

## 1.2.1 Bacterial Gill Disease

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### A. Name of Disease and Etiological Agent

Bacterial gill disease was first reported by Davis (1926); however, it was not until 1978 that the causative agent was isolated on a bacteriological medium (Kimura et al. 1978). Wakabayashi et al. (1989) conducted physiological and phenotypic characterization studies using isolates recovered from fish from the United States, Japan and Hungary (Kimura et al. 1978; Wakabayashi et al. 1980; Farkas 1985) and named the bacterium *Flavobacterium branchiophila*. *Flavobacterium branchiophilum* was subsequently recognized as the proper and current nomenclature (von Graevenitz 1990).

Combined, the Flavobacterial diseases encompass a wide host range and geographic distribution. This is due, in part, to their ubiquitous nature and broad range in water temperatures in which they persist. Many species of fish worldwide are susceptible to one or more *Flavobacterium* spp. Other fish flavobacterial diseases involve pathology to the gills, for instance, columnaris disease caused by *F. columnare*. However, columnaris disease can be distinguished from BGD by characteristic cell “columns” observed microscopically in wet mount preparations; *F. columnare* is easily cultured and colony growth is unique- a pale yellow, sticky, rhizoid morphology. Ostland et al. (1999) recovered *F. psychrophilum* from the gills of intensively reared rainbow trout *Oncorhynchus mykiss* suffering from an atypical bacterial gill disease. This disease occurred at water temperatures below 10°C and clinical disease signs were distinct from BGD by *F. branchiophilum*. *Flavobacterium psychrophilum* can also be readily cultured and distinguished using standard testing. Abnormal gill pathologies may also occur from non-infectious agent causes, such as turbidity, high unionized ammonia, chlorine or other toxic agents in the water.

### B. Known Geographical Range and Host Species of the Disease

#### 1. Geographical Range

Generally considered worldwide and in conjunction with intensive culture of salmonids. Because BGD is primarily a disease related to environmental conditions and is common and relatively straightforward to control, both the geographic range and affected hosts are likely to be underreported. Reports of BGD caused by *F. branchiophilum* originate from the United States, Canada, Japan, Korea, Hungary, India and The Netherlands (Farkas 1985; Wakabayashi et al. 1989; Heo et al. 1990a; Ostland et al. 1994; Ko and Heo 1997; Swain et al. 2007).

## 2. Host Species

BGD caused by *F. branchiophilum* has been reported from rainbow trout *Oncorhynchus mykiss*, brook trout *Salvelinus fontinalis*, aurora trout *Salv. fontinalis timagamensis*, lake trout *Salv. namaycush*, splake *Salv. fontinalis* X *Salv. namaycush*, chinook salmon *O. tshawytscha*, steelhead trout *O. mykiss*, sheatfish *Silurus glanis*, Yamame *O. masou*, sockeye salmon *O. nerka*, Atlantic salmon *Salmo salar*, brown trout *Salm. trutta*, walleye *Stizostedion vitreum*, rohu *Labeo rohita*, catla *Catla catla* and silver carp *Hypophthalmichthys molitrix* (Nagel 1976; Wakabayashi et al. 1980, 1989; Farkas 1985; Ostland et al. 1994; Rach et al. 2000; Swain et al. 2007; Good et al. 2008, 2010). Additionally, Ostland et al. (1995) reported mortality in common shiners *Luxilus cornutus* using a bath challenge exposure to *F. branchiophilum*. Although bacterial gill disease affects all ages of fish, small fish are primarily affected. Disease outbreaks are common in spring and early summer as water temperatures increase and when hatcheries typically have their greatest inventories of small fish. BGD is widely considered to be a problem of cultured fishes, particularly salmonids, and is not recognized as being problematic to wild fish populations.

## C. Epizootiology

Transmission of *F. branchiophilum* is horizontal. The bacterium is highly contagious to fish. Attachment and colonization of trout gill tissues occurs in less than one hour following exposure to viable *F. branchiophilum* cells, and within a matter of hours of cohabitation with infected fish (Ferguson et al. 1991; Ostland et al. 1995). When rainbow trout were exposed to *F. branchiophilum* cultures by immersion, mortality was dose dependent (Ostland et al. 1995). Both virulent and avirulent strains of *F. branchiophilum* readily adhere to gill tissues; however, only the virulent isolates were capable of colonization (Ostland et al. 1995). Mortality can quickly increase, perhaps within a day or two, and the percent of dead fish in a lot can be high if husbandry is not improved and if a treatment is not promptly administered. In fact, Speare et al. (1991a, 1995) used the word “explosive” to describe the potential morbidity and mortality. At hatcheries that are continually plagued with BGD, *F. branchiophilum* is thought to be a ubiquitous organism in the water supplies with disease outbreaks typically occurring in conjunction with increased host stressors. Water temperatures for BGD range from 5–6°C to at least 19°C (Farkas 1985; Bullock et al. 1991, 1994).

Many researchers consider that BGD outbreaks typically occur in conjunction with or as a result of predisposing factors, which foster disease progression and severity (Bullock et al. 1994). Host and environmental predisposing factors that are thought to be important include overcrowding, low dissolved oxygen, reduced water flows, elevated unionized ammonia ( $\text{NH}_3$ ), and turbidity in water. Swain et al. (2007) reported that overcrowding and overfeeding were predisposing factors in natural outbreaks in rohu and catla. Following an epidemiological examination of factors related to BGD in salmonids, Good et al. (2008) identified brook trout (i.e., species or host) and spring time (March – May) as significant for diseases among salmonids in Ontario, Canada. Although anecdotal, the percent mortality lost to BGD often appears to be related to the extent of overcrowding. It has been stated that BGD naturally thins the fish loading to a comfortable density. Monetary losses at fish hatcheries due to BGD may be considerable even for small fish when time and effort for spawning are factored in. Cumulative monetary losses are greater for stocking-size and larger fish due to the long-term investments of rearing the fish. *Flavobacterium branchiophilum* does not produce diseases in other animals or humans.

*Flavobacterium branchiophilum* apparently persists in the water or sediment. Hatchery-resident fish and indigenous fish in water supplies may also contribute as reservoirs of infections. In a survey of fish hatcheries in North America that rear rainbow trout, a significant association of BGD outbreaks was found at those facilities that previously experienced BGD (Bebak et al. 1997). Hatcheries that had indigenous fish in hatch-house water supplies were also at a greater risk to experience BGD

outbreaks. At some facilities, heavy rains that disturb fine particulate material that had settled in supply pipes or spring water collection basins have been followed by subsequent BGD outbreaks occurring within a day or two (C. E. Starliper, unpublished observations). These sediment-related outbreaks have occurred at facilities without feral fish in their water supplies and among healthy fish with no apparent host stressors. It is thought that this particulate material irritates the gills in addition to the sediment potentially being a reservoir for *F. branchiophilum*.

## D. Disease Signs

### 1. Behavioral Signs

Unique clinical disease signs are associated with BGD. Disease signs include lethargy, loss of appetite, gasping at the top of the water, and sluggish response to external stimuli such as hitting the side of a tank or waving a hand over the fish. The fish will swim high in the water column and align in a "soldier-like" fashion near and into the incoming tank water flow in an apparent effort to reach the coolest and most oxygenated water. These signs indicate that the fish are under high duress in an effort to respire. Mortality will quickly increase in the succeeding hours and days and if left untreated.

### 2. Gross Signs

Bacterial gill disease affects the gills. Body surface lesions and internal pathology are typically not evident. The gill filaments will be pale and swollen (Figure 1) to the extent that they will be unable to lay down flat and as such, the dorsal ends of the opercula will be flared open to varying degrees depending on the severity of swelling. Swain et al. (2007) reported dark body coloration, congested eyes, and necrotic gills in rohu and catla.

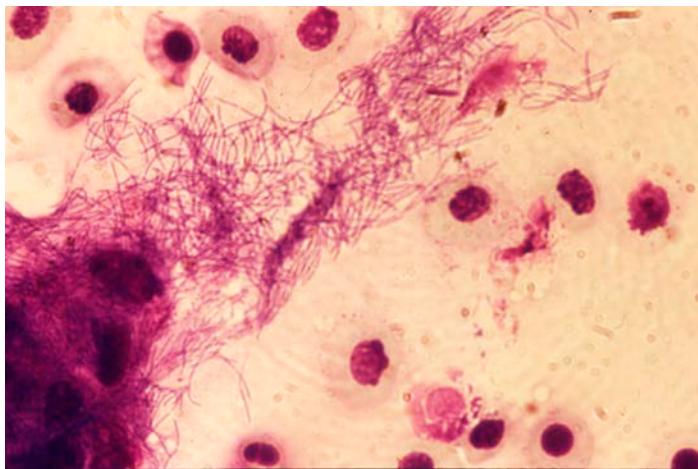


**Figure 1.** Fingerling rainbow trout (*Oncorhynchus mykiss*) affected with bacterial gill disease showing severely swollen gills. Photo courtesy of Dr. Chris Wilson, Utah Division of Wildlife Resources.

### 3. Microscopic Signs

Wet mounts (400  $\times$ ) will reveal masses of filamentous cells, hyperplasia of gill epithelium, fusion of gill lamellae, and clubbing of gill filaments (Bullock et al. 1991; Speare et al. 1991b). Bacterial

colonization is on the external gill tissues and is not restricted to certain areas (Speare et al. 1991b); however, with light microscopy the masses of cells will be particularly obvious along the periphery of affected tissues. Stained gill smears (e.g., simple-stain, Gram-stain; Section 2, 3.8.A “Gram Reaction”) will show numerous long, thin Gram negative cells, approximately 0.5 µm wide and 5 - 8 µm long (Figure 2).



**Figure 2.** Diff-Quik stained gill impression smear from a fingerling rainbow trout (*Oncorhynchus mykiss*) with bacterial gill disease showing masses of typical long, thin rods characteristic of *Flavobacterium branchiophilum*. 1,000 × magnification. Photo courtesy of Dr. Chris Wilson, Utah Division of Wildlife Resources.

## E. Disease Diagnostic Procedures

### 1. Presumptive Diagnosis

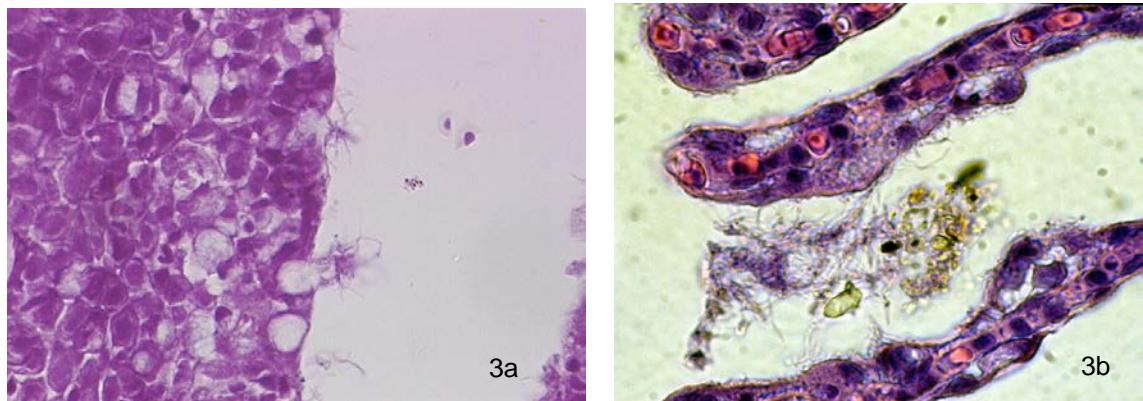
#### a. Microscopy and Case History.

A presumptive diagnosis can confidently be made by experienced personnel based on the characteristic behavior demonstrated by the fish, previous facility history and microscopy. Consideration should be given as to the susceptibility of the host species involved, previous BGD outbreaks to this or other fish lots, and seasonality and production cycle with peak numbers of small fish. Fish rearing conditions are important considerations, such as density or overcrowding, overfeeding, inadequate water flow, cleanliness of the tanks or raceways, and recent changes in a water supply, for example, to make up flow due to dry conditions. Inadequate water flow leads to reduced dissolved oxygen, increased ammonia-nitrogenous wastes, along with the potential loss of any self-cleaning benefit in tanks or raceways.

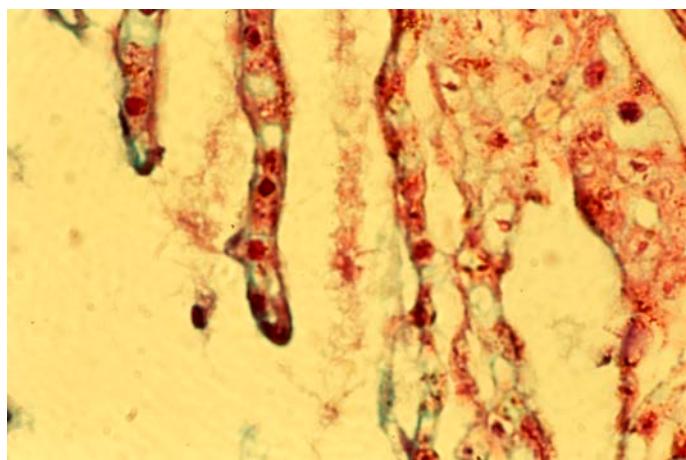
#### b. Histology

Gill tissues or gill arches may be placed in Bouin’s fixative or phosphate buffered 10 % formalin. After 24 hr. in Bouin’s, Ostland et al. (1990) transferred the tissues to 70 % isopropyl alcohol, whereas, Lumsden et al. (1993) transferred them to 70 % ETOH and Bullock et al. (1994) placed them into 65 % ETOH. Speare and Ferguson (1989) and Speare et al. (1991c) quickly fixed gills in Bouin’s fixative to minimize epithelial capillary separation and epithelial cell hypertrophy which are artifactual changes created by delays in transferring to either Bouin’s or phosphate buffered 10 % formalin. Gills need to be placed in fixative within 20 sec to avoid these changes. Gills may be stained with hematoxylin and eosin or periodic acid-Schiff.

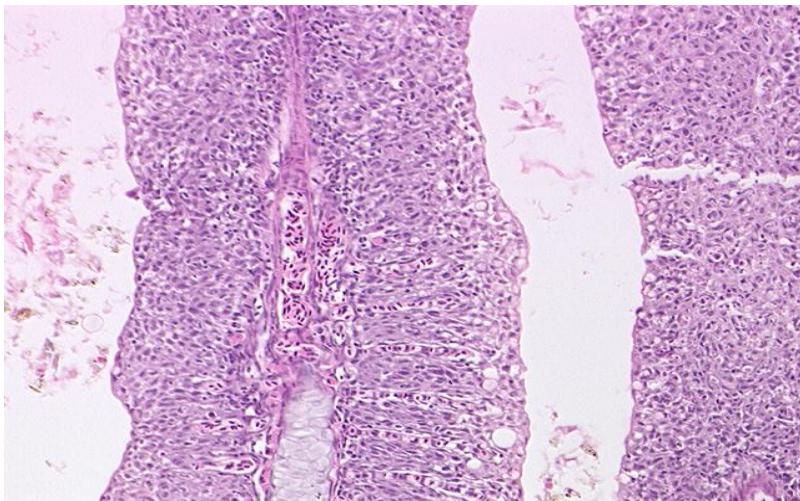
Histopathology to gills caused by BGD may be extensive with the severity of lesions being positively correlated with the extent of bacterial colonization (Ostland et al. 1990; Byrne et al. 1991). Noted pathological changes in addition to obvious presence of masses of filamentous bacteria (Figures 3a and 3b) include fusion of gill lamellae and filaments, hypertrophy (Figure 4) and hyperplasia (Figure 5) of pavement cells, lamellar epithelial and chloride cells, epithelial and chloride cell degeneration and necrosis, and mucus cell hyperplasia.



**Figures 3a,b.** Masses of bacteria, presumably *Flavobacterium branchiophilum*, attached to the epithelium of rainbow trout (*Oncorhynchus mykiss*) gill lamellae affected with bacterial gill disease. Stained with hematoxylin and eosin; 1,000 × magnification. Photos courtesy of Dr. Vicki Blazer, USGS Leetown Science Center (3a) and Dr. Chris Wilson, Utah Division of Wildlife Resources (3b).



**Figure 4.** Histologic section of fingerling rainbow trout (*Oncorhynchus mykiss*) gill lamellae affected by bacterial gill disease showing cellular hypertrophy. Stained with hematoxylin and eosin; 1,000 × magnification. Photo courtesy of Dr. Chris Wilson, Utah Division of Wildlife Resources.



**Figure 5.** Histologic section of rainbow trout (*Oncorhynchus mykiss*) gills affected by bacterial gill disease showing extensive hyperplasia and fusion of the secondary lamellae. Stained with hematoxylin and eosin; 1,000 $\times$  magnification. Photo courtesy of Dr. Vicki Blazer, USGS Leetown Science Center.

## 2. Confirmatory Diagnosis

### a. Bacterial Culture

Primary bacterial culture of *F. branchiophilum* is not routinely attempted by fish health diagnosticians. The slow-growing nature of the bacterium does not lend itself to identification using standard biochemical characterizations when the typically high mortality demands a quick response. The ability to accurately diagnosis BGD based on clinical disease signs and microscopy also provides that opportunity for a timely response.

When primary isolation of *F. branchiophilum* is done, cytophaga agar (Anacker and Ordal 1959) is typically used. The recipe for cytophaga medium is provided at Section 1, 1.1.1 General Procedures for Bacteriology. The best performance of this medium for *Flavobacterium* spp. is achieved using the paste (i.e., not the dried ingredient) form of beef extract (Lorenzen 1993) and 1.5 % agar may be used to prepare plates to better facilitate the streak-plate inoculation technique. Culture growth is aerobic at 10 - 25°C with optimal growth at 18°C (Wakabayashi et al. 1989). Aseptically collect gill tissue samples from diseased fish and inoculate media by streak plating or drop plate inoculating gill tissue dilutions, for example serial dilutions prepared in cytophaga broth. After 5 days of incubation, colonies are small (0.5 – 1.0 mm in diameter), pale yellow, smooth, with a slightly raised center and non-spreading, entire border (Wakabayashi et al. 1989). Ostland et al. (1994) reported 3 – 5 mm colony diameters after 6 - 7 days of incubation at 18°C. Section 1, 1.1.1 General Procedures for Bacteriology includes links to the individual tests in Section 2 used for bacterial identifications. Isolates do not grow in presence of NaCl greater than 0.1 % or on nutrient agar. Catalase and cytochrome oxidase are produced; gelatin, casein and starch are utilized, and acid is produced from glucose, fructose, sucrose, maltose, trehalose, cellobiose, melibiose, raffinose and inulin. Esculin is not hydrolysed, and isolates are negative for motility, indole, nitrate reduction, and DNase. Acid is not produced from arabinose, lactose, mannitol, rhamnose, salicin, and xylose. The DNA G+C content ranges from 29 to 31 mol %. Isolates from Ontario, Canada and Korea shared biochemical and physiological characteristics; however, cell lengths of up to 15  $\mu$ m were observed (Ostland et al. 1994; Ko and Heo 1997).

### b. Polymerase Chain Reaction

Polymerase chain reaction (PCR) is currently not in wide use for diagnosis of BGD or for detection of *F. branchiophilum*. However, a number of assays have been reported. Toyama et al.

(1996) identified *F. branchiophilum* using a primer set (BRA1-1500R) that targeted 16S ribosomal DNA (rDNA). The primer set was specific for the six *F. branchiophilum* strains in the study and did not react with rDNA of related fish pathogens, namely, *Tenacibaculum maritimum* (formerly *Flexibacter maritimus*) and *Flavobacterium columnare*. Warsen et al. (2004) developed methodology to identify a variety of fish pathogens simultaneously, including *F. branchiophilum*, *F. columnare*, *F. psychrophilum* and *T. maritimum*, through PCR amplification of 16S rDNA coupled with an oligonucleotide microarray. In another study that developed PCR detection of *F. columnare*, universal primers (16S-14F and 23S-1R) were used to amplify the 16S-23S rDNA intergenic spacer region (ISR) of *Flavobacterium* spp., including *F. branchiophilum* and *F. psychrophilum* in addition to *F. columnare* (Welker et al. 2005).

### c. Serological methods

Serologic assays using antisera produced to detect *F. branchiophilum* cells and antigen have been developed and used; however, antisera are not currently commercially available for serodiagnosis of BGD. In those reports that used serodiagnostic assays for BGD and *F. branchiophilum*, the antisera were developed (or acquired) by the respective authors of the studies (Wakabayashi et al. 1980; Huh and Wakabayashi 1987, 1989; Heo et al. 1990b; Bullock et al. 1994; Ostland et al. 1994; MacPhee et al. 1995a, 1995b; Ko and Heo 1997; Swain et al. 2007).

## F. Procedures for Detecting Subclinical Infections

A procedure is not specifically described to detect subclinical infections, nor is testing done for *F. branchiophilum* since it is not a pathogen targeted by hatchery inspections. However, the sensitive assays reported in the literature could be used to detect subclinical infections if necessary biologics were commercially available. For example, the indirect fluorescent antibody test (IFAT) has been used to detect *F. branchiophilum* cells on gill tissues (Huh and Wakabayashi 1987; Heo et al. 1990b; Bullock et al. 1994; Ostland et al. 1994) and the enzyme-linked immunosorbent assay (ELISA) has been used to identify and quantify *F. branchiophilum* antigen (MacPhee et al. 1995a, 1995b; Swain et al. 2007).

## G. Procedures for Detecting Prior Exposure

As a disease of hatchery-reared fishes, case records should show past BGD outbreaks. Diagnostic procedures to detect prior *F. branchiophilum* infections in hatchery lots of fish are not routinely done; however, most of the evidence indicates immunity to BGD is probable and past infections with *F. branchiophilum* can be detected (Lumsden et al. 1993, 1995; Bullock et al. 1994).

A vaccine to afford protection against BGD outbreaks is not currently available.

## H. Procedures for Transportation and Storage of Samples

Fresh moribund specimens should be used for wet mount microscopy and bacterial culture primary isolations. Gill smear slides for staining (i.e., simple or Gram) and microscopy could be prepared in advance and stained and examined at a later time. Tissues for histology can be placed in Bouin's fixative, Z-fix or 10 % buffered formalin. Tissues for PCR may be stored in 95% ethanol or frozen for later DNA isolation.

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