The LD_{50} of *S. difficilis* in tilapia by IP injection, recorded over a 6-week period, was 10^7–10^8 colony-forming units (CFU) for cultures that had been passed repeatedly on culture plates and 10^2 CFU for cultures that had been passed 3× in fish. Rasheed and Plumb found the 96-hour LD_{50} for group B type Ib streptococci to be 1.4×10^4 CFU and the 7-day LD_{50} to be 7.5×10 CFU by IP injection, in bullminnows, but could not establish experimental infections by oral intubation with 10^6 cells or bath immersion in 10^{10} CFU/mL (137). When fish were injured by scratching the skin with a scalpel blade prior to immersion, mortality rates of 75–100% resulted after 7 days.

e. Geographic range and host susceptibility—*S. difficilis* and other group B type Ib streptococci are known in Israel as pathogens of tilapia and mullet, in Japan as pathogens of yellowtail, and in the U.S. as pathogens of the following wild marine and estuarine species: menhaden (*Brevoortia patronus*), sea catfish (*Arius felis*), striped mullet (*Mugil cephalus*), pinfish (*Lagodon rhomboides*), Atlantic croaker (*Micropogonias undulatus*), spot (*Leiostomus xanthurus*), stingray (*Dasyatis* sp.), silver sea trout (*Cynoscion nothus*), spotted sea trout (*Cynoscion nebulosus*), bluefish (*Pomatomus saltatrix*), red snapper (*Lutjanus campechanus*), striped bass (*M. saxatilis*), and weakfish (*Cynoscion regalis*) (7). Susceptible species in U.S. aquaculture/mariculture are tilapia, hybrid striped bass, bullminnows, and golden shiners. Freshwater species that have been experimentally infected are bluegill (*Lepomis macrochirus*), green sunfish (*Lepomis cyanellus*), buffalo (*Ictiobus cyprinellus*), goldfish (*C. auratus*), black crappie (*Pomoxis nigromaculatus*), and largemouth bass (*M. salmoides*). Channel catfish are refractory to experimental infection (133). American toads (*Bufo americanus*) were susceptible to experimental infection, and 80% mortality was reported in bullfrogs (*Rana catesbeiana*) cultured in Brazil (141).

f. Clinical signs of disease and treatment—The clinical signs of streptococcal disease in tilapia and hybrid striped bass are similar to those of *S. iniae* infection. Bacterial septicemia in the acute phase and meningoencephalitis in the chronic phase are the most common pathological conditions. Eye disease characterized by exophthalmia, corneal opacity, hemorrhage, and rupture of the globe are commonly observed in chronic infections. Death does not always result following infection, and a certain percentage of fish become blind as a result of infections of the eye or of the optic nerve. Enlargement of the spleen, exophthalmia, and focal necrosis of the liver are characteristic of the disease in bullminnows. Group B streptococci have been shown to be sensitive to terramycin, erythromycin, ampicillin, and amoxicillin, but difficulties with recurrent infections, similar to those encountered with *S. iniae* following antibiotic treatment,

are common. Erythromycin-medicated feed, 25–50 mg/kg (11–23 mg/lb/day) of body weight per day for four to seven days, was effective in controlling *Streptococcus* in yellowtail in Japan. Vaccines have been used experimentally to control the disease in tilapia, yellowtail, and hybrid striped bass.

***III. Other Streptococcus* sp.** Other species of *Streptococcus* have been implicated as fish pathogens, but information about these infections is limited; therefore, they will only be listed, along with their hosts and geographic locations:

Streptococcus dysgalactiae — tilapia; U.S.
S. sp. *similar to dysgalactiae* — ayu (*P. altivelis*); Japan.
S. parauberis — turbot (*S. maximus*); Spain.

B. *Streptococcus* — Like Bacteria

I. Lactococcus garvieae ("Enterococcus seriolicida"). Causative agent of *Lactococcus* septicemia. Synonyms: streptococcosis, enterococcosis, enterococcal infection.

a. History — Gram-positive cocci, producing -hemolysis on blood agar plates, have been implicated as causative agents of fish disease since the first description from rainbow trout in Japan by Hoshina in 1958 (116). The streptococcus of Hoshina has never been classified properly, on account of the lack of complete phenotypic data; however, it shares many characteristics with the streptococcus strains isolated from yellowtail mariculture farms near Shikoku Island, Japan by Kusuda in 1974 and from eels in Japan in 1977 (142,143). Since 1974, the disease has spread throughout Japan and has become the most economically important disease in Japanese mariculture (88). In 1989, the reported losses were 8,240 tons of yellowtail and 180.8 tons of other maricultured species such as sea bream, saurel (*Trachurus japonicus*), and flounder. The classification of the bacterium has gone through several changes in recent years. Initially it was referred to as *Streptococcus* sp., in the Japanese literature, because it did not conform to any of the described species as determined by biochemical and cultural characteristics or by Lancefield serotyping (144). In 1991, the organism was described by Kusuda as a new species, *E. seriolicida*, on the basis of DNA–DNA hybridization, despite its lack of a Lancefield group D antigen (145). In 1996, during studies of the DNA relatedness between strains of enterococci from various sources and lactococci isolated from water buffalos with mastitis, *L. garvieae* and *E. seriolicida* were found to be related at the species level. Because *L. garvieae* was the senior synonym of *E. seriolicida*, the name was retained (146).

b. Culture — Primary isolation is on TSA with 5% sheep blood, or BHIA, and incubation is at 30–35 °C in a normal atmosphere. Growth is rapid, but 48 hours may be required for full visualization of the small (1.0 mm diameter) white colonies. Selective isolation is achieved on phenyl ethyl-alcohol (PEA) blood agar or on blood agar containing .01% sodium aside.

c. Description — *L. garvieae* is a Gram-positive ovoid coccus (1.4 × 0.7 μm) forming short chains. The bacterium is nonmotile, and colonies on blood agar are α-hemolytic. Growth occurs between 10 and 45 °C, between 0 and 6.5% NaCl, and between pH of 4.5 and 9.6. Optimum growth is at 37 °C, 0% NaCl, and pH 7.5. The bacterium is fermentative in GMD, negative for catalase and hippurate hydrolysis, and positive for Voges–Proskauer, arginine dihydrolase, tetrazolium reduction, and bile-esculin hydrolysis. Biochemical tests useful in differentiating *L. garvieae* from other gram positive cocci are found in Table 4.

d. Epizootiology — *L. garvieae* may be detected in the aquatic environment year-round in Japan, but the highest counts are detected in sea water in the summer months in the vicinity of sea cages. Transmission of *L. garvieae* is horizontal, from one infected fish to another, in high-density aquaculture; however, the feeding of contaminated raw fish was determined to be a common source of infection (147). The bacterium was shown to survive for up to six months in frozen sandlance, a food commonly used in yellowtail culture. The bacterium has also been isolated from muds in the vicinity of sea cages during the cooler months of the year; these muds serve as a reservoir for infection (148). Wild fish such as sardine, anchovy and round herring are known to harbor the bacterium, and asymptomatic yellowtail can serve as carriers (147).

e. Geographic range and host susceptibility — *L. garvieae* is primarily a disease that affects cultured yellowtail and eel in Japan. Although the history is difficult to trace, because of problems with the taxonomy of streptococci pathogenic to fish, the initial outbreaks are believed to have been in the populations of yellowtail cultured near Shikoku Island from July to September of 1974 (88). Since 1974, the disease has occurred with increasing frequency and has become a disease of great economic importance in Japanese aquaculture. In cultured eels, the disease was first reported (by Kusuda) in 1978 (143).

f. Clinical signs of disease and treatment — Clinical signs of *L. garvieae* infections are similar to those due to other streptococcal infections in fish. The typical gross external clinical signs are exophthalmia, petechiae on the inside walls of the operculum, and congestion and hemorrhage in the intestine. Necrotic areas may be noted in the enlarged spleen and kidney. The first effective chemotherapeutant for the treatment of *L. garvieae* infections was erythromycin [25 mg/kg (11 mg/lb) fish/day for four to seven days in medicated feed], and, since 1984, erythromycin and spiramycin have been used to control the disease in Japan. Resistant strains have emerged in recent years; to combat infections due to them, josamycin has been used effectively at 30 mg/kg/day (13.6 mg/lb) for three days (149). Experimental vaccines have been effective at inducing protection, by immersion or by injection, but an effective vaccine has not been produced on a commercial scale. Nonspecific immunostimulants, such as the β-1,3 glucans, have produced increased resistance to infection by lactococci when injected at 2–10 mg/kg fish (150).

II. Lactococcus piscium, Carnobacterium piscicola ("Lactobacillus piscicola") and Vagococcus salmoninarum. Causative agents of pseudokidney disease in salmonids and of bacteremia in striped bass and channel catfish.

a. History—These three species are placed together on account of the similarity of the disease condition that they produce in salmonid fishes. Pseudokidney disease was named for the propensity to misdiagnose it as kidney disease, a condition in salmonids caused by the gram-positive bacterium *R. salmoninarum*, when only gram-stained tissue smears are examined. The latter is a common method of diagnosis, because *Renibacterium* is fastidious, and so special media and prolonged incubation times are required for isolation. Pseudokidney disease was described by Ross and Toth in 1974 (151) as a disease of adult salmonid broodfish that had undergone stress associated with handling or/or spawning. The causative organism was originally isolated and identified as *L. piscicola* by Hui, 1984 (152), but other unidentified coccobacilli were also isolated from certain fish. *L. piscicola* was renamed *C. piscicola* by Collins et al., in 1990 (153), who used molecular taxonomic methods. A new species, *V. salmoninarum*, was described in 1990 by Wallbanks et al. (154), from two cultures originally considered atypical lactobacilli isolated from diseased trout in Oregon and Idaho 1968 and 1981. *V. salmoninarum* infections were not again reported until yearly outbreaks of disease were recorded on a trout farm in the southwest of France from 1993–1997 (155). Another bacterium associated with pseudokidney disease of salmonids, *L. piscium*, was described as a new species by Williams et al., in 1990 (156), but very little information concerning pathogenesis or range of occurrence is available. Isolates of *Carnobacterium* spp., were reported by Baya et al., in 1991 (157), from cultured populations of striped bass and channel catfish and from wild populations of brown bullheads.

b. Culture—*Carnobacterium*, *Vagococcus*, and *Lactococcus* are not fastidious and are easily isolated on standard media such as TSA, TSA with 5% sheep blood, or BHIA. Incubation is between 20–25 °C, and small, white, round, entire, 1-mm colonies appear within 48 hours.

c. Description—1. *V. salmoninarum* is a short, ovoid, nonmotile, gram-positive coccobacillus (0.5 × 2 μm) occurring singly, in pairs, or in short chains. The bacterium is facultatively anaerobic; it grows between 5 and 37 °C but fails to grow at 40 °C. Tests for esculin hydrolysis, hippurate, and H_2S are positive; tests for arginine dihydrolase (ADH), catalase, oxidase, and urease are negative. Positive results are obtained in tests for the enzymes alkaline phosphatase (PAL), leucine arylamidase (LAP), pyrrolidonyl–arylamidase (PYR), and para–nitrophenyl–galactosidase (PNPG). Most strains are weakly α-hemolytic after two to three days of incubation. Growth occurs at pH 9.6 and in the presence of 40% bile, but not in 6.5% NaCl.

2. *C. piscicola* is a short, nonmotile, gram-positive rod (0.5 × 1.5 μm) occurring singly, in pairs or in short chains. The bacterium is facultatively anaerobic; it grows between 10 and 37 °C but fails to grow at 42 °C. Tests for esculin hydrolysis, ADH, and PYR are positive; tests for hippurate, urease, and H_2S are negative. Most strains are α-hemolytic and grow at pH 9.6 and in 6.5% salt but not in the presence of 40% bile. Results of PAL and LAP enzyme tests are not available.

3. *L. piscium* is a nonmotile, gram-positive coccus (0.5 × 1.0 μm) occurring singly, in pairs or short chains. The bacterium is nonhemolytic and facultatively anaerobic; it grows between 5 and 30 °C but fails to grow at 40 °C or at pH 9.6. Tests for esculin hydrolysis and starch hydrolysis are positive; tests for ADH, urease, H_2S, hippurate, and PYR are negative. Test results for growth in 40% bile are not available. Biochemical tests useful in differentiating this group of gram-positive organisms are included in Table 4.

d. Epizootiology—Pseudokidney disease is normally a chronic condition in salmonids, one resulting in low mortality rates and generally regarded as an opportunistic infection caused by one or more of the gram-positive organisms listed in this section. The bacteria have traditionally been considered to be normal components of the aquatic microflora and to gain entrance into the fish following trauma associated with spawning. Losses can be substantial, however, as is evidenced by mortality rates as high as 50% on trout farms in France caused by *V. salmoninarum* infections. The affected fish were adult trout weighing 600–4000 g (1.3 lb to 8.8 lb), and peak mortality followed the handling associated with the sorting and stripping of fish for spawning (155). Water temperatures during the outbreaks were lower than in other cases of pseudokidney disease, with peak mortality at around 9–10 °C (48–50 °F). A report by Michel in 1986 (158) indicated that young salmonids and carp reared in western Europe were susceptible to infection with *C. piscicola* and *L. piscicola*, although they noted that adult fish were the ones most commonly affected.

e. Geographic range and host susceptibility—*C. piscicola* is most commonly associated with adult salmonids with pseudokidney disease from the Pacific Northwest region of North America (152,159) and from Newfoundland, Canada (160); it has also been reported from brown trout (*Salmo trutta*), rainbow trout (*O. mykiss*), and common carp (*C. carpio*) cultured in France and Belgium (158). Isolates of *V. salmoninarum* have been obtained (*1*) from salmonids from the Pacific Northwest of the U.S. (154) and (*2*) from the southwest of France from diseased rainbow trout (155). The *L. piscium* type culture, HR1A-68, was isolated by R.A. Holt from rainbow trout at the Hood River Hatchery, Oregon (156). *Carnobacterium* spp., similar to *C. piscicola* were isolated from cultured striped bass and channel catfish and from wild populations of brown bullheads from Chesapeake Bay, Maryland (157). Attempts to confirm Koch's postulates with Maryland strains were successful in trout, but striped bass and catfish seemed to enter into a carrier state after being injected with the bacterium. Difficulty in recreating pseudokidney disease by experimental infection has been noted by several authors;

it is probably due to the low virulence of the bacterial strains (158,159).

f. Clinical signs of disease and treatment—*Carnobacterium* infections in salmonids are systemic and usually chronic in nature, with one or more of the following pathological signs: abdominal distension due to ascites, splenomegaly, muscle granulomata, internal hemorrhages, subcutaneous sanguineous vesciculation, and renal granulomata (152,158). In trout infected with *V. salmoninarum*, the prominent pathological manifestations were septicemia (with epicarditis) and meningitis (155). Treatment of pseudokidney disease is not well documented; however, strains of *Carnobacterium* have been shown to be susceptible to tetracycline, ampicillin, and erythromycin. Treatment of *V. salmoninarum* infections in France with ampicillin, amoxicillin, or erythromycin failed to prevent or decrease trout mortality. Vaccines for pseudokidney disease have not been considered, because of the opportunistic nature of the disease and the variety of causative bacteria.

***III. Enterococcus faecium, E. faecalis and Enterococcus* spp.** Causative agents of enterococcosis of rainbow trout and other species.

a. History—Enterococcus-like bacteria were recorded as fish pathogens as early as 1974 by Boomker et al., in 1979 (161) from rainbow trout cultured in South Africa; additional reports from this same geographic area were published in 1986 by Bragg and Broere (162). Carson and Munday described a disease in farmed rainbow trout in Australia caused by a similar organism, from outbreaks between 1982 and 1990 (163). Today, the disease is considered one of the major diseases of farmed fish in Australia and South Africa. Other published accounts of organisms presumptively identified as *Enterococcus* sp., or *E. faecalis* have been described from farmed rainbow trout in Italy by Ceschia (164) and Ghittino (cited in 164). In an unpublished case report, *E. faecium* was determined to be the causative agent of enterococcosis in hybrid striped bass cultured in semiclosed intensive culture systems in the U.S. (7), and unidentified enterococci have been isolated from diseased channel catfish (165). An organism presumptively identified as *Enterococcus* sp., has been described from diseased turbot cultured in Galicia, northwest Spain (166).

b. Culture—Primary isolation is on TSA with 5% sheep blood or BHIA and incubation at 30–35 °C in a normal atmosphere. Growth is relatively fast, but 48 hours may be required for formation of typical 1 mm, smooth, white colonies. Selective isolation is achieved on aside blood agar (0.2 g/L sodium aside) (Difco Laboratories, Detroit, Michigan) or by using a selective isolation procedure outlined by Bragg (167): In this method, tissue samples are placed in nutrient broth containing 100 μg/mL nalidixic acid and 160 μg/mL oxolinic acid or 200 μg/mL of sodium aside, and they are incubated for three days at room temperature; samples from the broth are then plated on tetrazolium agar plates, and small red colonies appearing on this medium are presumptively enterococci. Cephalexin–Aztreonam–Arabinose Agar (CAA) has also been used for the selective isolation and differentiation of *E. faecium* (168).

c. Description—The genus *Enterococcus* was created in 1984 to include the enteric streptococci *Streptococcus faecalis* and *S. faecium*, and, since that time, 17 additional species have been proposed for inclusion in the genus. The enterococci are gram-positive cocci occurring singly, in pairs, or short chains. The organisms are facultatively anaerobic and catalase-negative, and optimum growth occurs at 35 °C, however, most strains grow at 10 and 45 °C. The bacteria also grow in 6.5% NaCl and at pH 9.6 and hydrolyze esculin in the presence of 40% bile salts. Most hydrolyze pyrrolidonyl–naphthylamide (PYR), produce leucine arylamidase (LAP), and possess the Lancefield group D antigen. Biochemical tests useful in differentiating *Enterococcus* spp., from other gram-positive cocci are included in Table 4. Recent isolates from Italy (164), Spain (166), Australia, and South Africa (169) may ultimately be identified as biotypes or serotypes of *L. garvieae*, because they fail to grow at 45 °C, do not possess the group D antigen, and are otherwise biochemically identical to *L. garvieae*. This problem cannot be solved until genetic analysis is done on the strains in question. Strains from Spanish turbot differ serologically from the Italian trout strains as visualized by immunoblots and ELISA, and trout strains are not immunogenic in turbot (170).

d. Epizootiology—Temperatures were in the range of 19 °C (66 °F) on South African farms where outbreaks first occurred, and temperatures did not fluctuate due to the groundwater source; however, in subsequent cases reported in 1986 by Bragg and Broere, temperatures ranged from 18 to 25 °C (64–77 °F) and were believed to be a stress factor. Other factors such as water quality, feed, and feeding practices were found to be acceptable, but temporary overcrowding during periods of new pond construction did seem to be a predisposing factor to disease. On Italian farms, poor water quality was implicated as a factor leading to increased susceptibility to infection. The source or reservoir of the pathogen has not been determined, but, once it is established in a fish population, horizontal transmission can occur through damaged skin or by the fecal-oral route. Infectivity studies with turbot (mean weight 10 g) produced an LD_{50} in the range of 10^4 cells by intraperitoneal injection. Chronic disease in hybrid striped bass occurred at temperatures between 26 and 28 °C (79–82 °F) in fish stocked at high densities; this case was complicated by a parasitic infection with *Epistylis* sp.

e. Geographic range and host susceptibility—Thus far, enterococci have been implicated as causative agents of disease for rainbow trout in Australia, South Africa, and Italy, for turbot in Spain, and for hybrid striped bass and channel catfish in the U.S. Outbreaks in South Africa have occurred in cultured populations of rainbow trout in the Eastern Transvaal area; however, an extensive survey of fish populations in eight river systems in Natal yielded no isolations of enterococci or streptococci (171).

f. Clinical signs of disease and treatment—In trout, turbot, and hybrid striped bass, the clinical signs are reminiscent of other streptococcal diseases in fish.

Bilateral exophthalmia, corneal opacity, subcutaneous abcesses in periorbital spaces and at the base of fins, and caudal peduncle are common external signs. Internal clinical signs include pale liver, dark red enlarged spleen, hemorrhage in the intestine, and ascites in the peritoneal cavity. Treatment depends on the sensitivity of the individual strain isolated, because enterococci are often resistant to many of the common antibiotics used in aquaculture. Antibiotics that have been used in various cases include oxytetracycline, erythromycin, ampicillin, and enrofloxacin; however, the disease is chronic in nature, and short term medicated feed treatments are rarely effective. Vaccines are not yet available for enterococcal infections in fish.

C. Other Aerobic and Facultatively Anaerobic Gram-Positive Cocci. Organisms in this group have uncertain status as fish pathogens and will therefore just be listed with their hosts:

Micrococcus luteus—rainbow trout; UK.
Aerococcus viridans—lobsters.
Planococcus sp.—Atlantic salmon rainbow trout; UK.
Staphylococcus aureus—silver carp; India.
S. epidermidis—yellowtail, red sea bream; Japan.

D. Aerobic Gram-Positive Rods

I. Renibacterium salmoninarum. Causative agent of bacterial kidney disease. Synonyms: Dee disease, corynebacterial kidney disease, salmonid kidney disease.

a. History—Bacterial kidney disease (BKD) was first described in the early 1930s in wild populations of Atlantic salmon from the Dee river in Scotland and was thus named "Dee disease" (172). It was subsequently found in rainbow trout, brook trout (*Salvelinus fontinalis*), and brown trout (*Salmo trutta*) in the U.S.: in Massachusetts by Belding and Merrill, and in California by Wales (as cited by Earp (173)). The first isolations from Canada were in 1937, in cutthroat trout (*Salmo clarki*), by Duff (as cited by Evelyn et al., in 1986 (174)). Now BKD is known from hatchery and farmed salmonid populations in many parts of the world, from both fresh and marine waters, but only rarely has it been observed causing mortality in wild fish stocks. The causative agent of BKD was originally classified as a *Corynebacterium* (175), on the basis of morphology and staining. As additional information was gathered following the first successful culturing of the organism, investigators realized that it belonged in a new, previously undescribed genus. The new genus and species was described, and it was named *R. salmoninarum* by Sanders and Fryer in 1980 (176).

b. Culture—Culture of the fastidious causative agent of bacterial kidney disease was not achieved until Earp used a specially formulated nutrient-rich medium. Growth on this medium was poor, requiring more than 14 days to obtain small colonies. Continued improvements were made on the isolation medium, but most significant was the discovery by Ordal and Earp in 1956 (175) that L-cysteine added to nutrient-rich blood agar provided a significant boost in growth. This medium (cysteine blood agar) was used until the development of KDM2 medium by Evelyn in 1977 (177), which utilized the addition of fetal calf serum and replaced blood and many of the other nutrients with peptone, cysteine, and yeast extract. KDM2 and a selective medium, SKDM, derived from it by Austin et al., in 1983 (178) are currently in use for the primary isolation of *R. salmoninarum*. At 15 °C incubation, the organism requires 20 days for the appearance of smooth, white, creamy, 2-mm colonies. Because the organism grows slowly, plates must be kept moist during the incubation process. Research on improved culture techniques is ongoing, and rich, serum-free, semidefined media have been developed by Embley (179) and Sheih (9).

c. Description—*R. salmoninarum* is a gram-positive rod (0.5 × 1.0 μm) occurring singly or in pairs. The bacterium is a nonmotile, not spore-forming, not acid-fast, fastidious bacterium that grows best at 15–18 °C. The bacterium fails to grow at temperatures above 25 or below 5 °C. *R. salmoninarum* strains from different geographic locations are homogeneous, typically being positive in tests for catalase, litmus milk, alkaline phosphatase, caprylate esterase, glucosidase, leucine arylamidase, α-mannosidase, and trypsinase. The organism fails to liquefy gelatin and does not grow in 1% sodium chloride. Serologically, isolates appear to be homogeneous when tested using polyclonal antisera, however, differences among strains can be detected with monoclonal antibodies (180). All strains apparently possess a heat-stable 57-kd surface protein. Antibodies to this antigen have been useful for detecting *Renibacterium* by ELISA, and it has been determined that the observed strain differences result from the recognition by monoclonal antibodies of different epitopes on the 57 kd surface protein (181).

d. Epizootiology—It is generally felt that *R. salmoninarum* is an obligate pathogen of salmonids and that its reservoir of infection is other infected salmonids. Outbreaks of BKD have not been reported from nonsalmonids. Survival of the pathogen can occur outside the host, but it is short-lived in water and mud. Bacterial kidney disease can be present as an overt infection, or it can exist in a carrier-only state. Water quality seems to influence cumulative mortality and severity of disease; BKD is more common at soft-water hatcheries than at those with high total hardness. A serious problem is the effect of BKD on the survival of salmon smolts as they are moved from freshwater to seawater. Mortality rates as high as 17% have occurred in smolts moved to saltwater, as against 4% in those kept in freshwater (182). BKD can occur over a wide range of temperatures, but most epizootics occur in the fall between 12 and 18 °C (54–64 °F) and in the winter between 8 and 11 °C (46–52 °F). Water temperature influences the time between experimental exposure and death. Mortality occurs at 30–35 days postexposure at 11 °C (52 °F) at 60 to 90 days postexposure at 7–10 °C (45–50 °F). Bullock, in 1978 (183), was able to demonstrate vertical transmission of BKD and theorized that the bacterium was carried within the egg, because surface disinfection of the egg

had no effect on transmission efficiency. This hypothesis was later proven to be true by Evelyn et al. (174), who demonstrated that *Renibacterium*-infected coelomic fluid is the source of infection for the egg. Several studies have shown that the dietary composition of feed influences the susceptibility of fish to *Renibacterium* infections (8).

e. Geographic range and host susceptibility — BKD has been reported from most areas of the world where salmonids occur, with the exception of Australia, New Zealand, and the Soviet Union. The disease has been reported from the United Kingdom, Europe, Japan, North America, and South America (Chile). All salmonids are considered susceptible, but brook trout and chinook salmon are the most susceptible. Natural outbreaks have been documented only in salmonids; however, a few fish species, such as the sable fish, the Pacific herring, the shiner perch, the common shiner, and the fathead minnow, have been infected experimentally.

f. Clinical signs of disease and treatment — External clinical signs of BKD are uncommon, except in the terminal stages of disease. The most common early signs of infection are dark pigmentation, exophthalmia, hemorrhages at the base of fins, and abdominal distension associated with a widely disseminated bacteremia. As the disease progresses, pale gills, exophthalmia, cutaneous blisters, and ulcerative abcesses may be seen externally; internally, cavitations in the musculature, bloody/turbid fluid, and creamy-white granulomatous lesions are common in the kidney. Treatment of BKD by chemotherapy is not particularly effective, presumably because of the intracellular nature of the pathogen. Erythromycin is the antimicrobial of choice for treatment and prophylaxis against BKD. Treatment recommendations vary from oral administration in medicated feed at 100 mg/kg (45 mg/lb/day) of fish/day for 21 days for fingerlings [Wolf and Dunbar 1959 (184)] to injection of prespawn female salmon with 10–20 mg/kg (4.5–9.0 mg/lb) body weight and 1–2 mg/L (ppm) for 30 min as an additive during water-hardening of eggs. Disinfection of egg surfaces using iodophors at 25–100 mg/L (ppm) for 5 minutes has proven beneficial in reducing transmission of the disease. Although research on vaccination of salmonids for BKD has been ongoing since 1971 (185), there has been only limited success, and a commercial vaccine is currently not available.

II. Other Aerobic Gram-Positive Rods. Several other species of aerobic gram-positive, rod-shaped bacteria have been implicated as pathogens of fish, but because of uncertainty as to their significance, they are only mentioned here along with their hosts:

Corynebacterium sp. — rainbow trout (186).
Corynebacterium aquaticum — striped bass (187).
Rhodococcus sp. — chinook salmon (188).

E. Gram-Positive, Acid-Fast Rods

I. Mycobacterium marinum ("Mycobacterium piscium, M. platypoecilus, M. anabanti"), M. fortuitum, M. chelonei ("M. chelonei* subsp. *piscarium, M. salmoniphilum"). Causative agent of mycobacteriosis of fish. Synonym: fish tuberculosis.

Three species of mycobacteria are recognized as fish pathogens; they are discussed together here because of the similarities in the pathologic condition they produce in fish.

a. History — Acid-fast staining bacteria were first described as fish pathogens of common carp in 1987 in Europe by Bataillon (189); this is one of the oldest known fish diseases. The name "fish tuberculosis" was coined to reflect the similarity to human tuberculosis: the acid-fast staining reaction, and the granulomatous lesions commonly seen in infections. This old name has given way over the years to the less threatening name "mycobacteriosis." The first isolation of an organism in this group was reported by Bataillon in 1902 (190), but the cultures of that organism, named *Mycobacterium piscium*, have been lost, and the name lacks validity. *Mycobacterium marinum* was described by Aronson in 1926 (191) from a tropical coral fish kept at the Philadelphia Aquarium. *M. marinum* was originally thought to cause disease only in marine fish; however, it has subsequently been reported from many freshwater species as well, and the original isolates of *M. piscium* are believed to have been a strain of *M. marinum*. *Mycobacterium fortuitum* was isolated from diseased neon fish in 1953 (192) and continues to occur commonly in tropical aquarium species. Mycobacteriosis of Pacific salmon was originally described by Earp et al., in 1953 (173), and the causative organism, *Mycobacterium salmoniphilum*, was cultured, described, and named by Ross in 1960 (193). *M. salmoniphilum* was later shown to be a strain of *M. chelonei* (194), with the species name being corrected to *M. chelonei* (195).

b. Culture — Isolation and culture of the mycobacteria is difficult due to their fastidious nature and slow growth rate. Primary isolation is best achieved on Dorsett egg, Lowenstein–Jensen, Petragnani, or Middlebrook 7H10, with subcultures maintained in capped tubes. If the cell number per gram of tissue is high and contamination is low, general-purpose media such as TSA with 5% sheep blood may be used for primary isolation. Incubation should be at 20–30 °C for 2–30 days. *Mycobacterium fortuitum* is classified as a rapid grower, and colonies should appear in less than seven days on culture media. *M. chelonei* is also classified as a fast grower on subculture, but primary isolation may take several weeks. *M. marinum* is a slow grower, requiring 7–10 days for visible growth and several weeks for typical colonies to develop at 25 °C. Of the three species, only *M. fortuitum* is capable of growth at 37 °C. Selective isolation from contaminated sites on or in the fish may be enhanced by homogenizing the tissue in a grinder and treating it with 0.3% solution of the disinfectant Zepheran® (benzalkonium chloride 17%) for 20 minutes, prior to the adding of several drops of the homogenate to culture media (196). Because of the extended incubation times, care must be taken to keep plated media moist, by incubating in a humid atmosphere.

c. Description — Representatives of all the species of mycobacteria pathogenic to fish are Gram-positive, acid-alcohol-fast, non-motile, non-sporing, pleomorphic rods ($1.0–4.0 \times 0.2–0.6$ μm), with occasional filamentous or

coccoid forms. Colonies of *M. marinum* may vary in their appearance, depending on the culture medium and incubation time. *M. marinum* is said to be photochromogenic, because cultures incubated in the dark will produce white colonies, whereas cultures exposed to light will form yellow to yellow/orange colonies. Other mycobacteria can be photochromogenic, so this characteristic alone cannot be used for identification; however, *M. marinum* does not reduce nitrate and cannot grow at 37 °C, to separate it from similar organisms. Both *M. fortuitum* and *M. chelonei* are nonchromogenic and grow more rapidly than *M. marinum*, producing smoother, white- to buff-colored colonies, and are differentiated from other mycobacteria by their ability to grow on MacConkey agar. *M. fortuitum* is positive on iron-uptake, sucrose-utilization, and nitrate-reduction tests; they separate it from *M. chelonei*, which is negative on these tests. In addition to pigment and growth at 37 °C, *M. marinum* may be differentiated from *M. fortuitum* and *M. chelonei* by positive results for production of nicotinamidase and pyrazinamidase.

d. Epizootiology—Little is known about the epizootiology of mycobacterial infections in fish. The organisms mentioned in this section are all common in the soil and in freshwater and marine environments, and the factors that lead to the development and spread of disease are unknown. Mycobacterial infections are more common in aquarium fish and in food fish cultured in closed or semi-closed recirculating tank systems (197); they are very rare in pond fish. Stress due to overcrowding, to poor water quality, and to ingestion of contaminated food or aquatic detritus have all been mentioned as factors leading to mycobacterial infections. Feeding infected trash fish to cultured Pacific salmon (192) and snakehead (*Channa* sp.) (198) resulted in mycobacteriosis. Vertical transmission has been confirmed in the Mexican platyfish (*Xiphophorus maculatus*), but it has not been confirmed in salmonids (199). Historically, mycobacteriosis has not been the cause of disease in natural fish populations, and it has only rarely been seen as subclinical infections in feral fish (200). In 1997 and 1998, outbreaks of mycobacteriosis in wild populations of striped bass in Chesapeake Bay were documented by Vogelbein (201), with as many as 30 to 50% of the striped bass in certain tributaries reported as having skin lesions. As many as five different species of mycobacteria, including *M. marinum* were isolated from fish in this study, and the underlying cause of the epizootic is under investigation.

e. Geographic range and host susceptibility—Nigrelli and Vogel (202) published a list of 151 species of freshwater and marine fish that were susceptible to mycobacteriosis. That list has continued to grow, and the opinion is generally held that most, if not all fish species are susceptible to the disease. The occurrence of mycobacteriosis is worldwide in both marine and freshwater locations. *Mycobacterium marinum* and *M. fortuitum* are pathogenic to humans, and *M. marinum* causes the condition known as "fish handler's disease" or "swimming pool granuloma." The names result from the tendency for cutaneous granulomas to develop in the skin at the extremities of the body, such as the fingers, the toes, and the outsides of the elbows and the knees. Human infections are confined to the extremities by the optimum temperature range of the bacterium, which is normally 30–33 °C (86–91 °F).

f. Clinical signs of disease and treatment—Mycobacteriosis is a chronic, systemic, progressive, wasting disease that often goes unnoticed in a fish population until a high percentage of fish have become infected. Gross clinical signs include anorexia, emaciation, shallow hemorrhagic skin lesions, ulcers, and fin erosion. Cold-water salmonids may not exhibit all of these signs. Internally, greyish-white miliary granulomas are seen primarily in the spleen and the head kidney, but they may be found in most of the tissues of the fish. Organs such as the spleen and the kidney may be greatly enlarged and have a granular appearance. Treatment of mycobacterial infections, even when an early diagnosis is made, is not economically feasible for food fish. Infected stocks are best destroyed, and the systems disinfected, before new fish are brought in. Cleaning and disinfection of fish systems with HTH, chlorox solution, or chlorine dioxide-based sterilants is best. Care should be taken by fish culturists when handling infected fish, to avoid spine wounds, skin abrasions, and prolonged contact with water containing mycobacteria. More valuable ornamental fish may be treated in accordance with the antimicrobic susceptibility of the strain isolated.

II. *Nocardia asteroides, N. seriolae ("N. kampachi")*.

Causative agents of nocardiosis.

The species are discussed together because of the similarity in the disease condition they cause in fish.

a. History—Nocardiosis was first described (from the neon tetra, a tropical freshwater fish), in 1963, by Valdez and Conroy (203). Snieszko et al., described the disease in rainbow trout in 1964 (204) and Campbell and MacKelvie found the disease in brook trout in 1968 (205). The species responsible for these early cases was identified as *Nocardia asteroides*. A second species, *N. kampachi*, was described by Kariya (206) in 1968 as the causative agent of disease in cultured yellowtail in Japan. The name *N. kampachi* was never fully accepted by taxonomists, and in 1988 Kudo et al. (207), proposed the name *N. seriolae* for the causative agent of nocardiosis of Japanese yellowtail. Nocardiosis has become one of the more important diseases in Japanese mariculture, with 262 tons of yellowtail reported lost to the disease in 1989 (88).

b. Culture—Nocardiae are typically less fastidious than the mycobacteria, and they can be cultured on standard media such as TSA with 5% blood and BHIA, although they also grow on Lowenstein–Jensen and Ogawa egg media. Incubation should be between 20 and 30 °C. Colonies of *Nocardia asteroides* appear in 4–5 days; 10 days may be required for *N. seriolae* colonies to appear. Colonies of *N. asteroides* are ridged and folded, are pigmented pinkish-white to yellow-orange, and produce aerial hyphae along the colony margin. *N. seriolae* produces flat and wrinkled colonies.

c. Description—*Nocardia* sp., are gram-positive, weakly acid-fast, nonmotile, long-branching bacilli that, in tissue imprints, resemble mycobacteria. Identification of isolates as *N. asteroides* is based on the physiological and

biochemical description of Gordon and Mihm in 1962 (208). Acid is produced from glucose and glycerol but not from adonitol, arabinose, erythritol, inositol, lactose, maltose, mannitol, raffinose, sorbitol, or xylose. A characteristic of *N. asteroides* is the ability to grow at 37 °C; however, not all isolates of this species from fish are capable of growing at this temperature, and *N. seriolae* does not grow at 37 °C. *N. seriolae* is biochemically very similar to *N. asteroides*, but differs in the sugar fermentation pattern and organic acid utilization patterns reported by Kusuda (209).

d. Epizootiology — Norcardiosis is a slowly developing chronic infection, with accompanying low mortality. It is known to be a normal inhabitant of soil and water, and carrier fish may serve as a reservoir of infection. On rare occasions, mortality rates can be as high as 20% over a two week period, as was reported from cultured Formosan snakehead in ponds on Taiwan (210). Snieszko et al. (204), were unable to transmit *N. asteroides* to rainbow trout by feeding, but injection reproduced the disease in one to three months. Kusuda (209) also succeeded in transmitting the disease in yellowtail by injection or by smearing surface wounds with *N. seriolae*, results indicating a preference for this route of infection.

e. Geographic range and host susceptibility — Nocardiosis has been reported from the United States, Argentina, Germany, Japan, and Taiwan, and the potential exists for its occcurrence on a world wide basis. Thus far, infections with *N. asteroides* have been reported in rainbow trout, brook trout, neon tetra, snakehead, and giant gourami, and *N. seriolae* ("*N. kampachi*") has been reported in the yellowtail.

f. Clinical signs of disease and treatment — Nocardiosis shares many characteristics with mycobacteriosis, and the two diseases are often confused, particularly when histopathology is the only tool used for diagnosis. Young fish are the most vulnerable, but all ages may be affected, particularly in the late summer and early fall. In early stages of the disease in yellowtail, the clinical signs are emaciation, inactivity, and discoloration of the skin. Subcutaneous abcesses appear, later in the infection, in the skin and gill; they are white and about 5 mm (0.44 in.) in diameter. These lesions become histologically tuberculoid, with a fibrous capsule and bacterial filaments located in the center of the lesion. Abdominal distension resulting from internal granulomata may occur. Diffuse granulomatous lesions may be found in the skeletal muscle, in all of the visceral organs (particularly the spleen), and in the mesentery. Little is known concerning the treatment of norcardiosis. Food fish such as the yellowtail have been treated with 4–100 mg (1.8–45.4 mg/lb/day) of sulfamonomethoxine/kg/day for five to seven days, and mortality has been slowed; but a cure has not been reported. In most cases, removal and disposal of infected fish is the proper course of action followed by disinfection of facilities.

F. Gram-Positive Anaerobic Rods

I. Eubacterium tarantellus. Causative agent of eubacterial meningitis of marine and estuarine fish.

a. History — An anaerobic gram-positive rod was determined to be the causative agent of a large fish kill on Biscayne Bay, Florida in 1976 (211). The striped mullet (*M. cephalus*) was the species primarily affected; it exhibited a neurological condition resulting from a bacterial meningitis. In 1977, Udey et al. (212), described the bacterium as a new species, *Eubacterium tarantellus*. The *Catenabacterium* described from epizootics in red drum and grey mullet along the Texas coast is most likely the same organism (9,213).

b. Culture — The organism is readily cultured from the brain and liver on standard media, such as TSA with 5% sheep blood or BHIA plates with anaerobic incubation in a gas pack or other suitable anaerobic chamber at 20 °C for three to five days. Culture plates should be prereduced prior to primary isolation. Tissues from fish, including brain tissue, may be inoculated directly into fluid thioglycollate medium with 1% NaCl and 100 mcg/mL gentamycin incubated at 30 °C.

c. Description — *Eubacterium tarantellus* is a long, unbranched, filamentous, gram-positive, asporogenous rod that can fragment into shorter bacilli of 1.3–1.6 × 1.0–17.0 μm. Good growth occurs between 20 and 37 °C under anaerobic conditions, and colonies on agar media are flat, translucent, approximately 2–5 mm in diameter, colorless, rhizoid, and slightly mucoid. Colonies on blood agar are surrounded by a zone of β-hemolysis.

d. Epizootiology — Under laboratory conditions, the disease cannot be transmitted by direct fish-to-fish contact but can be reproduced by intraperitoneal injection of susceptible hosts, such as the mullet, with the bacterium. This fact had led some investigators to suspect that transmission occurs via external parasites. The organism has an affinity for the brain, the spinal cord, and the liver, regardless of the mode of infection. The salinity tolerance of the organism is 2 ppt, so it would be restricted to low-salinity environments. The bacterium has been found in several species of marine and estuarine fish showing no signs of disease, so some species may serve as carriers of the organism.

e. Geographic range and host susceptibility — The bacterium has been found in a variety of estuarine fishes, including the striped mullet, the snook (*Centropomus undecimalis*), the red drum, and the Gulf flounder (*Paralichthys albigutta*) (213). The range is not well known, but outbreaks of the disease have been restricted to estuaries of the Gulf of Mexico along the coasts of Florida and Texas.

f. Clinical signs of disease and treatment — Eubacterial meningitis is characterized by a neurological impairment that causes erratic swimming behavior of fish near death. Following experimental infection, the progress of the disease is slow, with clinical signs not appearing until 14 to 30 days postinoculation. Affected fish show darkened pigmentation, uncoordinated swimming movements, and inability to maintain proper orientation in the water column. Moribund fish may hang vertically, lie still on the bottom of the tank, swim slowly while rotating about their long axis, or whirl. Little is available in the literature concerning treatment of this disease; however, suggestions include the controlling of ectoparasites and

the use of erythromycin- and oxytetracycline-medicated feed at 100 mg/kg/day (45.4 mg/lb/day) for 5 days.

***II. Clostridium botulinum, Clostridium* spp.** *Clostridium botulinum* and other *Clostridium* species have been isolated from diseased cultured fish on several occasions, but their significance in aquaculture is uncertain and therefore is not covered in detail. Isolation of strictly anaerobic, gram-positive rods with subterminal or terminal ovoid endospores would indicate the possibility of *Clostridium* spp.

G. Miscellaneous Bacterial Agents: Gliding, Flexing, and Yellow-Pigmented Gram-Negative Bacteria

I. Flavobacterium columnare ("Bacillus columnaris, Chondrococcus columnaris, Cytophaga columnaris, Flexibacter columnaris"). Causative agent of columnaris disease.

a. History—Columnaris disease was first described by Davis in 1922 (214) in warm-water fish from the Mississippi River, but it was not until the work of Ordal and Rucker in 1944 (215) that the organism was isolated, characterized, and named *Chondrococcus columnaris*. Davis originally named the organism *Bacillus columnaris*, inspired by the formation of columnar masses of cells that were visible microscopically on wet mounts of infected fish tissue. A year following the work of Ordal and Rucker, Garnjobst published an account of isolation of the bacterium and assigned it to the genus *Cytophaga* (216). The bacterium has been renamed and reclassified several times over the years on the basis of morphological and biochemical features, having been referred to at various times as *Chondrococcus columnaris*, *Cytophaga columnaris*, and *Flexibacter columnaris* (9). The currently accepted name for the organism, *Flavobacterium columnare*, was adopted in 1996, as suggested by Bernardet (217); however, total agreement has not been achieved, as evidenced by the opinion of Bader and Shotts, following their work on sequence analysis of the 16s ribosomal-RNA genes of *F. columnare* and other closely related organisms in this group (218), that the genus *Flexibacter* should be retained.

b. Culture—Culture of *F. columnare* may be accomplished on a variety of low-nutrient, low-agar-content media. The commonly used medium is the cytophaga agar (CA) of Anacker and Ordals (219); however, shorter incubation times and higher yields may be obtained in media containing salts, such as Shieh medium (220). Selective isolation of columnaris can be achieved from contaminated external sites such as the skin and gills on selective cytophaga agar (SCA) (221) or on Hsu-Shotts (HS) medium (222). *Flavobacterium columnare* may be incubated at 10–33 °C, but primary isolation plates are typically incubated at 28–30 °C. Pale yellow rhizoid colonies are present after 48 hours of incubation. A dilute form of Mueller–Hinton medium must be used for antimicrobic susceptibility testing (with disc diffusion methods) or for determination of minimal inhibitory concentration (MIC) in broth (221).

c. Description—Long, slender, gram-negative rods (0.3–0.5 × 3–10 μm), motile by gliding on surfaces, nonmotile in suspension except for flexing movement, with formation of columnar aggregates of cells on infected tissue (often referred to as "haystacks"). The physiological characteristics of *F. columnare*, as described by Bernardet and Grimont (223), are the following: strict aerobic growth; no acid produced from carbohydrates; positive for cytochrome oxidase and catalase; nitrate reduced to nitrite; positive for hydrogen sulfide; cellulose, chitin, starch, esculin, and agar not hydrolyzed; gelatin, casein, and tyrosine hydrolyzed; arginine, lysine, and ornithine not decarboxylated; flexirubin pigments produced. The NaCl tolerance is reported as 0.5%, but this may be variable. Griffin (224) devised a simple method of identifying *F. columnare*, using five characteristics that separate it from other yellow-pigment-producing aquatic bacteria:

1. Ability to grow in the presence of neomycin sulfate and polymyxin B
2. Colonies on CA plates typically rhizoid and pigmented pale yellow
3. Production of gelatin-degrading enzymes
4. Binding of congo red dye to the colony
5. Production of a chondroitin sulfate-degrading enzyme.

d. Epizootiology—Columnaris disease is one of the most common diseases in freshwater aquaculture worldwide, affecting a wide variety of species. It may occur as a secondary infection following stress or injury or as a primary infection. Columnaris disease most often occurs as an external infection of the skin, the fins, or the gills, but it has been isolated systemically from fish showing no external clinical signs. These internal isolates may indicate a systemic form of the disease; however, histopathological lesions are often lacking. Mixed infections involving other bacterial pathogens are also common. Hawke and Thune (221) found from 99 different cases of bacterial disease in catfish ponds in Louisiana,U.S.A. that columnaris disease was diagnosed in 53.5% of the submissions. In this study, *F. columnare* was the sole etiological agent in 7.0% of the cases; it was present in mixed infections with other pathogens (i.e., *Aeromonas* spp., *E. ictaluri*, and *E. tarda*) in 46.5% of the cases. Transmission of columnaris disease is typically from fish to fish via the water, but stress resulting from handling and poor water quality exacerbates the disease. Survival of *F. columnare* is poor in water with pH less than 7.0, with hardness less than 50 mg/L (ppm), or with low organic matter. Only 35% of inoculated cells survive for one week in sterile pond water at 20 °C (68 °F); this result indicates a need for carrier fish to maintain the organism in the aquatic environment (225).

e. Geographic range and host susceptibility—Columnaris exists worldwide in a variety of freshwater habitats. The channel catfish is the most severely affected aquaculture species in the U.S.; however, golden shiners, fathead minnows, hybrid striped bass, and goldfish are susceptible. In Europe and Asia, columnaris is a problem in cultured eels and in common carp. Salmonids and cultured

centrarchids are also susceptible under permissive water temperatures. Anderson and Conroy (226) listed 36 species of fish from which columnaris disease had been described, and most species of fish are considered susceptible to infection.

f. Clinical signs of disease and treatment—Columnaris disease is characterized by shallow, white-to-yellowish focal patches of necrosis in the skin, often extending around the dorsal fin (hence, the term "saddleback"). Necrotic foci in the gills may also be observed, and lesions often appear brown or muddy, a coloration due to clay particles or detritus trapped in the slime secreted by the bacteria. Secondary infection of skin lesions by *Aeromonas* spp., is common and results in deeper, liquefactive lesions in the muscle. On catfish, the infected skin loses its natural sheen, and a grey-to-white margin surrounds the lesion. The mouth and inner walls of the oral cavity may be covered with a yellowish mucoid material. Scraping those areas and observing wet mounts of the tissue microscopically at 400× allows the investigator to observe the typical thin, filamentous, flexing rods and "haystack" formation described previously. This method of diagnosis is commonly used, although it is advisable to isolate the bacterium in pure culture and to run confirmatory biochemical tests or serology, for reliable identification. Improved husbandry and water quality conditions can help prevent columnaris disease, but, once an outbreak has occurred, only two basic methods of treatment are available. External columnaris may be treated by adding potassium permanganate to the water at 2 ppm over the permanganate demand. Potassium permanganate imparts a red color to the water and this color should persist for four to five hours for an effective treatment. Potassium permanganate is on deferred status by the USFDA: though currently permitted for use on food fish, it is not yet fully approved, and it may be made illegal in the future if new evidence finds it to be unsafe. Feeds medicated with Romet® or Terramycin® are effective for controlling columnaris disease, although they are not labeled specifically for treatment of this disease in the United States (227). Maintaining a salinity of 5 ppt is inhibitory to the development of columnaris disease.

II. Flavobacterium branchiophilum ("F. branchiophila"). Causative agent of bacterial gill disease.

a. History—Bacterial gill disease (BGD) of salmonids was originally described by Davis in 1926, from a salmonid fish hatchery (228). The primary etiological agent of BGD, *Flavobacterium branchiophila*, was described in 1989 by Wakabayashi (229), and the name of the bacterium was revised in 1990 to *F. branchiophilum* (230). *Cytophaga aquatilis* was also described as a causative agent of BGD by Strohl and Tait (231); this bacterium has been renamed *Flavobacterium hydatis* (217). It is suspected that other related *Flavobacterium* spp., *Cytophaga* spp., and *Flexibacter* spp., play an opportunistic role in BGD (7). The disease is now known to be multifactorial; degraded environmental conditions work in concert with bacteria to produce the classic lesions of BGD.

b. Culture—Primary isolation of *Flavobacterium branchiophilum* and related organisms associated with BGD is on cytophaga agar (CA). *Flavobacterium branchiophilum* is somewhat fastidious and grows slowly, producing 0.1–1.0 mm yellow, round, transparent, colonies on CA after two to five days of incubation at 25 °C. The organism grows between 10 and 30 °C, but not at 37 °C.

c. Description—Long, thin, gram-negative rods (0.5 × 5 to 8 μm); nonmotile by any mechanism; oxidase-positive; gelatin, casein, and starch hydrolyzed, but not chitin or esculin; acid produced from glucose, fructose, sucrose, maltose, and trehalose.

d. Epizootiology—The epizootiology of BGD is confusing, because many different etiologic agents have been implicated, and attempts to fulfill Koch's postulates with various isolates have failed. *Flavobacterium branchiophilum* has been successfully transmitted, and BGD was experimentally induced in the laboratory, but extreme environmental stress was required to initiate infection. It is now accepted that BGD is initiated following injury to the gills by chemical or physical irritants (7). If excess feed or detritus is present in the water column, these particles may become impinged in the hyperplastic and mucus-slime-covered gills and cause asphyxiation. Salmonid fry and fingerlings less than 5 cm (2 in.) in length are most susceptible to BGD, and mortality rates in affected populations can be as high as 80% over a one-week period. The survivability of *F. branchiophilum* in water is unknown, but infection is believed to be transmitted from fish to fish. Disease outbreaks commonly occur between 12 and 19 °C.

e. Geographic range and host susceptibility—Bacterial gill disease has been found in most parts of the world where fish are cultured in intensive freshwater systems. *Flavobacterium branchiophilum* has been reported from Japan, the United States, Hungary, the Netherlands, and Canada. Many species of fish are susceptible to BGD; however, the cultured species most affected are all of the salmonids, the common carp, goldfish, channel catfish, eels, and fathead minnows (232).

f. Clinical signs of disease and treatment—Bacterial gill disease is diagnosed by microscopic examination of diseased gill tissue. The presence of tufts of filamentous bacteria between hyperplastic secondary lamellae are indicative of the disease. Culture on CA is recommended for isolation and identification of *F. branchiophilum* and other related organisms. Bacterial colonization and fusion of the gill lamellae begins on the distal end of filaments and results in "clubbing" of the gills. Maintenance of good water quality and reduction of stress is the best method for controlling BGD. Disinfectants such as benzalkonium chloride have been used successfully at 1–2 mg/L (ppm) for one hour for treating infected salmonids in raceways, but the margin of safety is small. Chloramine-T at 8–10 mg/L (ppm) for one hour is the most effective treatment for BGD; however, neither of these compounds is currently approved by the USFDA for treatment of food fish (7,8).

III. Flavobacterium psychrophilum ("Cytophaga psychrophila, Flexibacter psychrophilus"). Causative agent of bacterial cold-water disease. Synonym: peduncle disease.

a. History—A disease of salmonids originally referred to as "peduncle disease" was described by Davis in 1946

from the eastern United States (233). This observation of a characteristic open lesion on or near the caudal peduncle led to the common name first used for the disease. The causative bacterium was isolated, was described, and was named *Cytophaga psychrophila* by Borg in 1960 (234); however, Bernardet and Grimont, using DNA relatedness techniques, renamed the organism *Flexibacter psychrophilus* (223). The bacterium has recently received additional reclassification; it is currently named *Flavobacterium psychrophilum* (217).

b. Culture—Primary isolation is on cytophaga agar (CA) incubated at 15–20 °C. After 48–96 hours incubation, 1–5 mm, bright yellow, raised, convex colonies with a thin spreading irregular edge are visible. Improved growth is reported for this organism in Shieh broth and Shieh agar media (220) and in tryptone-yeast extract (TYE) (8). Generally, growth is limited on standard media such as TSA. The organism grows at temperatures between 4 and 25 °C. No growth is detectable at 30 °C.

c. Description—Long, slender, Gram-negative rods, (0.3–0.75 × 2–7 μm), but filamentous forms 10–40 μm in length are occasionally observed. The bacterium is motile by gliding on solid surfaces, nonmotile in suspension. The physiological characteristics of *F. psychrophilum*, as described by Bernardet and Grimont, are: strict aerobic growth; no acid produced from carbohydrates; oxidase-negative; catalase-positive; negative for hydrogen sulfide; hydrolysis of casein, gelatin, albumin, elastin, tyrosine, and collagen; no hydrolysis of agar, cellulose, starch, or chitin; flexirubin pigments are produced. The NaCl tolerance is 0.5 to 1.0%.

d. Epizootiology—Bacterial cold-water disease (BCWD) appears in the early spring at hatcheries, when water temperatures are between 4 and 10 °C (39–50 °F), and the severity of the disease depends on the age and development of the affected fry. In sac fry, the mortality rate may be as high as 50%, but, when the disease occurs in fingerlings in rearing units, losses are usually less than 20%. Experimental infection was induced by Holt (235) in coho and chinook salmon and in rainbow trout at temperatures of 3 to 15 °C (37–59 °F), but, above 15 °C, the severity of the disease was greatly reduced. The natural reservoir of the bacterium is not understood, but it is generally believed that transmission is from fish that serve as carriers of the organism.

e. Geographic range and host susceptibility—Bacterial cold-water disease occurs throughout the trout- and salmon-growing regions of North America, Europe, and Japan. It is a serious problem in the northwestern United States and western Canada. All salmonid species are believed to be affected, but coho salmon are particularly susceptible (8). The nonsalmonids affected include European eels, carp, tench (*Tinca tinca*), and crucian carp (*Carassius carassius*).

f. Clinical signs of disease and treatment—The clinical signs of BCWD vary with the size and the age of affected fish. When the disease occurs in sac fry, the primary sign is erosion of the skin covering the yolk sac. Older fish exhibit lethargy, spiral swimming behavior, and the classic shallow peduncle lesion. The caudal peduncle of affected fingerlings first turns white; the skin then becomes necrotic and is sloughed off, leaving exposed underlying muscle tissue. Lesions can also occur laterally, dorsally, or on the isthmus. Internally, petechiae may be observed in the liver, pyloric cecae, adipose tissue, heart, swim bladder, and peritoneal lining. Outbreaks of BCWD are difficult to treat, because the infection is systemic and because affected fry do not feed. Bath treatments with water-soluble Terramycin® at 10–50 mg/L (ppm) and with quaternary ammonium compounds at 2 mg/L (ppm) are effective when infections are confined to the skin. For systemic infections, oxytetracycline in the diet at 50–75 mg/kg of fish/day (23–34 mg/lb) for 10 days has been found to be effective (7,8).

IV. Flexibacter maritimus ("Cytophaga marina"). Causative agent of salt water columnaris. Synonym: black-patch necrosis of Dover sole (*Solea solea*).

a. History—A marine form of columnaris disease was described as early as 1960 by Borg (234), and a detailed account of a columnaris-like disease in juvenile red sea bream in Japan was given by Masumura and Wakabayashi (236). *Flexibacter maritimus* was first described in 1986 from a variety of marine fish species in Japanese aquaculture by Wakabayashi (237). An organism described as the causative agent of "black patch necrosis" of cultured Dover sole in Scotland (238) was later determined to be *F. maritimus*. Recently, *F. maritimus* has been found to be the cause of mortality in farm-reared sea bass in France (239) and in turbot, Atlantic salmon, and coho salmon cultured in Spain (240). Chinook salmon reared in sea cages in southern California, as well as white sea bass (*Atractoscion nobilis*), northern anchovy (*Engraulis mordax*), and Pacific sardine (*Sardinops sagax*), have been diagnosed with *F. maritimus* infections (241).

b. Culture—This bacterium must be cultured on cytophaga agar (CA) prepared with at least 30% sea water. Addition of sodium chloride to CA is not sufficient to culture the organism. Incubation is at 25–30 °C; however, growth can occur between 15 and 34 °C (8). A selective medium for the isolation of *F. maritimus* from external lesions has been developed in Spain (240).

c. Description—Long, slender, gram-negative rods, 0.5 × 2.0–10.0 μm, with occasional filamentous forms 30 μm in length; motile by gliding on solid surfaces, nonmotile in suspension except for flexing; and formation of columnar aggregates of cells on infected tissue. *Flexibacter maritimus* produces catalase and cytochrome oxidase and hydrolyses casein, gelatin, tributyrin, and tyrosin. Hydrogen sulfide is not produced; acid is not produced from carbohydrates and agar; cellulose, chitin, starch, and esculin are not degraded. Nitrate is reduced to nitrite. Flexirubin pigments are not produced.

d. Epizootiology—Saltwater columnaris of red sea bream occurs in the spring after transfer of juveniles from the hatchery to inshore net cages. The disease rarely affects sea bream greater than 60 mm (2.3 in.) in length. Stress occurring during the transfer period is believed to be responsible for initiation of infection. Black patch necrosis occurs in juvenile Dover sole between 60 and 100 days of age and occurs more frequently in the summer.

e. Geographic range and host susceptibility—*Flexibacter maritimus* is confirmed from red sea bream, black sea bream, and flounder in Japan and from the Dover sole in Scotland; however, it is speculated that many species of marine fish are susceptible to the disease. Sea-cage reared salmonids are also susceptible on the Pacific coast of the United States (241) and off the coast of Spain (239).

f. Clinical signs of disease and treatment—The clinical signs of saltwater columnaris and of black patch necrosis are similar to those of columnaris disease in fresh water species. (See sections G, I, and F of this entry). Avoiding overcrowding, overfeeding, and stress is the best means of managing the disease; antibiotic feeds have met with little success. Addition of a sand substrate to tanks containing juvenile Dover sole greatly reduced the incidence of black patch necrosis (238).

H. Obligate Intracellular Bacteria

1. Piscirickettsia salmonis. Causative agent of salmonid rickettsial septicemia. Synonyms: coho salmon syndrome, Huito disease.

a. History—Salmonid rickettsial septicemia (SRS) of coho salmon was first noticed in Chile as early as 1981, but it was not until 1991, in the published account of Cvitanich (242), that a formal description of the disease was given and the disease was formally named. The incidence of the disease has increased over the years; currently, it is the major problem affecting salmonid aquaculture in Chile. The organism was formally recognized as belonging to a new taxon, and the name *Piscirickettsia salmonis* was proposed by Fryer (243). This name currently has valid standing. Since 1988, the disease has been recognized as a problem on 51 farms on the west coast of Norway in cage-reared Atlantic salmon (244).

b. Culture—Attempts to culture *P. salmonis* on artificial media have been unsuccessful. The organism must be grown in cell culture, on cell lines of salmonid and non-salmonid origin; chinook salmon embryo CHSE-214 is the cell line of choice for isolation from infected kidney tissue. Incubation is at 15–18 °C, and cytopathic effect is witnessed within five to six days.

c. Description—*Piscirickettsia salmonis* is an obligate intracellular pathogen. The cells are pleomorphic but are predominantly gram-negative cocci (0.5 × 1.5–2.0 μm) that are nonmotile.

d. Epizootiology—Salmonid rickettsial septicemia was first reported from the southern coast of Chile in salmonids reared in sea cages. There are no indigenous salmonids on the Pacific coast of South America; it is theorized that *P. salmonis* originated from local marine fish species and infected the introduced salmon. Samples of various ectoparasites of Chilean salmon revealed positive identification of *P. salmonis* from *Cerathothoa gaudichaudii*, a hematophagous external parasite now believed to be a vector for the disease (245).

e. Geographic range and host susceptibility—Once thought to be restricted to salmonids in Chile, the disease has now been reported from Atlantic salmon in Norway (244). Experimental infections have been achieved in coho salmon and rainbow trout (246).

f. Clinical signs of disease and treatment—Infected fish gather at the surface of cages and exhibit lethargy and inappetence. External signs include darker-than-normal coloration, pale gills, and hemorrhagic skin lesions. Internally, the fish exhibit ascites, peritonitis, and pale discoloration of swollen organs. The gastrointestinal tract, swim bladder, and fat deposits may have petechial hemorrhages. The disease is not apparent in the freshwater stage of culture; it does not appear until 6–12 weeks after transfer to seawater. The organism is sensitive to some antibiotics, among them clarithromycin, chloramphenicol, erythromycin, gentamycin, oxytetracycline, sarafloxacin, and streptomycin, but it is not sensitive to penicillin (8,9).

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See also ANTIBIOTICS; CHLORINATION/DECHLORINATION; DISEASE TREATMENTS; DRUGS.

BAITFISH CULTURE

NATHAN STONE
University of Arkansas at Pine Bluff
Pine Bluff, Arkansas

OUTLINE

Development of Baitfish Farming
Culture Methods
- Facilities
- Propagation
- Fry Transfer Method
- Fertilization
- Natural Foods, Nutrition, Feeds, and Feeding
- Water Quality
- Diseases
- Other Pond Management Problems
- Annual Production Cycle in Arkansas
- Harvesting
- Holding, Grading, and Transport

Marketing
Economics
Bibliography