A Review of *Flavobacterium Psychrophilum* Biology, Clinical Signs, and Bacterial Cold Water Disease Prevention and Treatment

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Abstract: Bacterial coldwater disease and other infections caused by *Flavobacterium psychrophilum* are a worldwide concern, particularly for freshwater salmonid hatcheries. *F. psychrophilum* infections can be difficult to control; antibiotic resistance is common and no effective vaccines are currently available. This review summarizes the biology and characteristics of this important pathogen, as well as the techniques required for isolation and identification. In addition, the epidemiology, clinical signs, treatment, and possible preventative measures of bacterial coldwater disease are discussed.

Keywords: Bacterial coldwater disease, *Flavobacterium psychrophilum*, salmonid, pathogenic bacteria.

INTRODUCTION

Flavobacterium psychrophilum is a ubiquitous bacterium in the aquatic environment, particularly in freshwater [1]. As the etiological agent of bacterial coldwater disease, it is a serious fish pathogen causing substantial economic losses and rearing difficulties to both commercial and conservation aquaculture [2]. This review paper describes the epidemiology, clinical signs, prevention, and treatment of the fish diseases attributed to this pathogen, which are similar despite the different geographic labels. In addition, the basic biology of *F. psychrophilum* and the techniques required for successful bacterial culture, isolation, and identification are discussed.

EPIDEMIOLOGY

Davis [3] first named an infection due to *Flavobacterium psychrophilum* as peduncle disease. According to Wood [4], a similar infection was called low temperature disease in 1949 because of its propensity to occur at cooler water temperatures. *F. psychrophilum* infections have also been labeled as fin rot disease [5], saddleback disease [6], fry mortality syndrome [7, 8], rainbow trout fry syndrome [9, 10], rainbow trout fry mortality syndrome [11], bacterial disease of cold water [12], coldwater disease [13] and bacterial coldwater disease [14, 15]. Bacterial coldwater disease (BCWD) has become the established name in North America, where *F. psychrophilum* infections were first reported, whereas rainbow trout fry syndrome is the common disease name in Europe, where the disease etiology was not initially known [16, 17]. BCWD will be used in the remainder of this paper to denote any of these infections caused by *F. psychrophilum.*

Flavobacterium psychrophilum infections are found throughout the world [18, 19]. BCWD has been identified throughout North America [14, 20], nearly every country in Europe [8, 21-25], Australia [26], Chile [27], Peru [12], Japan [27, 28], Korea [29], and Turkey [30, 31].

Juvenile rainbow trout and coho salmon are particularly susceptible to BCWD [1, 2, 15, 32, 33]. However, *F. psychrophilum* infections have been reported in a wide range of both anadromous and non-anadromous salmonids of various sizes [34-42]. In addition, *F. psychrophilum* has either caused disease or been detected in Japanese eel *Anguilla japonica* [43], European eel *Anguilla anguilla* [44], common carp *Cyrpinus carpio*[44], crucian carp *Carassius carassius* [44], tench *Tinca tinca* [44], ayu *Plecoglossus altivelis* [27, 29], pale chub *Zaco platypus* [28], perch *Perca fluviatilis* [45], and roach *Rutilis rutilis* [45].

PATHOGENESIS

The biology of infection by *Flavobacterium psychrophilum* begins with the presence of the pathogen. *F. psychrophilum* is most likely in the aquatic environment and can likely survive for several months or even years in fresh water outside a fish host [46-50]. It may also be present *via* a fish reservoir [25], with live infected fish shedding 10 x 10^3 to 10 x 10^7 bacterial cells/fish/h into water [45]. Dead fish release even greater numbers of bacteria [51]. While there is some discrepancy over the presence of the bacteria on healthy fish skin, breaks in the tegument are the most likely invasion routes into the fish [45, 52, 53]. Madetoja *et al.* [45] observed that an abrasion of the skin and associated mucus greatly increased *F. psychrophilum* invasion. Likewise, Miwa and Nakayasu [53] recovered the bacteria only from damaged skin, even if inflicted injuries were only micro-

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scopic, and emphasized that skin injuries are a major portal for *F. psychrophilum* entry. The bacterium likely has an affinity for the lower jaw, fin, and caudal peduncle [54, 55]. The infectivity of *F. psychrophilum* may be influenced by the presence of other infectious or noninfectious organisms [52]. Busch *et al.* [56] suggested that ectoparasites may enhance the invasiness of *F. psychrophilum*. Degraded water chemistry, such as high organic loads or elevated nitrite concentrations, may also play a role [57, 58].

Vertical transmission from the brood female is highly probable [59, 60]. This was not supported by Madsen and Dalsgaard [61] however, who could not recover *F. psychrophilum* within the egg and questioned the Taylor [60] methodology. Madsen and Dalsgaard [61] believe *F. psychrophilum* to be intimately connected with the egg membrane, but not within the egg itself. Numerous researchers have noted *F. psychrophilum* on the exterior of the egg, in milt, and in ovarian fluid [36, 59-65]. Ekman *et al.* [10] mimicked vertical transmission by injecting *F. psychrophilum* into adult rainbow trout. Kumagai [66] recovered *F. psychrophilum* cells within salmonid eggs by experimentally immersing them in a *F. psychrophilum* suspension prior to water-hardening. Resistance to vertically-transmitted *F. psychrophilum* may also be influenced by the maternal transfer of contaminants such as PCBs [67].

Upon entry into the fish, *F. psychrophilum* secretes psychrophilic protease [68-70]. Nematollahi *et al.* [58] noted a negative relationship between temperature and bacterial attachment. The bacteria create tubular boreholes to obtain Ca for further protease activation [55, 70]. After entering the dermis, *F. psychrophilum* spreads through collagenous connective tissue, for which it has a high affinity, and further expands into the musculature [53, 71]. Open ulcers are created, with subsequent lesions on the internal organs [53, 71].

Flavobacterium psychrophilum strongly suppresses the nonspecific humoral defense mechanisms of the infected fish [72]. Lammens *et al.* [73] observed that in the presence of phagocytes bacterial numbers decreased, but this decrease was not due to phagocytic action. Viable bacteria have been noted inside spleen phagocytes [52, 74], which may protect them against humoral defense mechanisms such as complement and lysozyme activity [52]. Wiklund and Dalsgaard [75] observed that high complement activity in rainbow trout sera did not reduce the number of *F. psychrophilum* cells. Because of reduced reactive oxygen species, spleen macrophages might provide a safe location for *F. psychrophilum* to minimize exposure to the immune system of the infected fish [76]. LaFrentz *et al.* [77, 78] noted that both non-specific immune actions and a specific antibody are needed to induce an effective immune response against *F. psychrophilum*.

F. psychrophilum has at least three main serotypes [18, 24, 43]. There are also a number of distinct genetic lineages, with the number appearing to increase with the refinement of molecular DNA techniques [19, 20, 40, 79] with considerable genetic variation and variation in virulence among strains [19, 25, 45, 80, 81]. Strain virulence may also be fish species specific [42]. Based on RAPD (Random Amplification of Polymorphic DNA) results, Chakroun *et al.* [83] suggested that North America was the source of *F. psychrophilum* in Asia, and a separate strain from Europe was transferred to Australia and then back to Europe. However, using nucleotide polymorphisms from *F. psychrophilum* worldwide, Nicolas *et al.* [19] found no evidence that North America was the original source of *F. psychrophilum* and that there was a wide diversity of strains in Europe, particularly in wild, non-salmonids. Nicolas *et al.* [19] suggested that human activities likely facilitated the spread of the main two *F. psychrophilum* clonal complexes.

CLINICAL SIGNS AND DIAGNOSIS

The erosion of tissue, particularly involving the caudal peduncle or caudal fin, is a classic characteristic of BCWD [3, 6]. Martínez *et al.* [55] noted that one of the first signs of an infection is the development of whitish material along a fin margin, followed by progressive necrosis. However, even if the fin erosion or characteristic peduncle necrosis is not evident, other clinical signs of BCWD are numerous. Signs such as lower jaw skin ulcerations, pale or necrotic gills, epidermal hyperplasia, increased mucus production, increased pigmentation (particularly posteriorly, resulting in "black tail"), ascites, lethargy, scleritis, blindness, anemia, enlarged speen, intestinal inflammation, exophthalmia, pale liver and kidney, nervous disorders, spinal abnormalities, hemorrhagic and protruding anus, and spiral swimming behavior have also been reported [7,12, 14, 22, 34, 54, 84-94]. Larger fish may be more likely to exhibit necrotic lesions associated with classic BCWD [17, 95], where as histological indications are similar among all external manifestations of *F. psychrophilum* infection [17]. Nuerological symptoms, including spiral swimming, or deformities such as spinal compressions may be present long after an infection [1, 85, 93].

Histologically, necrosis of most of the internal organs has been observed [7, 17, 46, 63, 87, 96, 97]. *F. psychrophilum* is strongly associated with phagocytes in the kidney and spleen [74, 97, 98]. The spleen is particularly affected, with hemosiderosis [87], hemorrhages [17], necrosis [17, 74, 97], and the presence of numerous bacteria [17, 74, 97, 99]. *F. psychrophilum* has also been observed in retina and choroid gland of the eye [71].

Reported mortality from BCWD has varied. The highest reported mortality rate has been 90% in rainbow trout [25]. Mortality rates of 85% in steelhead (anadromous rainbow trout) have been reported by Brown *et al.* [59] with up to 70% mortality in rainbow trout from Western Europe by Santos *et al.* [22], Lorenzen *et al.* [7] and Bruno [87], and in Turkey by Kum *et al.* [31]. In contrast, Jensen *et al.* [100] reported an average of 34% rainbow trout mortality from BCWD in Denmark, Bruno [87] estimated mortalities between 10 and 30% in the UK, and Gultepe and Tanrikul [30] reported 20% mortality in Turkey. Wood [4] and Holt [14] noted mortality rates of up to 50% in coho salmon fry. Mortalities ranging from 5 to 30% are typically experienced in slightly larger coho fingerlings [4, 14, 16, 101]. Losses from BCWD in cutthroat trout *Oncorhynchus clarki* have been reported to range from 30 to 45% [94, 102], and Schachte [34] noted 25% mortality in lake trout *Salvelinus namaycush*. Post [5] indicated that mortality could be very low (1%) and continuous, but could reach up to 75% in a severe epizootic.

The observed differences in fish mortality rates are likely due to a number of reasons. Water temperature is one key factor [103]. Although the disease mostly occurs from 4 to 10°C [4, 5], it is most severe at 15°C [1]. Wood [4] noted increasing mortality in coho salmon with increasing water temperatures from 10 to 16°C. Slighter higher water temperatures from 16 to 21°C are typical in BCWD outbreaks in ayu in Japan [104].

Bacterial virulence is also extremely important in determining the severity of BCWD epizootics [103]. There are large numbers of serologically and genetically different *F. psychrophilum* strains with highly variable virulence [19, 45, 105], perhaps due to plasmid or siderophor influences [106]. The pathogenic strains themselves are also heterogeneous [20]. Different strains have exhibited different resistance to rainbow trout macrophage activity [76]. The genetics of the fish themselves also contributes to different mortality rates. Johnson *et al.* [107] noted a large variability in mortality after challenging 71 full sib families with *F. psychrophilum*.

BACTERIAL CULTURE AND PATHOGEN IDENTIFICATION

Davis [3] first observed bacterial rods from scrapings of rainbow trout *Oncorhynchus mykiss* caudal penduncle lesions. Borg [108], observing similar clinical symptoms in coho salmon *O. kisutch*, isolated a bacterium from lesions and the kidney. Further describing the rod-shaped bacteria as gram-negative with gliding motility, no fruiting bodies or microcysts, and an inability to grow on culture media above 25°C, Borg [6] classified it with myxobacteria and named it *Cytophaga psychrophila*. Lewin [109] suggested renaming it *Flexibacter aurantiacus*, and this name was used approximately 20 years later by Starliper *et al.* [110]. Based on DNA homology, Bernardet and Grimont [111] contended that this species should be reclassified and renamed *Flexibacter psychrophilus*, order Cytophagales. Subsequently, it was again reclassified into the family Flavobacteriaceae and renamed *Flavobacterium psychrophilum* [112]. These taxonomic changes have not been without some confusion and controversy however [113-115].

Pacha [84], Holt [14], and Madetoja *et al.* [41] describe *F. psychrophilum* as a weakly refractile, slender, gram-negative, flexible rod-shaped bacterium. Gliding motility occurs [84, 111], but is strongly influenced by nutrient concentrations [116]. In addition, the extent of gliding motility varies considerably, with substantial variation between strains [82, 117]. Pacha describes its size as approximately 0.75 μm in diameter and 1.5 to 7.5 μm in length. In contrast, Bernadart and Kerouault [21] describe the bacteria range in length as 3 to 10 μm, and Post [5] described it having a diameter from 0.7 to 1.5 μm, and length from 5 to 100 μm. Age may affect the length of this species [118]. Optimal incubation temperature is 18 to 20° C, with no growth occurring at temperatures of 30° C or greater [21, 84, 119]. *F. psychrophilum* is strictly aerobic and exhibits variable colony morphology [11, 84]. On cytophaga agar, *F. psychrophilum* colonies appear as bright yellow colonies with thin spreading margins [18, 84].

Flavobacterium. psychrophilum is only weakly reactive to chemical tests [120], however it is highly proteolytic, and can hydrolyze casein, digest albumin, hydrolyze tributyrin, and peptonize litmus milk [68, 69, 106, 111]. It will produce catalase [84, 101] and oxidase [111, 117]. None of the *F. psychrophilum* strains evaluated thus far can hydrolyze starch or utilize carbohydrates [21, 84]. The bacterium does not produce hydrogen sulfide, reduce nitrite to nitrate, produce cytochrome oxidase, degrade chitin, decompose cellulose, or hydrolyze xanthine [84]. Pacha [84] noted that *F. psychrophilum* would grow in 0.8% NaCl, but growth was inhibited at concentrations of 2.0%. However, Bernardet and Kerouault [21] observed no growth at NaCl concentrations greater than 0.5%. This discrepancy could be possible explained by differences between *F. psychrophilum* strains.

Being somewhat fastidious, *F. psychrophilum* can be difficult to both culture and isolate [117, 121, 122]. Several different agars have been used to grow *F. psychrophilum* in the laboratory, such as numerous variations of cytophaga agar [8, 21, 84, 96, 123-127]. Tryptone-yeast extract and tryptone-yeast extract-salts agar have also been used [45, 121]. Antibiotic-containing media has also been used [128, 129]. Comparing several media, Cepeda *et al.* [119] found that tryptone-yeast extract-salts agar with added glucose was the most effective medium at 18°C for isolating *F. psychrophilum* from diseased fish tissues. Antaya [2] also successfully used tryptone-yeast extract-salts agar in conjunction with incubation times of 72 h, while Álvarez and Guijarro [122] noted improved culture results with the addition of activated charcoal to several types of media.

Other methods have improved the speed, sensitivity, and precision of detecting and identifying *F. psychrophilum*. Lorenzen and Karas [130] used immunofluorescence to rapidly diagnose *F. psychrophilum* infections. Madetoja and Wiklund [131] considered an immunofluorescent antibody technique an improvement over traditional plate culture. ELISA (enzyme-linked immunosorbent assay) and fluorescent antibody techniques have also been used as both an identification and screening tool for *F. psychrophilum* [18, 132]. Álvarez *et al.* [133] considered ELISA to be the best diagnostic technique. An agglutination assay was used by Misaka and Suzuki [134], and Misaka *et al.* [135] detected and quantified viable *F. psychrophilum* using colony blotting and immunostaining.

Nakagawa and Yamasota [136] developed polymerase chain reaction (PCR) primers for *F. psychrophilum.* PCR was also used by Toyama *et al.* [137], Izumi and Wakabayashi [138, 139] and Bader and Schotts [140], although Toyama *et al.* [141] noted that the technique lacked sensitivity. Urdaci *et al*. [142] and Wiklund *et al.* [129] used PCR to detect *F. psychrophilum* in samples of infected fish tissue. Cepeda and Santos [143] described a fast and reliable PCR method specific to *F. psychrophilum* using relatively non-toxic chemicals. *F. psychrophilum* has been identified from formalin-fixed and wax-embedded tissue [144] using PCR. Ramsrud *et al.* [145] differentiated strains using a simple PCR assay. Tiirola *et al.* [146], Izumi *et al.* [147], and Soule *et al.* [79], used PCR-RFLP (restriction fragment length polymorphism), whereas del Cerro *et al.* [148] developed a multiplex PCR method combining the use of 16S rDNA with *gyr*B based primers to improve reliability and accuracy.

Misaka and Suzuki [134] also used PCR targeting the *gyr*B gene in conjunction with nested PCR.

It is difficult to compare agar plate culture directly to molecular identification techniques, because the results are often strikingly different [146]. For example, *F. psychrophilum* was identified using PCR from trout tissues where no bacteria had been cultured using cytophaga agar [144]. Taylor and Winton [149] noted the improvement in speed, specificity, and sensitivity of nested PCR in comparison to agar plate culture. Nested PCR was also used by Baliarda *et al.* [120] to report viable *F. psychrophilum* cells which could not otherwise be cultured, and by Izumi *et al.* [150] to detect *F. psychrophilum* in environmental samples containing other organisms. Madetoja and Wilklund [131] suggested the use of bot nested PCR and an immunofluorescence antibody technique. More recently, Fujiwara-Nagata and Eguchi [151] described a simple and quick loop-mediated amplification assay (LAMP) method. The complete genome of *F. psychrophilum* has been sequenced [95].

TREATMENT

Antibiotics are the treatment method of choice during BCWD epizootics. Nifurpirinol is effective [4, 5], but is not registered in the US for food fish use because it, like other nitrofurans, is carcinogenic [16]. Sulfonamides are also effective [4, 5, 152], but like nitrofurans are not registered for use on food fish in the US. Oxytetracycline has been widely used around the world for the control of BCWD [1, 5, 13, 15, 35, 99]. Amoxycillin and oxolinic acid were widely used in Europe [99, 153]. However, antibiotic resistance to oxytetracycline (OTC), amoxycillin, and oxolinic acid developed relatively quickly [2, 32, 153, 154], leading to therapeutic treatments with florfenicol [13, 30, 153], which was only recently approved in the US [155].

Antibiotic resistance to *F. psychrophilum* is a major challenge [156]. In 1979, Wood concluded that there were no OTC-resistant strains of *F. psychrophilum*. Brunn *et al.* [153] reported that oxolinic acid was initially used to control BCWD in Denmark starting in 1986, but by 2000 there was 100% resistance to the antibiotic, and the use of OTC became more prevalent. From 1994 to 1998, 60 to 75% of the *F. psychrophilum* isolated from Danish trout farms were OTC resistant, and amoxicillin resistance was also observed [153]. Only three years later, Brunn *et al.* [154] reported that OTC was rarely used with BCWD in Denmark because of antibiotic resistance. Kum *et al.* [31] reported *F. psychrophilum* resistance to florfenicol. Bruun *et al.* [153] speculated that *F. psychrophilum* may have instrinsic resistance to potentiated sulfonamides, and Álvarez *et al.* [133] reported the mutation of antibiotic-resistant genes in the bacteria. The ability of *F. psychrophilum* to produce extensive antibiotic resistant biofilms likely leads to the rapid development of antibiotic resistance, as well as recurrent infections [157].

Other authors have reported BCWD therapies involving multiple chemicals or even non-antibiotics. Gultepe and Tanrikul [30] described a treatment protocol in Turkey in which a hydrogen peroxide bath was followed by feeding florfenicol-medicated diets. Post [5] described a similar treatment regime, combining an antibiotic followed by quaternary ammonium baths every three days. Shachte [35] combined a potassium permanganate treatment in conjunction with antibiotics. The purpose of the potassium permanganate immersion treatment was two-fold, reduce the *F. psychrophilum* load and expedite mortality of infected individuals, thereby eliminating them as bacterial reservoirs [45]. Potassium permanganate was also mentioned by Groff and LaPatra [1] and La Frentz and Cain [15]. In addition, they listed the use of a 10 to 30 minute, 3% salt bath as a treatment for BCWD.

Despite considerable effort, there is not yet a viable commercial vaccine for BCWD. Live attenuated strain vaccines may be possible [158, 159]. Virulence attenuation does occur with different strains of *F. psychrophilum* [82], which allowed Álvarez *et al.* [158] to obtain significant disease resistance by injecting attenuated live bacteria. Kondo *et al.* [160] significantly improved survival following a subsequent challenge with a virulent strain by injecting formalin-killed *F. psychrophilum* into fish. Bath vaccination with heat-inactivated *F. psychrophilum* worked successfully, but had to occur at least 50 d post-hatch [96]. Immunity has also been induced by using a non-attenuated *F. psychrophilum* bath treatment [161], and Nikoskelainen *et al.* [162] included *F. psychrophilum* in a polyvalent vaccine trial. Compared to using the entire *F. psychrophilum* cell, better protection was achieved by the using part of, or all, of the antigenic outer membrane [163]. Thus, the identification of specific *F. psychrophilum* antigens by Crump *et al.* [164] and La Frentz *et al.* [165, 166], may enhance vaccine development. With the successful isolation of several *F. psychrophilum* bacteriophages, phage therapy is also being investigated [105, 167]. The use of probiotic bacteria has also shown promise *in vitro* [168].

PREVENTION

Egg disinfection with iodophor is frequently listed as a preventive measure against BCWD [1, 15, 16, 35, 60, 94, 99]. However, *F. psychrophilum* is very iodine tolerant, and can survive treatments of at least 100 mg/l active iodine for at least 30 minutes [59, 66]. The relative ineffectiveness of current egg disinfection protocols against *F. psychrophilum* is evident in the spread of virulent strains around the world [66, 83]. Cipriano [64] also noted that standard iodophor disinfection is ineffective, and experimentally showed that even triplicate iodophor treatments did not eliminate *F. psychrophilum* within the egg. Non-chemical disinfection is also possible, although Hedrick *et al.* [169] noted that ultraviolet doses of 42 mWs/cm^2 do not kill *F. psychrophilum*, and that higher doses of 126 or 256 mWs/cm^2 are required.

Avoiding or minimizing physical handling and stress is highly recommended to prevent BCWD outbreaks [1, 15, 16]. Not only does stress and physical handling cause immunosuppression [170], physical handling also likely leads to cutaneous lesions, providing the ideal point-of-entry for the pathogen [45, 52, 53]. Ryce and Zale [94] described the typical pattern of BCWD occurrence in a Montana hatchery, and noted that outbreaks typically occurred three weeks after fish handling or moving. Reducing rearing densities is also recommended [16, 60].

The use of high quality diets may help prevent BCWD. Post [5] listed malnutrition as a probable primary cause underlying BCWD. A link between diet and BCWD was dem-

onstrated by [171]. In their study, rainbow trout fed a diet with high oxidized lipid concentrations, compared to fish receiving control diets, experienced elevated mortality after a *F. psychrophilum* challenge.

Poor water quality was also listed one of the primary causes contributing to outbreaks of BCWD by Post [5]. Groff and LaPatra [1] and Taylor [60] both listed optimum water quality as a BCWD preventative measure. Reduced organic loads and decreased nitrite concentrations may help reduce *F. psychrophilum* infectivity [57, 58]. Using pathogen-free water supplies, either through natural sources or *via* filtering, ultraviolet treatment, or ozonation, was suggested by Cipriano and Holt [16]. Elevating water temperatures, if possible, may also serve to prevent *F. psychrophilum* infections [1, 33].

Broodstock screening has been mentioned as a tool to prevent BCWD [1, 35]. Lindstrom *et al.* [132] suggested using ELISA and filtration-based fluorescent antibody tests for broodstock selection. However, Lumsden *et al.* [13] questioned the practicality of broodstock screening if the rearing environment is already heavily contaminated with *F. psychrophilum*.

Hadidi *et al.* [155] suggested genetically selecting for resistance in brood fish. Henryon *et al.* [172] also noted that in the absence of effective vaccines, selective breeding showed considerable promise. Resistance to BCWD in rainbow trout is moderately heritable and not adversely correlated with growth, thereby allowing for genetic improvements [173], assuming no further detrimental bacterial mutations occur.

Because dead fish are a reservoir for *F. psychrophilum* [51], removal of mortalities from rearing units is highly recommended [13, 15]. Avoiding the introduction of wild or novel fish into existing fish stocks is another preventative measure [1, 5]. Other preventative measures, such as maintaining infected stocks downstream in the production system, and routine equipment sanitation are also recommended.

CONCLUSION AND RECOMMENDATIONS

Despite considerable study, *F. psychrophilum* remains a serious pathogen causing substantial mortality during hatchery rearing worldwide. There are several areas for productive future research. Additional work is needed to develop rapid, accurate, and definitive diagnostic tests so that existing and novel therapies can be started as quickly as possible. While getting additional antibiotics registered for legal use would be desirable, given the financial constraints of aquaculture drug registration and the rapid development of antibiotic resistance by *F. psychrophilum*, research into the use of probiotics or immunostimulants may likely be more productive. More study of combination therapies or non-antibiotic chemical treatments would also be beneficial. Several areas of dietary research are also warranted. Nutritional improvements with enhanced dietary formulations built on established formulations may lead to changes in BCWD susceptibility. In addition, the effects of novel dietary ingredients, such as the substitution of fish meal with plant-based proteins in carnivorous fish diets, should be evaluated. Development of BCWD-resistant brood stocks should continue. Lastly, controlled research examining hatchery management techniques to reduce stress and minimize the creation of *F. psychrophilum* entrance portals, should be conducted.

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