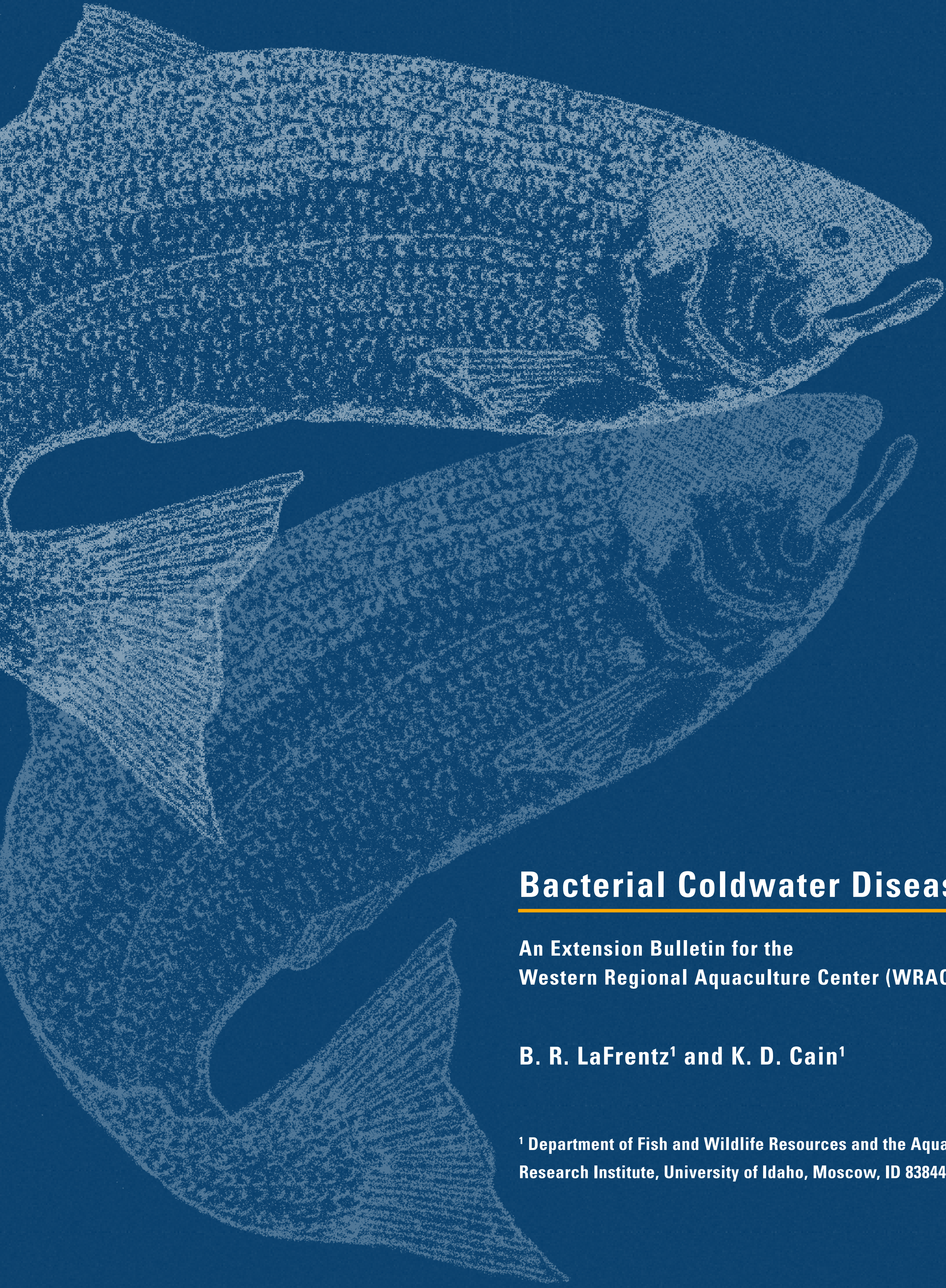

Bacterial Coldwater Disease

An Extension Bulletin for the
Western Regional Aquaculture Center (WRAC)

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Table of Contents

Introduction	1
Distribution	1
Host Susceptibility	2
Clinical Signs	2
Diagnosis	4
Transmission	4
Prevention and Treatment	5
Eggs	5
External Infections	5
Internal infections	6
Management of Coldwater Disease in the Future	6
References	6
Acknowledgments	inside back cover

Cooperative State Research Education & Extension Service (CSREES)

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Introduction

Bacterial coldwater disease (CWD) was first described in 1948 (Borg 1948). The causative agent of CWD, *Flavobacterium psychrophilum*, is a gram-negative bacterium that produces an acute septicemic infection in salmonids (Wood & Yasutake 1956) and a few other species (Lehman *et al.* 1991). The disease typically occurs at low temperatures and infected fish may exhibit a range of clinical signs, including large open lesions on the caudal peduncle (tail area). Hence, this disease has been referred to as peduncle disease and low-temperature disease. In Europe, the disease is referred to as rainbow trout fry syndrome (RTFS) due to impacts related to early life stage mortalities in trout.

Flavobacterium psychrophilum is considered one of the most important salmonid pathogens worldwide (Michel *et al.* 1999) because of the severe mortalities caused by infection with this pathogen and the resulting economic impact among commercial aquaculture producers and conservation hatcheries. In recent years, the rainbow trout (*Oncorhynchus mykiss*) industry in the Hagerman Valley of southern Idaho has experienced severe disease problems associated with this bacterium. In this region, major outbreaks are often associated with co-infections of both *F. psychrophilum* and infectious hematopoietic necrosis virus (IHNV). Conservation aquaculture in the Pacific Northwest of the United States also suffers large losses primarily in coho salmon (*O. kisutch*) and steelhead (*O. mykiss*).

The following information provides an overview of CWD. The distribution, host susceptibility, signs of infection, and modes of transmission are discussed along with the current treatment practices and future of vaccine development.

Distribution

Originally isolated in Washington state in 1948 (Borg 1948), the distribution of *F. psychrophilum* was believed to be limited to North America. However, the bacterium has since been isolated from many salmonid-producing regions of the world, indicating a much broader distribution. *F. psychrophilum* has been isolated in Denmark (Dalsgaard & Madsen 2000), France (Bernardet & Kerouault 1989), England (Austin and Stobie 1991), Australia (Schmidtke & Carson 1995), Chile (Bustos *et al.* 1995), Finland (Wiklund *et al.* 1994), Japan (Wakabayashi *et al.* 1991; Wakabayashi *et al.* 1994), the United Kingdom (Santos *et al.* 1992), Canada (Lumsden *et al.* 1996), and the United States (Pacha 1968; Kent *et al.* 1989). Presently, it is believed that the bacterium is ubiquitous in freshwater and eventually will be identified in most salmonid-producing regions of the world.

Host Susceptibility

All species of salmonids are believed to be susceptible to CWD, but coho salmon and rainbow and steelhead trout appear to suffer the greatest losses in the Pacific Northwest. Considered to be primarily a disease of young fish, the greatest mortality occurs in fry and fingerlings, although older fish can also be affected. Coldwater disease has also been reported in Atlantic salmon *(Salmo salar*), chinook salmon (*O. tshawytscha*), sockeye salmon (*O. nerka*), chum salmon (*O. keta*), cutthroat trout (*O. clarki*), brook trout (*Salvelinus fontinalis*), and brown trout (*S. trutta*) (Rucker *et al.* 1953; Bullock *et al.* 1971; Holt *et al.* 1993). Additionally, non-salmonid fishes such as eel (*Anguilla anguilla*), carp (*Cyprinus carpio*), tench (*Tinca tinca*), crucian carp (*Carassius carassius*), and ayu (*Plecoglossus altivelis*) are also susceptible (Lehmann *et al.* 1991; Wakabayashi *et al.* 1994).

Clinical Signs

Outbreaks of CWD typically occur at temperatures ranging from 39-50°F (4-10°C). However, over the past decade the commercial trout industry of the U.S. has experienced severe disease at constant temperatures of 59°F (15°C). The severity of disease has been found to decrease with increasing temperature above 59°F (Holt *et al.* 1989). Clinical signs vary between outbreaks and age of fish affected. In most cases, the younger the fish, the greater the severity of disease. Mortality among alevins (sac fry) may range from 30% to 50% and typical signs may include erosion of the skin covering the yolk sac (Holt *et al.* 1993). Infected fry, fingerlings, and adult fish generally become lethargic and may exhibit poor appetite and dark coloration (Evensen & Lorenzen 1996). *F. psychrophilum* has an affinity for skin and muscle tissue, and at the onset of feeding, lesions with yellow colored edges may form on the caudal peduncle region (Holt *et al.* 1993; Lumsden *et al.* 1996). If lesions appear in the caudal region, necrosis may progress deep into the muscle tissue and erode down to the vertebrae (Figure 1). Lesions on other parts of the fish and fraying and erosion of fins are also common.

Of particular concern is that fish surviving an epizootic of coldwater disease may develop spinal deformities (Madsen *et al.* 2001). The most common deformities are spinal compressions in the anterior, mid, or posterior regions of the fish (Figure 2). Spiral swimming behavior and dorsal swelling behind the skull have also been reported in survivors (Kent *et al.* 1989). This poses a problem in market-size fish as the product quality is downgraded and efficiency of automated processing equipment is affected. The development of such deformities and the impact on survival in fish stocked for conservation efforts is unknown.

In heavily infected fish, large numbers of bacteria can be found in the spleen, liver, intestine, air bladder, peritoneum, pancreas, and heart indicating the septicemic nature of this disease (Wood & Yasutake 1956; Evensen & Lorenzen 1996). Petechia (spotted or

Figure 1.
The characteristic yellow-edged lesions of the caudal peduncle caused by *F. psychrophilum*
Photo: Benjamin LaFrentz

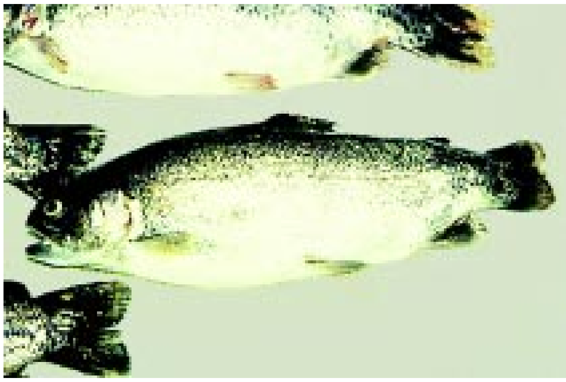

Figure 2.
This rainbow trout survived an epizootic of coldwater disease and exhibits spinal compression of the caudal peduncle region.
Photo: Benjamin LaFrentz

pinpoint hemorrhaging) may be present on pyloric cecae, adipose tissue, heart, swim bladder, and the peritoneal lining. The spleen of infected fish may also be grossly enlarged (Lumsden *et al.* 1996; Figure 3, page x). Renal elements of the kidney are usually severely damaged, and *F. psychrophilum* has been found to attack muscle fibers of the heart (Wood & Yasutake 1956). A small percentage of affected fish can exhibit lesions on the hindbrain, which may explain the spiral swimming behavior (Kent *et al.* 1989). Eye lesions have also been reported (Ostland *et al.* 1997), and it is speculated that such conditions may lead to blindness.

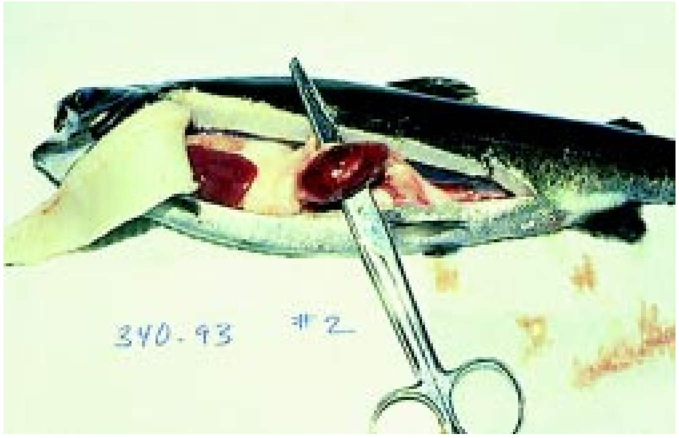

Figure 3.
Swollen spleen from a fish infected with coldwater disease.

Photo courtesy of Clear Springs Foods, Inc.

Diagnosis

Clinical signs and the development of characteristic yellow-edged lesions if the caudal peduncle is affected are usually indicative of an infection with *F. psychrophilum.* To obtain a presumptive diagnosis, one should submit moribund fish to a diagnostic fish lab, or examine imprints of the spleen or anterior kidney stained with Diff Quik® or Gram stain. *F. psychrophilum* cells are gram-negative, long, thin rods approximately 0.3-0.75 µm x 2-7 µm in size (Holt *et al.* 1993; Figure 4). For definitive diagnosis, bacteria should be isolated and identified on specialized low nutrient media. Tryptone-yeast extract (TYE) that may contain salts (TYES) is routinely used to culture *F. psychrophilum* and is the media of choice for diagnosis. Bacterial colonies (1-5 mm diameter) will grow in approximately two to four days at 59-68°F (15-20°C), appearing bright yellow with thin, spreading edges (Holt *et al.* 1993) sometimes referred to as a "fried-egg" colony morphology.

Transmission

Laboratory studies have shown that coldwater disease can be spread horizontally from fish to fish (Madsen & Dalsgaard 1999; Madetoja *et al.* 2000). However, it appears that horizontal transmission is much greater when there is a predisposing condition such as a lesion or damage to the skin (Madetoja *et al.* 2000). Stress of any kind may also increase susceptibility.

In addition, there is growing evidence of vertical transmission (from adult to progeny) of *F. psychrophilum* (Holt *et al.* 1993; Rangdale *et al.* 1996; Brown *et al.* 1997; Ekman *et al.* 1999). Bacteria can be isolated from ovarian fluid and milt from sexually mature fish and from egg surfaces (Holt *et al.* 1993; Rangdale *et al.* 1996). Another critical aspect is that it appears that *F. psychrophilum* may be transmitted within eggs. Such transmission can make control through standard iodophore disinfection difficult. Reports have indicated that the bacterium can be isolated from inside newly fertilized eggs, eyed eggs, and newly hatched sac fry (Brown *et al.* 1997), but true vertical transmission may not occur in every situation.

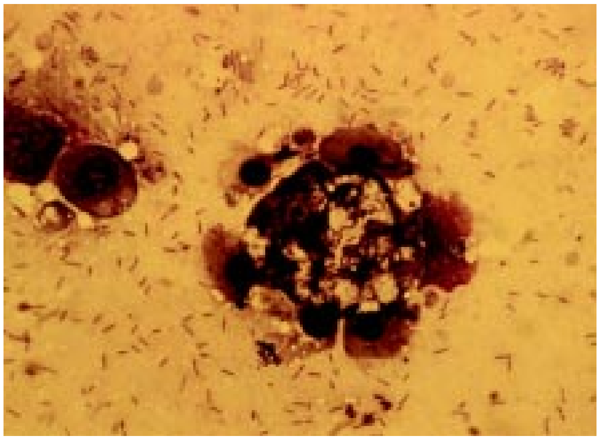

Figure 4.
Long thin gram negative rods of *F. psychrophilum* in an imprint of the spleen of an infected fish
Photo courtesy of Clear Springs Foods, Inc.

Prevention and Treatment

Prevention of coldwater disease is difficult due to the ubiquitous nature of *F. psychrophilum*, but centers around good fish culture management practices. The best prevention measure is to reduce stress and minimize damage to the skin since these two factors tend to increase disease transmission. Removal of mortalities and sick or dying fish from ponds is extremely important, helping to reduce the bacterial load shedding into the water and the risk of disease transmission.

Obtaining an accurate diagnosis is important before treating any fish for coldwater disease, and treatment requires consultation with a licensed veterinarian. There are no approved drugs available for the treatment of coldwater disease; however, extra-label use under veterinarian care is possible.

Eggs

Iodophore disinfection of eggs at water hardening and/or just prior to hatch has been shown to be a prudent management strategy to minimize egg-associated transmission risks. Because *F. psychrophilum* has been detected in fluid surrounding the eggs in sexually mature salmonids, iodophore treatment of eggs is generally used to reduce microbial contamination of the egg surface. Eggs should be treated at 100 parts per million active ingredient (iodine) for 10 to 30 minutes.

External Infections

Salt used as a bath solution for treating external bacteria can be effective. Salt (sodium chloride-NaCl) is listed by the Food and Drug Administration (FDA) as "low regulatory priority," which means that use of this drug generally will not be a problem if used responsibly and according to the conditions of the FDA. A salt solution of 3% (25 pounds per 100 gallons), if possible, should be applied for 10 to 30 minutes or until fish show signs of stress.

Potassium permanganate ($KMnO_4$) is another treatment used for control of external bacteria and is listed by the FDA as "deferred regulatory status." This means that regulatory action has been deferred pending further study and a veterinarian and/or the FDA should be contacted prior to use of this chemical as a drug.

Internal infections

Because there are no FDA approved antibiotics for CWD, it is essential to get proper veterinary approval as the use of antibiotics to control CWD in foodfish is considered an extra-label use of the drug. Internal infections are best treated with Terramycin® (Oxytetracycline; Pfizer, Inc.) medicated feeds administered at 2.5 to 3.75 grams of active ingredient per 100 pounds of fish for ten days. Treatments should always be the maximum recommended dose and should be fed for the total number of days recommended even if the fish appear to have recovered. Feeding less than the recommended dosage or number of days can lead to bacterial resistance to the antibiotic, which could render future treatments ineffective. A 21-day withdrawal period must occur before fish can be sold for human consumption.

Management of Coldwater Disease in the Future

The responsible use of available treatments and good management practices continue to be the only methods for control of CWD. Vaccination is another possible preventative measure, however, there is no commercial vaccine available at this time. Research for the development of a vaccine to prevent CWD in juvenile fish is progressing. An important aspect in the development of a vaccine is that fish develop significant antibody responses to *F. psychrophilum* (LaFrentz *et al.* 2002). With an increased understanding of the protective immune response to *F. psychrophilum* it may be possible to formulate vaccines that can provide protection during certain stages of development. Since CWD management strategies are limited, an effective vaccine still holds the greatest chance of preventing and minimizing CWD in rainbow trout and other salmonids.

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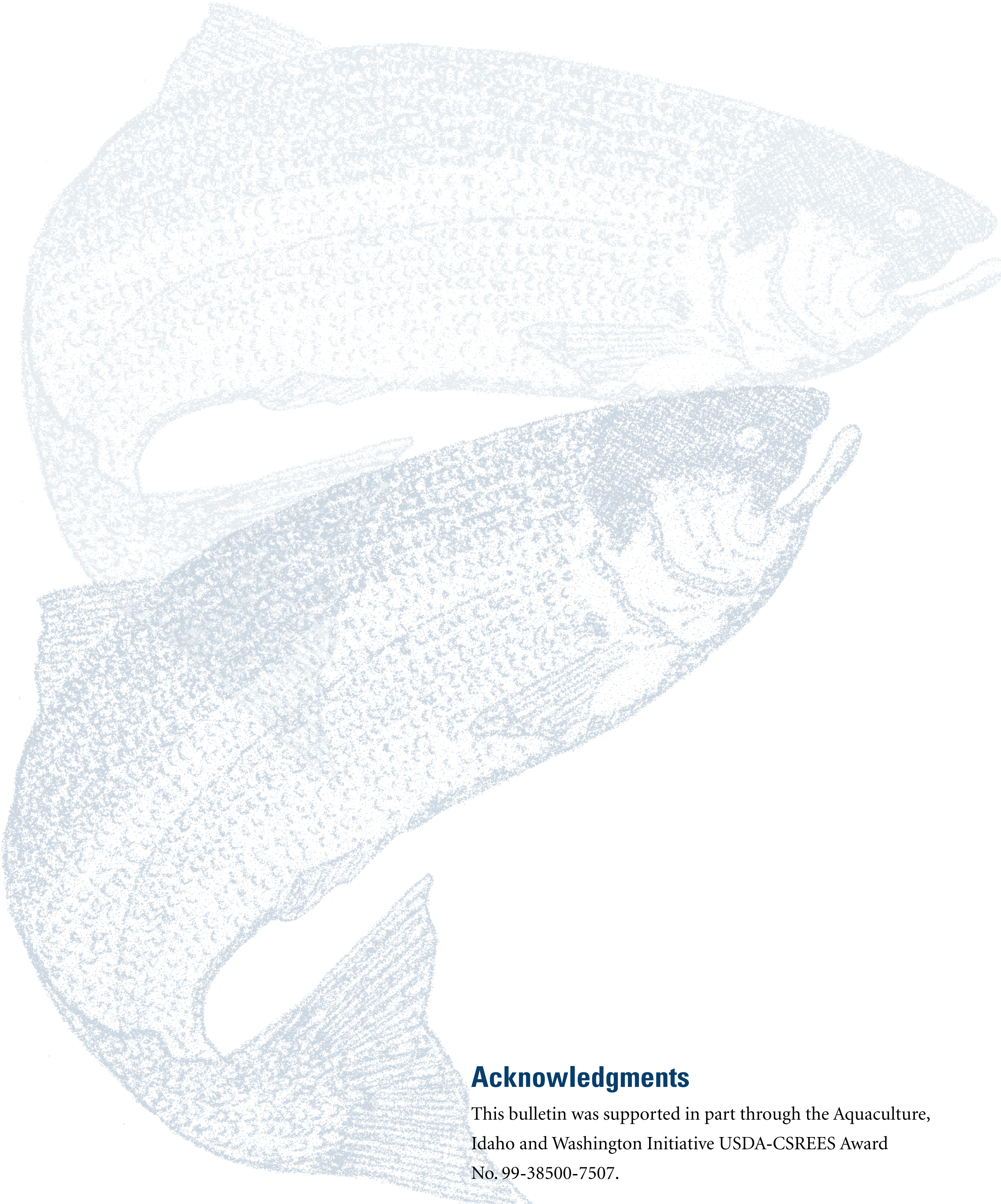

Acknowledgments

This bulletin was supported in part through the Aquaculture, Idaho and Washington Initiative USDA-CSREES Award No. 99-38500-7507.