Fish Disease Leaflet No. 86

2005

Flavobacterium psychrophilum, cause of Bacterial Cold-Water Disease and Rainbow Trout Fry Syndrome

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Suggested citation: Cipriano, R.C. and R.A. Holt. 2005. *Flavobacterium psychrophilum*, cause of Bacterial Cold-Water Disease and Rainbow Trout Fry Syndrome. Fish Disease Leaflet No. 86. United States Dept. of the Interior. U.S. Geological Service, National Fish Health Research Laboratory, Kearneysville, WV.

Introduction

During 1941 and then again in 1945, two epizootics occurred among fingerling rainbow trout (*Oncorhynchus mykiss*) at the National Fish Hatchery in Leetown (West Virginia), which Davis (1946) described as "Peduncle Disease," based upon the characteristic pathology that was associated with the peduncle and caudal fins of the affected fish. Although Davis could not isolate the etiologic agent, he observed masses of long thin bacilli within the lesions of affected fish. In 1948, similar pathology was noted among several populations of silver (coho) salmon (*Oncorhynchus kisutch*) from the Pacific Northwest (Borg 1948, 1960). Despite the significantly low water temperatures (6-10^{o}C), mortality was severe. Borg noted that both the pathology and the morphological characteristics of the bacteria associated with lesions in the coho salmon were similar to those that were described among rainbow trout by Davis (1946). The bacteria were retractile, displayed gliding motility, and appeared to be similar to *Flavobacterium columnare* (syn. - *Chondrococcus columnaris)*, the cause of Columnaris Disease, which was reported by Davis (1922) and further characterized by Ordal and Rucker (1944). Unlike *F. columnare*, however, Borg's bacteria did not "swarm" or produce "haystacks" that were characteristic of Columnaris infections. Using a nutrient-dilute medium suitable for the cultivation of "myxobacteria," Borg (1948) cultivated the etiologic bacterium, reproduced the disease, and fulfilled Koch's postulates. Based upon this bacterium's proclivity to induce epizootics at low water temperatures, the disease became known as Bacterial Cold-water Disease (BCWD) or Low-Temperature Disease (Wood 1974).

Hosts and Geographic Range

Bacterial Cold-water Disease was exclusively reported from salmonid hosts within North America (Anderson and Conroy 1969), until it was diagnosed among diseased rainbow trout in Germany (Weis 1987) and France (Bernardet et al. 1988; Bernardet and Kerouault 1989). In most European countries, the disease in rainbow trout is referred to as Rainbow Trout Fry Syndrome (Lorenzen et al. 1991; Rangdale 1999), Fry Mortality Syndrome (Bernardet 1997) or visceral myxobacteriosis (Baudin-Laurencin et al. 1989). Subsequent isolations of *F. psychrophilum* in additional hosts and from other geographic regions (Table 1) have substantiated that this pathogen affects fish worldwide. Although outbreaks commonly occur among salmon and trout (Rucker et al. 1953), the disease also affects European eels *Anguilla anguilla*, carp *Cyprinus carpio*, Crucian carp *Carassius auratus*, and tench *Tinca tinca* (Lehmann et al. 1991). A more complete list of hosts and the reported geographic range of *F. psychrophilum* is presented in Table 1.

Taxonomy and Classification

The confusion associated with the taxonomy of the yellow-pigmented gliding bacteria (Reichenbach and Dworkin 1981) has affected the nomenclature of this pathogen. The etiologic agent of BCWD, *F. psychrophilum*, was originally believed to be a myxobacterium and Borg proposed the name "*Cytophaga psychrophila*" (Borg 1948, 1960; Pacha 1968; Pacha and Ordal 1970). Such classification was considered to be inappropriate because *"C. psychrophila"* did not produce fruiting bodies or degrade complex polysaccharides (Leadbetter 1974). Thus, Leadbetter categorized "*C.*

psychrophila" as a *species incertae sedis* within the *Cytophagales*, family *Cytophagaceae*, and suggested that it most likely belonged within the genus *Flexibacter*.

Table 1: Geographic range and host distribution of *Flavobacterium psychrophilum*.

Salmonid Hosts:

Coho salmon, *Oncorhynchus kisutch* (USA - Borg 1960; Japan – Wakabayashi et al. 1991)
Chinook salmon, *Oncorhynchus tshawytscha* (USA - Rucker et. al 1953; Canada – Ostland et al. 1999)
Sockeye salmon, *Oncorhynchus nerka* (USA- Rucker et al. 1953)
Chum salmon, *Oncorhynchus keta* (USA – Holt 1987)
Amago, *Oncorhynchus rhodurus* (Japan - Furutsuka-Uozumi et al. 1996)
Masou salmon, *Oncorhynchus masou* (Japan – Iida and Mizokami 1996)
Atlantic salmon, *Salmo salar* (USA – Schneider and Nicholson 1980; Australia - Schmidtke and Carson 1995; Sweden - Ekman et al. 1999; Canada – Ostland et al. 1999)
Brown trout, *Salmo trutta* (Japan - Wakabayashi et al. 1991; Finland - Madetoja et al. 2001; Norway - Lorenzen and Olesen 1997)
Sea trout, *Salmo trutta (*Finland and Sweden - Madetoja et al. 2001)
Rainbow trout, *Oncorhynchus mykiss* (USA - Davis 1946; France - Bernardet et al. 1988; Bernardet and Kerouault 1989; Germany - Weis 1987; Italy – Sarti et al. 1992; Canada – Ostland et al. 1999; Finland – Madetoja et al. 2001; United Kingdom - Santos et al. 1992, Bruno 1992, Austin 1992; Spain – Toranzo and Barja 1993; Finland - Wiklund et al. 1994; Chile - Bustos et al. 1995; Denmark – Lorenzen et al. 1991; Switzerland and Northern Ireland - Lorenzen and Olesen 1997)
Steelhead, *Oncorhynchus mykiss* (USA - Brown et al. 1997, Canada – Ostland et al. 1999)
Cutthroat trout, *Oncorhynchus clarki* (USA – Holt 1987; Finland - Crump et al. 2001)
Brook trout, *Salvelinus fontinalis* (USA - Bullock 1972; Finland - Madetoja et al. 2001)
Lake trout, *Salvelinus namaycush* (USA - Schachte 1983)
Arctic char, *Salvelinus alpinus* (Finland - Madetoja et al. 2001)
Grayling, *Thymallus thymallus* (Estonia - Madetoja et al. 2001)

Non-Salmonid Hosts:

Ayu, *Plecoglossus altivelis* (Korea – Lee and Heo 1998; Japan-Wakabayashi et. al. 1994
Carp, *Cyprinnus carpio* (Germany - Lehmann et al. 1991)
Crucian carp, *Carassius carassius* (Germany -Lehmann et al. 1991)
Eel, *Anguilla anguilla* (Germany - Lehmann et al. 1991)
Forktongue goby, *Chaenogobius urotaenia* (Japan - Amita et al. 2000)
Japanese dace, *Trybolodon hakonensis* (Japan - Amita et al. 2000)
Lake goby, *Rhinogobius brunneus* (Japan - Amita et al. 2000)
Pale chub, *Zacco platypus* (Japan – Iida and Mizokami 1996)
Perch, *Perca fluviatilis* (Finland - Madetoja et al. 2002)
Roach, *Rutilus rutilus* (Finland - Madetoja et al. 2002)
Tench, *Tinca tinca* (Germany - Lehmann et al. 1991)

Based on phenotypic characterizations specifically related to the inability of isolates to degrade polysaccharides, Bernardet and Grimont (1989) concluded that the BCWD organism should be provisionally classified as *Flexibacter psychrophilus*, depending

upon further reorganization of the entire *Cytophaga-Flexibacter-Flavobacterium* phylogenetic branch. In support of their conclusions, Bernardet and Grimont noted several inconsistencies that did not enable them to satisfactorily speciate these bacteria. For example, the BCWD isolates had a DNA composition that was 32-33 mol% G+C, which was dissimilar to the ~48% DNA G+C content of *Flexibacter* species (Reichenbach and Dworkin 1981). Although the DNA G+C contents of the BCWD isolates were more closely associated with those of *Flavobacterium* species, the BCWD isolates displayed gliding motility, which was inconsistent with the description of the genus *Flavobacterium*. Bernardet and Kerouault (1989) noted this bacterium has "poor gliding ability" that is difficult to observe, which is a striking characteristic when compared to other fish pathogenic members of the Order Cytophagales. The description of the *Flavobacterium* genus was later emended by Bernardet et al. (1996), which allowed for all BCWD isolates to be taxonomically identified as *Flavobacterium psychrophilum*. Molecular analysis of 16S ribosomal RNA indicated that *F. psychrophilum, F. columnare* and *Flexibacter maritimus* were closely related, shared a common descent, and represented a distinct group within the *Bacteroides-Flavobacterium* (Bader and Shotts 1998a). Subsequently, Suzuki et al (2001) reported findings of phylogenetic, chemotaxonomic and phenotypic characteristics for marine *Cytophaga*-like bacteria and proposed a new genus *Tenacibaculum* to include the marine fish pathogen *Flexibacter maritimus* changing the name to *T. maritimum*. *Flavobacterium psychrophilum* (Figure 1) is a long and thin (0.2-0.75 microns in diameter by 1.5-7.5 microns long) gram-negative rod (Pacha 1968; Bernardet and Kerouault 1989; Lorenzen

et al. 1997), but its morphology may be affected by conditions of culture (Kondo et al. 2001).

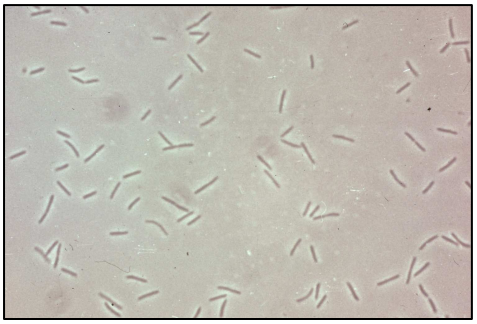

Figure 1. Microscopic photo showing *Flavobacterium psychrophilum* in a wet mount at 1000X. Photograph by R. A. Holt

The bacterium is refractile, does not form microcysts or fruiting bodies, but produces bright yellow colonies not greater than 3 mm in diameter with thin spreading margins (Figure 2) on specialized agar (Borg 1960; Pacha 1968). Additional phenotypic characteristics are presented in Table 2.

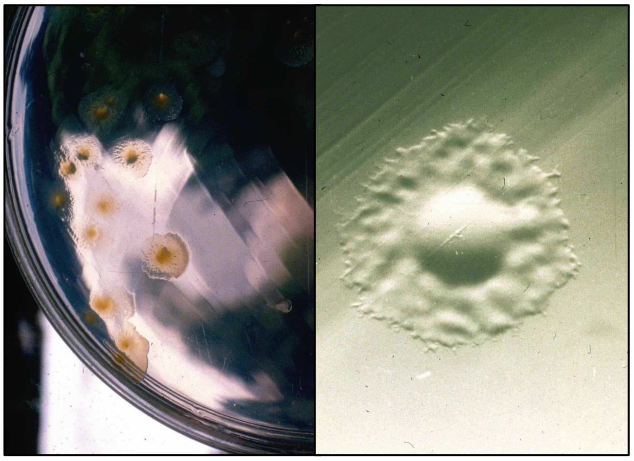

Figure 2. *Flavobacterium psychrophilum* colonies with thin spreading periphery on Cytophaga Agar. Photograph by R. A. Holt.

Epizootics of BCWD commonly occur when water temperatures range between 4 -10^{o}C (Borg 1960), but mortality generally abates as temperatures approach 15 - 18^{o}C (Rucker et al. 1953; Holt et al. 1989; Santos et al. 1992). In recent years at some fish hatcheries in the Pacific Northwest, this disease has been observed to persist and cause death at water

temperatures of 15-18°C in juvenile trout. Although the disease is generally not associated with warmer water temperatures, Iida and Mizokami (1996) observed BCWD occurring within feral ayu (*Plecoglossus altivelis*) and pale chub (*Zacco platypus*) at river temperatures up to 18°C.

Table 2: Phenotypic characteristics of *Flavobacterium psychrophilum*[1]

Characteristic	Reaction	Characteristic	Reaction
Gelatin hydrolysis	Positive	ONPG test	Negative
Albumin digestion	Positive	Nitrate reduced	Negative
Tributyrin hydrolysis	Positive	Pigment on tyrosine agar	Negative
E. coli cell autolysis	Positive	Tyrosine hydrolysis	Variable
Chitin hydrolysis	Negative	Growth on TSA[3]	Negative
Agar hydrolysis	Negative	Catalase	Weak positive
Casein hydrolysis	Positive	Flexirubin pigment	Positive
Starch hydrolysis	Negative	Gliding motility	Positive
Xanthine hydrolysis	Negative		
Tyrosine hydrolysis	Variable	Growth at 15°C	Positive
Cellulose decomposition	Negative	Growth at 20°C	Positive
Glucose oxidation	Negative	Growth at 25°C	Variable
Cellobiose oxidation	Negative	Growth at 30oC	Negative
Glucose fermentation	Negative	Growth at 37oC	Negative
Cellobiose fermentation	Negative		
Hydrogen sulfide	Negative[2]	Growth in 0.0% NaCl	Positive
Indole	Negative	Growth in 0.5% NaCl	Positive
Cytochrome oxidase	Weak positive	Growth in 1.0% NaCl	Variable
Congo red absorption	Negative	Growth in 2.0% NaCl	Negative

[1] Data from Borg (1960), Pacha (1968), Bernardet and Grimont (1989), Lorenzen et al. (1997).
[2] Lorenzen et al. 1997 found weak to clearly positive depending on test method.
[3] Trypticase soya agar, infrequently some growth observed on moist plates of TSA.

In addition to the effect of water temperature, the severity of BCWD epizootics also depends upon the host's stage of development. Coho salmon sac fry can experience greater than 50% mortality with little evidence of clinical disease other than destruction of the thin epithelial layer that protects the yolk (Wood 1974). If the disease does not

occur until the coho salmon fingerlings have been placed in ponds and have fed for several weeks, mortality may be less severe often with total losses ranging from 5-20%. In these fingerlings, the bacterium is prone to attack the peduncle and tail producing the classic skin and muscle lesions of BCWD (Figure 3). Epizootics of RTFS are usually most severe

Figure 3: Juvenile coho salmon with classic cold-water disease lesions of the peduncle and caudal area. Photo by R. A. Holt.

among 0.5 to 5.0 gram fish cultured in water at 10^{o}C or lower, about five weeks after the fish begin to feed (Bernardet et al. 1988; Branson 1995). The penchant for *F. psychrophilum* to induce mortality in very young fish was clearly demonstrated by the work of Decostere et al. (2001), who injected three groups of rainbow trout with similar doses of the bacterium. Clinical disease and mortality developed only in the 1-gram (10-week old) group of trout and not in the 25-gram (10-week old) or 300-gram (15-month old) groups of fish. Furthermore, viable intracellular *F. psychrophilum* increased with time within spleen phagocytes of the fry but bacteria were not apparent in the phagocytes of the older fish. Both ribotype and plasmid profiles have been used to associate different isolates of *F. psychrophilum* with variations in serotype, virulence, species of fishes

infected, and geographic origins that have proven to be epidemiologically valuable at a local or regional scale (Cipriano et al. 1996; Chakroun et al. 1998; Madsen and Dalsgaard 2000).

Mixed infections of certain viral, bacterial or parasitic fish pathogens and *F. psychrophilum* are frequently observed in various salmonid fish species. Concurrent infections of rainbow trout with *F. psychrophilum* and Infectious Pancreatic Necrosis virus have been described by Evensen and Lorenzen (1997). In the Pacific Northwest this bacterium is often found in rainbow and steelhead trout in a dual infection with infectious hematopoietic necrosis virus (IHNV; La Frentz et al. 2004). An epizootic of IHNV, BCWD and external fungi was observed during several winters in two and three year old kokanee salmon (*O. nerka*) in a central Oregon reservoir (Engelking and Kaufman 1994). Mixed infections of *F. psychrophilum*, external fungi and the virus causing erythrocytic inclusion body syndrome (EIBS) occur in yearling coho and Chinook salmon during the fall and winter months (Leek, 1981; Piacentini et al. 1989). In fact, the detection of BCWD infections in yearling coho or Chinook salmon in the fall or winter should alert the fish health specialist to also test these fish for EIBS virus. Also, BCWD has been found in association with furunculosis among coho salmon (Cipriano et al. 1996) and with coho and Chinook salmon with bacterial kidney disease. Hansen (1990) has also noted that Fry Mortality Syndrome (syn. – RTFS) has occurred in conjunction with high prevalence of intestinal infestation of rainbow trout by *Hexamita salmonis* and ectoparasitism by *Costia necatrix* (*Ichthyobodo*). Mortality associated with *F.*

psychrophilum among rainbow trout was also affected by the severity of ectoparasitic monogenean infestations caused by *Gyrodactylus derjavini* (Busch et al. 2003).

Flavobacterium psychrophilum has been cultured from the water and from algae covering rocks in rivers (Amita et al. 2000), but it is highly susceptible to osmotic changes (Michel et al. 1999). The bacterium can be cultured from stream water for up to four months, after which "starved" bacteria remained viable but non-culturable for as long as nine months (Vatsos et al. 2003). The organism remained viable and culturable in sterilized 15^{o}C fresh water after starvation for as long as 300 days, but the addition of nutrient-soils increased bacterial survival in water microcosms (Madetoja et al. 2003). Isolates of *F. psychrophilum* retain their virulence for relatively short periods (up to 8 months) without special provisions and for even longer periods when isolates are either lyophilized or frozen at –80^{o}C (Michel and Garcia 2003).

Clinical Presentation and Pathology

In his original description of BCWD among fingerling rainbow trout, Davis (1946) found that affected fish displayed a remarkable pathology that began as a whitish discoloration along the peripheral margin of the adipose fin that progressed to eventually invest the entire caudal peduncle. In most cases, the adipose fin and the integument covering the dorsal area of the peduncle became necrotic, which revealed the underlying musculature. In latter stages of disease, tissue and muscle degeneration progressed to such an extent

that varying degrees of the skeletal processes of the caudal fins were fully exposed, yet remained attached to the vertebral column (Figure 4).

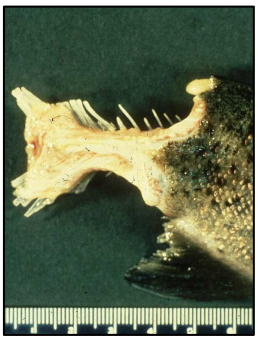

Figure 4. Classic peduncle lesion associated caused by *Flavobacterium psychrophilum* in a juvenile rainbow trout. In this instance, the integument and musculature have degenerated to such an extent that some of the underlying skeletal processes of the vertebral column are fully exposed. Photograph from the archive collection at the National Fish Health Research Laboratory

Coho salmon often display a progression of different disease signs when undergoing an epizootic of BCWD. Classic skin and muscle peduncle lesions (Figure 3) are observed early in epizootics of BCWD in juvenile coho salmon (Wood 1974). Later in the outbreak these lesions are found at various locations such as anterior to the dorsal fin, on the lateral side (Figure 5), ventrally, near the vent or on the lower jaw (Holt et al. 1993).

Figure 5. A deep dermal ulceration with necrosis of the underlying musculature caused by *Flavobacterium psychrophilum* in a juvenile coho salmon. Photograph by R. C. Cipriano.

Moribund coho salmon with no external skin lesions, but dark skin pigmentation especially in the peduncle area are observed late in the epizootic. In some outbreaks, affected salmon may lose their equilibrium, display dark pigmentation on one side of the

body, exhibit dorsal swelling just posterior to the skull, and swim in spiral motions when agitated (Kent et al. 1989). Periostitis, ostetitis, meningitis and ganglioneuritis were observed histologically with an attendant inflammation and periostreal proliferation of the vertebrae at the junction of the vertebral column and cranium. In this situation *F. psychrophilum* is isolated from the cranium cavity and the outbreak does not respond well to antibiotic therapy. There is a relationship between the severity of the BCWD epizootic in coho salmon and the occurrence of deformed fish in that population several months later (Conrad and DeCew 1967). Lordosis and scoliosis may become evident and is induced by the destruction of muscle fibers adjacent to the vertebral column (Conrad and DeCew 1967). When such spinal aberrations are pronounced, small hard cysts may overlay the fused caudal vertebrae. Wood (1974) referred to such clinical manifestation of pathology as "Crinkleback Disease," which generally presents among 3 to 4-month old coho salmon (Figure 6). The affected fish may be compressed lengthwise and assume an appearance

Figure 6. Pathological expression of "Crinkleback Disease" caused by *Flavobacterium psychrophilum* among juvenile coho salmon, as described by Wood (1974). Photograph by R. Cipriano.

that may resemble a "pumpkinseed" (Wood 1974). Often the same coho salmon populations that have undergone BCWD epizootics as juveniles will suffer chronic losses from this bacterium during the winter months as yearlings (Wood 1974). These affected fish will display lesions of the skin and muscle in the peduncle or caudal area, other areas

of the body or the head and snout (Figure 7). Among coho salmon, Wood and Yasutake (1970) noted that the epithelium in certain areas of the head was replaced by focal masses

Figure 7. Lesions on the snouts of juvenile coho salmon caused by *Flavobacterium psychrophilum*. Photograph by J. Evered.

of *F. psychrophilum* that extended into the musculature. Oral lesions were evident on the roof and floor of the mouth, as well as on the jaws and opercula. Within the gills, *F. psychrophilum* was evident on the central capillary of the respiratory platelets but was not found on the surface of the platelets or between them. An acute septicemia generally develops and the bacteria become abundant in most vascular tissues. Within the kidneys, renal dysfunction was characterized by bacterial necrosis of glomerular tubules and capillaries. The bacterium also caused necrosis and inflammation of cardiac muscle, causing lesions that were possibly the principal cause of death. In addition, the bacterium was evident in the spleen, wall of the intestine, swim bladder, liver and pancreas.

Clinical signs and pathology similar to many of those seen in coho salmon are observed in rainbow and steelhead trout and other salmonids. In rainbow trout, Lumsden et al (1996) found some of the fish developed large bullae along their lateral walls, which ulcerate and formed deep dermal lesions that expose the underlying musculature. Fish

may become melanose, have distended abdomens due to the accumulation of ascites, and exhibit an abnormal spiral swimming behavior (Bernardet and Kerouault 1989). The development of skeletal spinal column deformities is also consistent with the pathology associated with *F. psychrophilum* (Madsen and Dalsgaard 1999b; Madsen et al. 2001). Although some differences may be observed in the histopathological expression of BCWD among different species of fishes, Ekman and Norrgren (2003) were unable to document any statistically significant difference in the susceptibility or rainbow trout, sea trout, and Atlantic salmon to infection.

In RTFS, diseased fry may become inappetent, appear lethargic and weak, swim high in the water column, and exhibit exophthalmia as well as darken in color (Bernardet et al. 1988; Lorenzen et al. 1991). Fish may contain masses of the bacterium around the gill arch and secondary lamella. In some atypical cases, the presence of bacteria on the gill surfaces resulted in the formation of lamellar synechiae along entire gill filaments (Ostland et al. 1999). On rare occasions, *F. pyschrophilum* is found in macroscopic lesions on the gills of yearling rainbow trout similar in appearance to those caused by *F. columnare* (Holt 1993). They may also display deep necrotic skin lesions with lymphocyte infiltration into the dermis and underlying musculature (Bruno 1992), especially among fish larger than 10 grams (Wiklund et al. 1994; Branson 1995). The gills, kidneys and livers are usually pale, indicative of an anemia and the pathogen is consistently found in the spleen and kidney, but to a lesser extent within the liver and heart (Bruno 1992). The liver may whiten in appearance (Lorenzen et al. 1991) and have increased vascular degeneration with scattered necrosis of hepatocytes, eosinophilia

of the tubules, and a slight increase in hemosiderin content (Bruno 1992). There is also a consistent splenomegaly or hypertrophy of the spleen (Bernardet et al. 1988). In recent years in the Pacific Northwest, juvenile rainbow trout undergoing BCWD epizootics displayed hemorrhaged areas on the external body skin located near where the spleen is found internally and the spleen was enlarged (Figure 8).The presence of the bacterium in the retina and the subsequent inflammation associated with the infiltration of the retina and choroid gland by polymorphic granulocytes containing bacteria may account for why some of the rainbow trout that survive RTFS are blinded (Evensen and Lorenzen 1996).

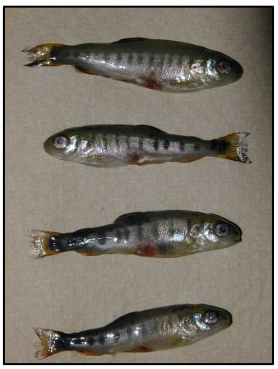

Figure 8. Juvenile rainbow trout from a hatchery outbreak of bacterial cold-water disease. Hemorrhaged area on the body is found over the spleen. The spleens were enlarged and contained many *Flavobacterium psychrophilum* cells. Photograph by C. Banner.

Pathogenicity

The virulence of different isolates of *F. psychrophilum* can vary extensively (Madsen and Dalsgaard 2000; Holt et al. 1993; Nematollahi et al. 2003a). Mortality in yearling coho salmon injected subcutaneously with viable cells of different strains of *F. psychrophilum* varied from 0-100% (Holt et al 1993). A number of virulence factors including adhesins, exotoxins, proteases and endotoxin contribute to the pathogenicity of *F. psychrophilum* (Dalsgaard 1993). Adhesins facilitate bacterial attachment to host cells and tissues,

therefore, enhancing the invasiveness of an organism. Holt (1987) could not demonstrate the presence of pili or fimbriae on *F. psychrophilum* isolates, but it was suggested that the bacterium has a surface polysaccharide extracellular layer, which facilitates motility and adhesion to host cells (Dalsgaard 1993).

During phagocytosis and the killing of ingested pathogens within a phagosome, releases of highly reactive oxygen metabolites are emitted, which can be assayed as chemiluminescence. Lammens et al. (2000) found that such a response occurred in rainbow trout kidney phagocytes stimulated by *F. psychrophilum*. The stimulatory effect was heat stable (therefore, not a protein), was not associated with bacterial lipopolysaccharides (unaffected by polymyxin B), and was impaired by Na-metaperiodate (a compound that modifies cell surface carbohydrates). Consequently, the chemiluminescent response was attributed to a carbohydrate moiety that was located on the surface of the bacterium.

Wiklund and Dalsgaard (2003) later observed that attachment of *F. psychrophilum* to the surface of rainbow trout kidney phagocytes was mediated by an opsonin independent cell-receptor adhesin, specifically affected by N-acetylneuraminic acid (sialic acid). Additional assay indicated that treatment of *F. psychrophilum* with Na-metaperiodate, significantly inhibited the association of pathogen and phagocyte. Differences in adhesive characteristics were noted between strains that could not be correlated with the degree of virulence or serotypic differentiation. Furthermore, all of the strains were not

cytotoxic for the rainbow trout kidney phagocytes, which suggested that although the adhesin may facilitate attachment, a phagocytic toxin was not necessary for virulence.

Borg (1960) originally concluded that *F. psychrophilum* did not produce extracellular toxins, because cell-free extracts that he injected into fish did not produce mortality. The proteolytic nature of *F. psychrophilum*, however, suggested that extracellular proteases may contribute to the pathogenicity of this organism (Pacha 1968). The ability to degrade elastin, for example, has been associated with virulence in some isolates (Madsen and Dalsgaard 1999a) and such a heat stabile metalloprotease was shown to induce severe muscle necrosis and necrotic myositis (Ostland et al. 2000).

Gross and microscopic lesions, which are similar to the lesions produced by injection of viable bacteria, have been observed among steelhead trout injected with extracellular products from this bacterium (Otis 1984). A 55-kDa psychrophilic metalloprotease (Fpp1), which depended upon calcium ion concentration for activity and thermal stability, was active between 25 to 40^{o}C but activity was greatly diminished at 45^{o}C (Secades et al. 2001). Cultures of *F. psychrophilum* produced lesser amounts of Fpp1 when incubated in the presence of calcium at 18^{o}C versus 12^{o}C; a relationship that directly mimics the correlation of temperature and the expression of this disease in nature. Furthermore, calcium concentrations necessary for optimal *in vitro* induction of Fpp1 were similar to those naturally found in the blood of fish. The protease cleaved gelatin, laminin, fibrnectin, fibrinogen, collagen type IV, and to a leaser extent collagen. It also degraded actin and myosin, which are basic elements of the musculature. Thus, Fpp1 may

significantly contribute to the pathogenicity of *F. psychrophilum* by allowing the bacterium to invade and colonize host tissues.

In addition to this metalloprotease, Bertolini et al. (1994) have found that *F. psychrophilum* actually produced two bacterial proteases (114- and 152-kDa), which degraded casein and gelatin, and eight other proteases (32- to 86-kDa), which degraded gelatin but not casein. Although some relationship was noted between protease composition and isolate virulence, the correlation was not absolute.

Madsen and Dalsdaard (2000) noted that all virulent Danish isolates carried a 3.3-kb plasmid. Unfortunately, a definitive correlation with virulence could not be established because the 3.3-kb plasmid was also detected within less virulent isolates.

Transmission

Flavobacterium psychrophilum is readily transmitted horizontally between fish via waterborne and contact exposure (Madsen and Dalsgaard 1999a; Madetoja et al. 2000). The bacterium is part of the flora associated with the skin, mucus, connective tissue of the fins, gills, and opercula of salmonid fishes (Nematollahi et al. 2003) and may systemically invade hosts that have been compromised by sub-optimal environmental condition (Roberts 1976). Abrasion of the skin and mucus enhanced invasion of the pathogen among fish challenged by either bath or cohabitation routes of infection (Madetoja et al. 2000). Additionally, dead rainbow trout shed significantly higher levels of this pathogen (10^4 - 10^8 cells fish^{-1} hour^{-1}) into the water column than live trout (10^3 - 10^6 cells fish^{-1} hour^{-1});

indicating the importance of removing dead and moribund fish from the general population to reduce the severity of contagion among cultured fish. Oral transmission has not been documented (Decostere et al. 2000; Madetoja et al. 2000)

The association of *F. psychrophilum* with early life stages of salmonid fish suggested that the bacterium might be transferred from parent to offspring via intra-ovum infection. The affinity of this bacterium with ovarian fluids, gametes (Holt 1987; Rangdale et al. 1996; Brown et al. 1997; Ekman et al. 1999; Rangdale et al. 1996) and its close association with eggs (Rangdale et al. 1997; Brown et al. 1997; Izumi and Wakabayashi 1997; Vastos et al. 2001) provide the linkages necessary for intra-ovum infection to occur. *Flavobacterium psychrophilum* has been introduced into facilities through the transport of contaminated eggs (Borg 1960, Kumagai 2001) and the pathogen has adversely impacted survival among eggs even after thorough disinfection in iodophor (Cipriano et al. 1995; Brown et al. 1997; Kumagai et al. 1998, 2000). The pathogen apparently entered the egg and resided within the perivitellin space where it was protected against the toxicity of iodophors (Kumagai et al. 2000). Because *F. psychrophilum* can resist lysozyme concentrations greater than those that typically occur within salmonid eggs (Brown et al. 1997), it may indeed survive within and consequently affect the survival of individual eggs (Ekman et al. 2003). Also, Brown et al (1997) found *F. psychrophilum* may not be very sensitive to iodophor reporting that in their tests 2% of the *F. psychrophilum* cells survived exposure to 100 ppm providone/iodine for 30 minutes.

Diagnosis

In association with clinical signs of disease as previously described, a presumptive culture-based diagnosis of *F. psychrophilum* may be accomplished by the isolation of moist, yellow, raised, convex colonies, with or without a thin, spreading, smooth or irregular margins on an appropriate medium after 3 to 6 days of incubation at 15-20^{o}C (Holt 1994). Isolations are usually made from the spleens, kidneys, and sometimes from the brains of affected individuals. Under field conditions a microscopic examination of an imprint of spleen tissue that has been air dried and stained with safranin for one minute often will reveal many cells with typical *F. psychrophilum* morphology.

Table 3: Basal media with per cent of ingredients used for cultivation of *Flavobacterium psychrophilum*, solidified with 0.9 to 1.5% agar according to the preference of the diagnostician.

Ingredient	A	B	C	D	E	F	G
Yeast Extract	0.05	0.20	0.05	0.05	0.04	0.04	0.05
Beef extract	0.02	0.05	-	-	-	-	-
Tryptone	0.05	-	-	0.20	0.40	0.40	0.20
Casitone	-	-	0.05	0.30	-	-	-
Gelatin	-	-	-	-	-	-	0.20
Glucose	-	-	-	-	-	-	0.05
Calcium Chloride	-	0.02	-	0.03	-	0.05	-
Magnesium sulphate	-	-	-	-	-	0.05	-
Sodium acetate	0.02	0.02	-	-	-	-	-

A – Cytophaga broth, Anacker and Ordal (1959)
B – Anderson and Conroy (1969)
C – Bootsma and Clerx (1976)
D – Hsu-Shotts medium, Shotts (1991)
E – TYE broth, Fujihara and Nakatani (1971)
F – TYE-S broth, Holt (1987)
G – TYG broth, Bullock et al (1986)

Growth of *F. psychrophilum* requires a specialized medium that is low in nutrients. Cytophaga Agar, as described by Anacker and Ordal (1959) is often used for the primary isolation and subsequent culture of *F. psychrophilum*; other modifications of this medium (Cipriano and Teska 1995) are also satisfactory (Table 3) and their use has expanded. Isolation of some strains may be enhanced by addition of 1-5% calf serum to Cytophaga Agar (Holt 1994). Furthermore, Daskalov et al. (1999) found that growth of the bacterium may also be enhanced by supplementing Cytophaga Agar or broth with 0.5 grams liter^{-1} of each of the following components: D(+) galactose, D(+) glucose, L-rhamnose and a protein source in the form of skimmed milk. Because the composition of beef extract that is required in some media is not a well-defined product, variations in the commercial sources and quality this ingredient may affect the success of isolation (Lorenzen 1993). Using a combination of methods, Michel et al. (1999) found that only about 25% of the bacteria within cultures of *F. psychrophilum* were successfully isolated on agar media because the cells are highly susceptible to osmotic conditions, which may require the addition of horse serum and trace elements as well as careful handling of the bacteria in isotonic suspensions. Further confirmation of the isolated bacteria may be made by serological analysis using either agglutination or fluorescent antibody assays (Holt 1994) followed by further phenotypic characterization (see Table 2).

Immunofluorescence has provided effective detection of the pathogen in spleen imprints from diseased fish, but absorption of *F. psychrophilum* antiserum with *Flavobacterium columnare* cells may be necessary in order to avoid cross-reactions with this closely related pathogen (Lorenzen and Karas 1992). Immunoperoxidase assays (Aikawa 1998;

Rangdale and Way 1995) and a biotin-avidin ELISA protocol, having a sensitivity of $1x10^4$ cells mL^{-1} have also been described (Mata and Santos 2001).

Non-culture based detection has also been accomplished through the use of molecular polymerase chain reaction (PCR) assays. These assays use a pair of oligonucleotide primers and a thermostabile polymerase to amplify target DNA sequences specific for a given organism. Using PSY1 and PSY2 (Toyama et al. 1994) or FP1 and FP2 (Urdaci et al. 1998) primers based on specific 16S ribosomal RNA gene sequences, molecular detection of *F. psychrophilum* has become somewhat routine (Toyama et al. 1994, Bader and Shotts 1998b; Urdaci et al. 1998). After sequencing the gyrB gene, which regulates the supercoiling of double-stranded DNA, Izumi and Wakabayashi (2000) developed PSY-G1F and PSY-G1R primers, which have a higher nucleotide substitution rate than the commonly used 16S rDNA and were, therefore, considered to be particularly advantageous for differentiation in epizootiological analyses.

Most PCR assays require the use of toxic elements such as organic solvents (e.g. chloroform, phenol) to extract DNA or ethidium bromide to visualize reactions. Using the PSY1 and PSY2 primers, a non-toxic based PCR was developed by Cepeda and Santos (2000) in which DNA was extracted using any one of three low-toxicity commercial systems (Chelex 100, Sigma; InstaGene Matrix, BioRad; DNA DIRECT; Dynal) in conjunction with visualization via vertical agarose electrophoresis and methylene blue staining (VAGE/MeB). Results were similar to those obtained using

more conventional toxic methods, but detection was on the order of 15 -150 cells for pure culture and 7,500 - 75,000 cells from seeded spleen tissue or ovarian fluids.

Greater sensitivity was needed, however, to detect subclinical or covert infections. Consequently, a nested PCR assay was described by Wiklund et al. (2000) who utilized two universal primers (20F and 1500R) that were complimentary to conserved regions of most eubacterial 16S rRNA in the first PCR step (PCR1) and the PSY1 and PSY2 primers in the second stage of PCR analysis (PCR2). Such assay detected as few as 17-colony forming units (cfu) mg^{-1} in brain tissue and 110-cfu mL^{-1} in water. Nested PCR proved to be more sensitive than either culture or the indirect immunofluorescent assay for the detection of *F. psychrophilum* from water samples (Madetoja and Wiklund 2002).

Using nested PCR assays, in which universal primers fD2 and rP2 were used in PCR1 followed by PSY1 and PSY2 primers in PCR2, Taylor and Winton (2002) similarly detected as little as 14 cfu per sample. Baliarda et al. (2002) used the 20F universal primer and a new primer (Fpsy-interR) during PCR1 in conjunction with the FP1 and FP2 primers described by Urdaci et al (1998) in PCR2. The resultant assay detected as few as 10 bacteria mg^{-1} of spleen and 5 bacteria mg^{-1} of ovarian fluid. Terminal restriction fragment length polymorphism (T-RFLP) analysis also enabled detection of as little as 30-cfu mg^{-1} of *F. psychrophilum* in kidney tissue (Nilsson and Strom 2002). Both a fluorogenic 5'-nuclease assay (TaqMan-based PCR) that detects as few as 4.7 cfu of *F. psychrophilum* per reaction (delCerro et al. 2002a) and a multiplex PCR that enables the simultaneous identification of *Aeromonas salmonicida*, *Yersinia ruckeri*, and *F.*

psychrophilum (del Cerro et al. 2002b) have also been developed. Warsen et al. (2004) developed a DNA microarray suitable for simultaneous detection and discrimination among 15 bacterial fish pathogens based on 16S rDNA polymorphisms using glass slides. This coupling of 16S rDNA PCR with a microarray detector appears suitable for diagnostic detection and surveillance.

Serology

The effectiveness of any serodiagnostic assay and the efficacy of vaccines may be affected by the intraspecific relatedness of *F. psychrophilum* isolates and the degree of interspecific antigenic similarity with other closely related bacteria (Faruk et al. 2002). Pacha (1968) observed that strong serological homogeneity existed among isolates of *F. psychrophilum* obtained from different epizootics in Pacific salmon and antisera used in those studies did not cross-react with other "myxobacteria" associated with fish. More recently it was reported to be necessary to absorb polyclonal antiserum prepared against this bacterium with *F. columnare* cells (Lorenzen and Karas 1992). While serologically comparing *F. psychrophilum* isolates from Denmark with those from other European countries, Lorenzen and Olesen (1997) noted that three serotypes existed; one major serotype "Th" that contained most of the Danish isolates. Serotype "Fd" was comprised of only a few isolates and serotype "$Fp^{T"}$ consisted of isolates that had not been obtained from clinical RTFS or BCWD situations. Interestingly, the type strain against which the Fp^{T} antiserum was developed originated from a strain of *F. psychrophilum* that was pathogenic for coho salmon in the United States (Holt 1987). Based on thermostabile antigens, Izumi and Wakabayashi (1999) also reported serotypic diversity among the

isolates that they had studied. Serotype O-1 contained isolates affecting coho salmon from both Japan and the United States, serotype O-2 contained strains from ayu, and an additional group of isolates (O3) from rainbow trout that could not be typed. Mata et al. (2002) published a more extensive serotypic analysis of *F. psychrophilum* in which they reported the characterization of seven host-specific serovars from isolates worldwide. How this differentiation relates to the studies performed by Lorenzen and Olesen (1997) and Izumi and Wakabayashi (1999) is presented in Table 4. Although Faruk et al. (2002) found evidence for a multiple number of serotypes, they could not establish correlations between serotypic differentiations, isolate virulence, geographic origins of the isolates, or species from which the isolate had been obtained.

Table 4: Serotypes of *Flavobacterium psychrophilum* based upon thermostabile antigens.

Species of isolation	Serotype as reported by:		
	Mata et al. (2002)	Lorenzen and Olesen (1997)	Izumi and Wakabayashi (1999)
Salmon	1	Fp^T	O1
Trout	2a	Fd	
Trout	2b	Th-2	
Trout	3	Th-1	O3
Eel	4		
Carp	5		
Tench	6		
Ayu	7		O2

Vaccination

By comparison to other bacterial fish pathogens, there is scant information regarding the immunogenicity of specific antigens and development of experimental vaccines against *F. psychrophilum*. This is partially due to the fact that the most serious outbreaks of the disease occur when fish are very young and often before they have developed a strong degree of immunocompetence. Consequently, there are still no commercially licensed vaccines available for use against this pathogen. Thus far, the best efficacy has been evoked using several experimental bacterins that have been delivered by intraperitoneal injection; a method that is not conducive to mass immunization of young fry. Holt (1987) established a relative percent survival (RPS) of 100% among 5.0 to 25.0 gram coho salmon following injection vaccination with a formalin-killed cellular bacterin administered in adjuvant. Heat inactivated whole cell bacterins have also produced a relative percent survival (RPS) of 80% against the visceral form of BCWD among 2.2-gram rainbow trout at 90 days post hatch (Obach and Baudin-Laurencin 1991). Similarly, Rahman et al. (2000) demonstrated that injection of formalin-killed *F. psychrophilum* administered in adjuvant evoked higher levels of efficacy (RPS = 60%) and humoral antibody production (average $\log_{(2)}$ titer = 26) in ayu than was observed among those fish that were immunized with a similar vaccine that was not emulsified in adjuvant (RPS = 40% and average $\log_{(2)}$ titer = 4). Other work has demonstrated that significant serum antibody responses have been evoked in rainbow trout at 6 – 9 weeks post vaccination regardless of whether or not the bacterins (whole cell or whole cell plus culture supernatant) were emulsified in adjuvant (LaFrentz et al 2002). Among ayu that had been

vaccinated by injection with an oil-adjuvanted whole cell bacterin, protection lasted up to 56 days post vaccination and was better than the degree of efficacy observed when fish were injected with either a water-soluble adjuvanted vaccine or formalin-killed whole cell bacterin (Masunari et al. 2001). Emulsification in adjuvant, however, was necessary to evoke mucosal antibodies that were detected at nine weeks post vaccination (LaFrentz et al 2002). In addition to the induction of specific antibodies, the lesser degree of protection that was afforded to trout sham-vaccinated with only saline plus adjuvant indicated that adjuvant vaccines also stimulate protective, albeit a non-specific immunity. Further enhancement of efficacy may require production of vaccines that also stimulate other non-specific humoral and cellular responses (LaFrentz et al. 2003).

Subcellular vaccines have been prepared from the outer membrane fraction (OMF) of *F. psychrophilum* cultures, which were more efficacious and evoked greater humoral antibody production than inactivated whole cell bacterins (Rahman et al. 2002). Crump et al. (2001) found that *F. psychrophilum* was surrounded by a loosely attached glycocalyx or slime layer that was highly antigenic and consisted of both carbohydrates and proteins. The predominant antigen recognized by convalescent rainbow trout immune serum was a low molecular mass (~16 kDa) lipopolysaccharide (LPS). High mass LPS (O-antigen) was not abundant on whole cells, but it was present in culture supernatants. In addition to these two antigenic compounds, immune serum from convalescent rainbow trout also recognized a 20-kDa protein associated with the cell surface. Electrophoretic characterization showed that the cell envelope consisted of more than 50 polypeptides, which included a dominant membrane-associated glycoprotein

termed “P60” (Merle et al. 2003). The immunogenicity of this glycoprotein awaits further clarification. LaFrentz et al. (2004) examined the immunogenic regions of *F. psychrophilum* corresponding 18-28, 41-49 and 70-100 kDa identified by western blot analysis using rainbow trout immune sera. The antigens within these regions were isolated by electro-elution and emulsified with Freunds complete adjuvant and used to immunize rainbow trout fry. It was demonstrated that the 70-100 and 41-49 kDa regions and *F. psychrophilum* treatments elicited significant protection when compared to saline control following subcutaneous challenge with virulent *F. psychrophilum* cells. Immunization with the 70-100 kDa region resulted in near complete protection in fish (mean RPS= 94%). Western blot analysis using sera from fish immunized with the 70-100 kDa region demonstrated that high molecular weight proteins and the O-polysaccharide component of lipopolysaccharide are recognized by serum antibodies. These antigens may serve as vaccine candidates.

An even more limited body of literature exists concerning the practical vaccination of fish by any means other than injection. Holt (1987) found that coho salmon vaccinated by direct immersion in formalin–killed whole cell bacterins conferred protection, but that protection was not strong when compared to the survival of fish that had been vaccinated by injection of the whole cell bacterin in adjuvant. In these studies, Holt concluded that in order to obtain a satisfactory degree of efficacy, coho salmon should be at least one gram in size prior to vaccination because estimates of RPS remained low among 0.5 g fish even when bacterins were applied in concentrated suspensions. Obach and Baudin-Laurencin (1991) similarly noted that heat inactivated vaccine administered via

immersion provided much reduced protection (RPS varied from 14 - 47%) as compared to the 80% RPS reported when injection was used to deliver the vaccine. Furthermore, protection was age and size dependent because it could only be established in 0.5 gram trout at = 50 days post hatch. Kondo et al. (2003) afforded modest to good levels of protection against experimental immersion challenges among juvenile ayu (also at 0.5 gram in weight, 75 days post hatch) that were orally vaccinated with formalin-killed whole cell bacterins. The oral bacterin was administered at 0.1 -2.0 grams of vaccine kg^{-1} of body weight and either offered to fish every day for 2 weeks or on 5 different days during a two week period.

Prevention and Control

Brood fish are potential carriers of *F. psychrophilum* and contaminated tissues, milt or eggs may cause either surface infection or intra-ovum infection of fertilized eggs. Although iodophor disinfections are ineffective if *F. psychrophilum* already resides within the perivitellin egg space, surface disinfection is routinely practiced with single or consecutive iodophor treatments at concentrations of 50 mg L^{-1} active I_2 for 30 minutes and 100 mg L^{-1} active I_2 for 10 minutes (USFWS 1995). Concentrations of iodophor should not be allowed to drop below recommended levels because *F. psychrophilum* appears to survive surprisingly elevated levels of this disinfectant (Brown et al. 1997). Disinfection of eggs may also be accomplished with either hydrogen peroxide at 100 mg L^{-1} for 10 minutes or with glutaraldehyde at 200 mg L^{-1} for 20 minutes (Branson 1995, Rangdale 1997). Because severe losses are induced by *F. psychrophilum* among early life stages of fish, eggs and emergent fry are often maintained in pathogen-free well or spring

water or filtered and ultraviolet-irradiated or ozonated water supplies prior to ponding or placement in production raceways.

Proper management of fish culture conditions, monitoring and maintenance of high standards for water quality, and adequate sanitation procedures are other essential requisites that may help to avoid and/or limit the severity of BCWD or RTFS epizootics. Equipment may be disinfected in 1-2% formalin for 10 minutes, in 0.5-1% chlorine for 2 minutes, or by exposure to alkaline conditions equal to pH 13 for 2 minutes (Branson 1995). Stresses associated with high water velocities and heavy rearing densities may elevate the level of mortality among cultured fishes and should be avoided (Wood 1974; Sarti and Giorgettii 1996; Iguchi et al. 2003). Wood (1974) has recommended that water flows in incubators should not exceed 4 - 5 gallons per minute and that the population of each tray should be adjusted in order to avoid oxygen deficiencies at this rate of flow. Substrates such as PVC netting material (Vexar) have been added to incubation trays to reduce sac-fry movement and mechanical abrasion (Leon and Bonney 1979). This has helped to reduce coagulated-yolk and BCWD losses in coho salmon fry. Other stresses normally associated with routine fish culture practices (e.g.- handling during grading, vaccination and transport, elevated rearing densities, poor water quality, and the presence of other pathogens or parasites) may precipitate epizootics (Branson 1995; Evensen and Lorenzen 1997). Nematollahi et al. (2003a) have demonstrated that adhesion of virulent *F. psychrophilum* to gill arches of rainbow trout can be facilitated by high levels of nitrites or organic materials in the water.

Treatment

Although many treatments are discussed within this section, licensed procedures vary within different countries. An individual must, therefore, become familiar with and utilize only those treatments, which have been officially sanctioned or licensed by the appropriate regulatory authority for use within a specific geographic region.

Chemical baths have limited impact against *F. psychrophilum* because the bacterium generally establishes systemic infections. Furanace, (generic name for this compound is nifurpirinol) however, is a nitrofuran that is rapidly absorbed from the water into fish tissues. Because therapeutic levels of this compound are readily absorbed within the fish, furanace has provided effective control of BCWD when administered to fry as a 60-minute bath repeated at three day intervals at 0.5 µg of drug mL^{-1} of water (Holt et al. 1975). Control was also achieved among fingerlings by application of similar baths administered twice weekly. Even just a single 60-minute bath treatment in furanace at 10-15 mg L^{-1} has been effective (Wood 1974). In another study, single 60-minute bath applications of nifurpirinol at 1 mg mL^{-1} also provided favorable control of *F. psychrophilum*, but the compound was even more effective when fish were treated with two baths, 24 hours apart (Amend 1972). Because nitrofurans are carcinogenic, these compounds are not approved for use on food fish within the United States.

In early stages of external infections, bath treatments with either water-soluble oxytetracycline at 10-50 mg L^{-1} or quaternary ammonium compounds at 2 mg L^{-1} have been recommended (Snieszko 1964; Schachte 1983). Such treatments are generally

ineffective once the erosion of the peduncle and caudal fin becomes evident. In such cases, Schachte (1983) recommended that oral antibiotics should be administered in association with 2 mg L^{-1} potassium permanganate flushes in conjunction with physical removal of the most seriously affected fish in order to reduce continued shedding of the pathogen into the water column.

Oral antibiotics do not always provide satisfactory control against *F. psychrophilum* because the most severe epizootics often occur among fry before they have begun to feed or within a few weeks of when the fry were placed in ponds. Often when water temperatures are very low it is difficult to achieve a therapeutic dose in fish and treatments must be repeated. In order to be effective, fish culture personnel should diligently monitor their stocks for any behavioral or clinical signs of disease to ensure that treatments are initiated early in the disease process; - before fish become inappetent and refuse to feed.

Sulfonamides, specifically sulfisoxazole and sulfamethazine, were among the first antibiotics investigated for the oral treatment of bacterial diseases among fish. Sulfisoxazole was effective against *F. psychrophilum* among feeding fry when administered either as a therapeutic treatment at 220 mg kg^{-1} day^{-1} for 10 days or as a prophylactic therapy offered at 88 mg kg^{-1} day^{-1} for 26 days (Amend et al. 1965, Amend 1970). The drug was also effective within ayu when administered at concentrations between 100 – 200 mg kg^{-1} day^{-1} for 7 days (Ninimiya and Yamamoto 2001). In addition, Wood (1968) indicated that sulfamethazine was also effective when administered at

concentrations from 220 - 440 mg kg^{-1} day^{-1} in starter diets and at concentrations of 110 mg kg^{-1} day^{-1}in pelleted feeds.

Oxytetracycline, however, has proven to be even more effective than sulfonamides for control of BCWD when administered in feed at 75 mg kg^{-1} day^{-1} for 10 days (Snieszko 1964, Wood 1968), but the minimum inhibitory concentration to oxytetracycline (MIC_{OTC}) may vary among different isolates and, therefore, affect the efficacy of treatment (Bruun et al. 2003). Although there are no drugs currently approved in the United States by the Food and Drug Administration for treatment of BCWD within food fish, special permission under an Investigational New Animal Drug permit may be sought in emergency situations for the use of terramycin administered as 3.5 g of oxytetracycline per 100 pounds of fish for 10 days (Warren 1991).

In Europe, RTFS has been successfully controlled using oxytetracycline at 75 - 300 mg kg^{-1} day^{-1} for 10 – 14 days; amoxicillin at 80 – 100 mg kg^{-1} day^{-1} for 7 days; and florfenicol at 10 mg kg^{-1} day^{-1} for 10 days (Branson 1995, Rangdale 1997, 1998; Rimaila-Parnanen et al. 1997). In a survey of antimicrobial resistance patterns conducted with Danish isolates of *F. psychrophilum*, Bruun et al. (2000) found that all of the isolates that they examined were sensitive to florfenicol and that resistance to oxytetracycline had remained stable over a period of five years, while resistance to amoxicillin and oxolinic acid was increasing. All isolates were also found to carry intrinsic resistance against the potentiated sulfonamide and sulfadiazine. Izumi and Aranishi (2004) reported finding a relationship between DNA gyrase (gyrA) mutations and quinolone resistance in *F.*

psychrophilum isolates. This suggests that as in other gram-negative bacteria, DNA gyrase is an important target for quinolones in *F. psychrophilum*.

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