1986

COLUMNARIS DISEASE OF FISHES

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FISH DISEASE LEAFLET 72

UNITED STATES DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Division of Fisheries and Wetlands Research
Washington, D.C. 20240

1986

[1] Revision of *Fish Disease Leaflet* 45 (1976), same title, by S. F. Snieszko and G. L. Bullock.

Introduction

Columnaris disease is an acute to chronic bacterial infection that affects anadromous salmonids and virtually all species of warmwater fishes. Davis (1922), who first described the disease, named it columnaris because the causal bacterial cells seen in wet mounts of affected gills and fins were arranged in columnar aggregations. Ordal and Rucker (1944) were the first to isolate the causal organism and, based on cellular morphology, identified it as a myxobacterium. Organisms classified in the order Myxobacterales are long, thin gram-negative rods that are motile on agar media by a creeping or flexing motion. They have a life cycle composed of vegetative cells, microcysts (resting cells), and fruiting bodies, or only vegetative cells and microcysts. Ordal and Rucker (1944) reported that the myxobacterium from columnaris disease produced both fruiting bodies and microcysts and named the organism *Chondrococcus columnaris*. Garnjobst (1945) studied strains of the columnaris bacterium and reported that microcysts were present but not fruiting bodies. Because fruiting bodies could not be demonstrated, she placed the organism in the genus *Cytophaga* and suggested *Cytophaga columnaris*. However, in the eighth edition of *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons 1974), it was stated that the columnaris bacterium produced neither fruiting bodies nor microcysts. Therefore it was removed from the Myxobacterales, placed in the order Cytophagales, and renamed *Flexibacter columnaris*.

Clinical Signs and Pathology

Columnaris disease begins externally on body surfaces and gills, and the lesions tend to vary with the fish. In scaleless fish such as the catfishes (*Ictalurus* sp.), initial lesions are small and circular and have gray-blue necrotic centers and red margins surrounded by a ring of inflamed skin. As the disease progresses, the lesions spread and may cover most of the body. In scaled fish such as the bluegill (*Lepomis macrochirus*), necrotic lesions begin at the outer margins of the fins and spread inward toward the body. In advanced body lesions, bacteria penetrate the dermis and cause necrosis of muscle fibers and destruction of capillaries.

Both in Pacific salmonids and in warmwater pondfishes, columnaris commonly causes extensive gill necrosis, which starts at the margins of the filaments and progresses inward toward the arches. Characteristic pathology associated with the gill lesions includes marked congestion of blood vessels, dissociation of surface epithelium of lamellae, and scattered hemorrhages.

As gill and muscle tissues are destroyed, the bacteria become systemic but internal pathology is limited to glomerular lesions. Cells of *Flexibacter* usually cannot be seen in stained smears, but the organisms can be readily cultured.

Etiology and Diagnosis

Flexibacter columnaris has been extensively studied and is the best known of the flexibacters pathogenic to fish. When grown on the medium described by Anacker and Ordal (1955) the bacterium forms pale yellow colonies that have a convoluted center, rhizoid edges, and tend to adhere to the medium. This type of colony has not been encountered in other flexibacters pathogenic to fish (Bullock 1972). The finding by Pyle and Shotts (1981) that *F. columnaris* showed little DNA binding with other flexibacters from diseased fish indicated that *F. columnaris* is not closely related to the other flexibacteria.

A selective medium (Fig. 1) for the isolation of *F. columnaris* has been developed (T. C. Hsu and E. B. Shotts, unpublished data) that is easier to work with in clinical situations than is the Anacker and Ordal medium (Fig. 1). It is composed of 2 g tryptone, 0.5 g yeast extract, 3 g gelatin, 15 g agar, and 1,000 mL deionized water. It is autoclaved at 15 psi for 15 min and cooled to 45 °C; filter decontaminated neomycin sulfate (4.0 μg/mL) is added and the medium is poured into petri dishes.

Presumptive diagnosis of columnaris disease is based on the presence of typical lesions containing long, thin gram-negative rods, and the formation of characteristic columns in infected tissues.

The appearance of the characteristic yellow, spreading colonies within 48 h on the Hsu-Shotts or Anacker and Ordal (1955) medium, and a positive slide agglutination test with *F. columnaris*

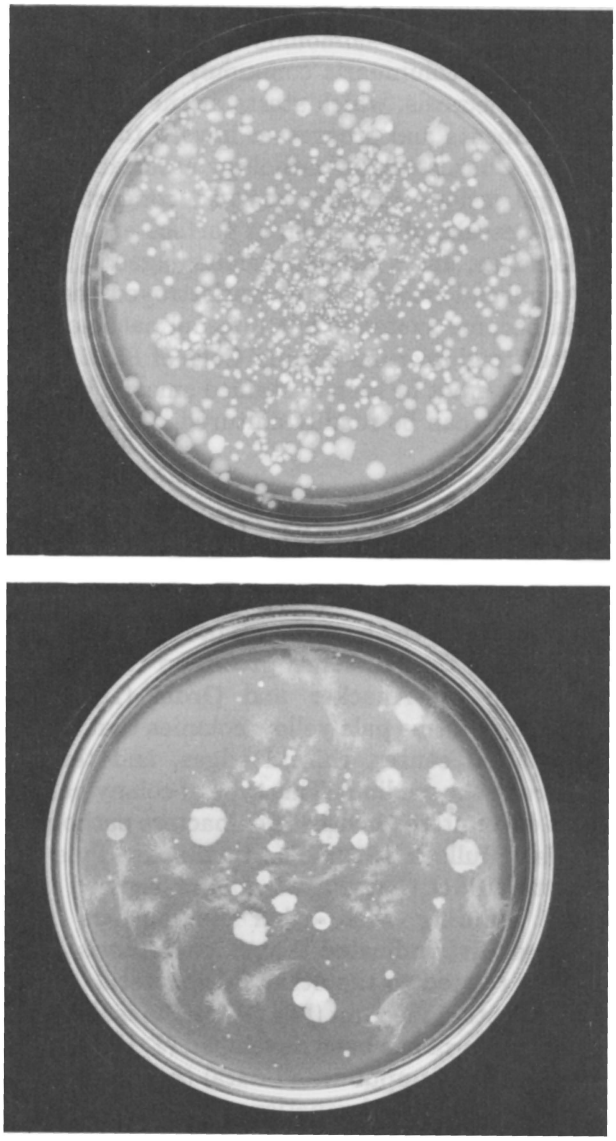

Fig. 1. Isolation of *Flexibacter columnaris* from infected tissue. *Upper photo*—Tissue streaked onto *Cytophaga* agar, which does not allow isolation of *F. columnaris* because it is overgrown by other bacteria. *Lower photo*—Tissue streaked onto Hsu-Shotts selective medium, which suppresses growth of most contaminating bacteria but allows growth of the characteristic spreading, rhizoid colonies of *F. columnaris*.

antiserum, constitute a confirmatory diagnosis. A direct or indirect fluorescent antibody test can also be used to identify *F. columnaris* (Amend 1983).

Virulence Factors

Pacha and Ordal (1962) determined the virulence of 500 strains of *F. columnaris*, using a bath exposure and 6- to 14-month-old sockeye salmon (*Oncorhynchus nerka*) or chinook salmon (*O. tshawytscha*). Test fish were held for 2 min in a 1:20 dilution of a 24-h broth culture and were then transferred to flowing water at 18 °C. Strains were designated as of high virulence if all exposed salmon died within 24 h, of moderate virulence if all salmon died within 48 h, of intermediate virulence if all salmon died within 96 h, and of low virulence if more than 96 h was required for all salmon to die. However, virulence among strains was neither stable nor uniform. For example, Pacha (1961) found that none of the 12 single-colony isolates from a high virulence strain were still virulent when salmon were challenged. The factors that contribute to this reduction or loss of virulence of *F. columnaris* strains are still being investigated. Morita (1975) reported that flexibacters from fish develop powerful proteolytic enzymes that lyse intact bacterial cells. Also, virulence may be reduced by the inactivation of certain enzymes that normally break down the integument and allow the spread of the bacteria—e.g., hyaluronidase (B. R. Griffin, U.S. Fish and Wildlife Service, Fish Farming Experimental Station, personal communication) and chondroitin sulfatase (E. B. Shotts, unpublished data).

Source and Reservoir of Infection

Although *F. columnaris* can survive in sterile water or mud, survival principally depends on temperature, water hardness, pH, and other factors. Becker and Fujihara (1978) reported that when *F. columnaris* cells were seeded in sterile mud for 77 h, 62% survived at 10 °C, but only 35% at 20 °C. Fijan (1968) reported that low pH, soft water, and low organic content decreased survival of *F. columnaris*. However, fish serve as the primary reservoir, and coarse fish such as suckers (Catostomidae) serve as reservoirs of infection for Pacific salmon. Bacteria are shed from lesions or from gills—only once by some fish, but several times by others. High temperatures and densities favor increased release of bacteria.

Transmission

Columnaris disease is principally transmitted from fish to fish, but many factors influence the release of cells. Holt et al. (1975) found that when steelhead trout (*Salmo gairdneri*) or coho salmon (*O. kisutch*) experimentally infected with columnaris were held in water at 12 to 20 °C, mortality increased with temperature. The disease was reported to be intensified by low dissolved oxygen and elevated ammonia by Chen et al. (1982), and by the addition of an organic material such as formulated feed (in Japanese eels, *Anguilla japonica*) by Sugimoto et al. (1981).

Host and Geographic Range

Columnaris disease is common in fresh and brackish (not marine) waters throughout the world, and affects virtually all species of freshwater fishes and anadromous salmonids. Catadromous fishes, such as eels, are highly susceptible when held in fresh or brackish water (Wakabayashi et al. 1970; Hine and Boustead 1974). The disease is common in pond fishes in the United States (Meyer 1970) and in common carp (*Cyprinus carpio*) in Europe (Spangenberg 1975).

Campbell and Buswell (1982) reported that black patch necrosis, a disease of dover sole (*Solea solea*), a cultured marine species, was caused by a bacterium that was similar to *F. columnaris*; this was the first report of an infection in a marine species caused by an organism resembling *F. columnaris*.

Control

Prevention

Columnaris disease is difficult to avoid because it is widespread among freshwater fishes and the bacterium is shed in the environment. However, temperature is a limiting factor and columnaris is not a problem among salmonids cultured at temperatures as low as 10 to 15 °C. In contrast, it is a major problem among cultured warmwater fishes and Pacific salmon returning to their spawning streams. In the late 1940's, the dramatic increase in the severity of columnaris in Pacific salmon led to the speculation that the operation of a plutonium plant complex at Hanford, Washington, was a contributing factor. However, studies conducted from 1965 to 1976 showed that the increased incidence and severity of the disease was caused by environmental modifications associated with hydroelectric development. Over a period of 30 years the Columbia River was transformed from a free-flowing stream to a series of impoundments. The resultant increased temperature, reduced flow velocity, and higher fish densities favored the proliferation of columnaris (Becker and Fujihara 1978).

Davis (1922, 1953) reported that after fish had been handled, the development or intensification of columnaris could be prevented by treating them with a 20-min copper sulfate bath at 37 ppm (1:30,000) or by adding copper sulfate to pond water at 0.5 ppm; he also recommended a dip of 1 to 2 min in a dilution of 1:2,000. Rogers (1971) recommended the addition of potassium permanganate to pond water at 2 ppm for an indefinite period.

Conrad et al. (1975) reported that ozone treatment of water significantly reduced the numbers of added *F. columnaris* cells, indicating that the application of ozone might be a practical method of prevention.

Although a potential for the development of a commercial vaccine exists, none is yet available. Channel catfish (*Ictalurus punctatus*) injected with *F. columnaris* produced an agglutinating titer of 1:4,337 (Schachte and Mora 1973), and Becker and Fujihara (1978) reported that rainbow trout injected with heat-killed cells produced an agglutinating titer of 1:5,120, and that 60–70% of the trout later survived an injection of 10^6 live *F. columnaris* cells. Additionally, the mortality of coho salmon fed a ration containing heat-killed *F. columnaris* was only 8%, compared with 48% in nonvaccinated controls. The effectiveness of bath, spray, or immersion vaccination has not been adequately tested but Amend (1983) reported that preliminary trials were not encouraging.

Treatment

Flexibacter columnaris is susceptible to a wide variety of drugs and antibiotics (Fijan and Vorhees 1969), and a number of chemicals and antibac-

terials have been found effective for the control of external or systemic columnaris infections; however, none are registered with the U.S. Food and Drug Administration. External treatments are possible only in the early stages of the disease, when infection is still external. One such treatment for salmonids is with the herbicide Diquat diluted to 8.4 ppm active ingredient (2 ppm of the Diquat cation). One-hour treatments should be repeated on each of 4 consecutive days.

Copper sulfate at 0.5 ppm (Davis 1922, 1953) and potassium permanganate at 2-4 ppm (Rogers 1971; Jee and Plumb 1981) are among the older chemicals used for treatment and for prevention of columnaris disease in pond fishes. These chemicals are added to ponds and allowed to dissipate with time.

The organic load in water affects the efficacy of potassium permanganate, and methods are now available to estimate that organic load and compensate by adjusting the level of the chemical (Avault 1985; Jee and Plumb 1981).

Oxytetracycline (Terramycin) given orally with food at a rate of 8 g per 100 kg of fish per day for up to 10 days is effective in both early and advanced outbreaks (Wood 1974).

Other effective new drugs, applied orally or as a bath, are nifurpirinol or Furanace (Amend and Ross 1970; Amend 1972; Ross 1972; Williams 1973); nifurprazine, known as Aivet in Japan and Carofur in Germany (Shiraka et al. 1970); and oxolinic acid (Endo et al. 1973). Nifurpirinol and nifurprazine are added to water at 1 ppm (in which fish are immersed for 5 to 10 min), or at 0.05 to 0.1 ppm in ponds for an indefinite exposure period. In oral administration, these nitrofurans are used at a rate of 2 to 4 mg/kg of fish with food for 3 to 5 days. Oxolinic acid is used as a bath at 1 ppm for 24 h. Sulfonamides such as sulfamerazine and sulfamethazine are used orally with food at a rate of 10 to 20 mg/kg of fish per day but are less effective than the other drugs.

Annotated Bibliography

Amend, D. F. 1972. Efficacy, toxicity, and residues of nifurpirinol in salmonids. U.S. Fish Wildl. Serv., Tech. Pap. 62. 13 pp.

Excellent control of columnaris was obtained with one or two daily 1-h treatments with 1 ppm nifurpirinol (Furanace) added to water.

Amend, D. F. 1983. Columnaris (*Flexibacter columnaris*) disease of freshwater fishes and a brief review of other flexibacterial diseases of fish. Pages 139-149 *in* D. P. Anderson, M. Dorson, and Ph. Dubourget, eds. Les Antigenes des micro-organismes pathogenes des poissons. Fondation Marcel Merieux, Lyon, France.

Description of epidemiology and control of columnaris, and characteristics of *Flexibacter columnaris*.

Amend, D. F., and A. J. Ross. 1970. Experimental control of columnaris disease with a new nitrofuran drug, P-7138. Prog. Fish-Cult. 32:19-25.

Treatment of experimentally induced columnaris disease with nifurpirinol (Furanace) was effective at a concentration of 1 or 0.5 ppm but not at 0.25 ppm.

Anacker, R. L., and E. J. Ordal. 1955. Study of a bacteriophage infecting the myxobacterium *Chondrococcus columnaris*. J. Bacteriol. 70:738-740.

The formulation of a medium used to grow the columnaris is given, as well as a description of a bacteriophage infecting *Flexibacter columnaris*.

Avault, J. W., Jr. 1985. Pond treatment with potassium permanganate. Aquacult. Mag. 11(1):45-46.

Organic matter in ponds often inactivates the oxidizing potential of potassium permanganate. A method is described for estimating that effect and the increase in potassium permanganate that must be made to ensure therapeutic levels for control of columnaris in channel catfish ponds.

Becker, C. D., and M. P. Fujihara. 1978. The bacterial pathogen *Flexibacter columnaris* and its epizootiology among Columbia River fish; a review and synthesis. Am. Fish. Soc. Monogr. 2. 92 pp.

Studies conducted on columnaris in resident and anadromous fishes of the Columbia River are reviewed. High-virulence strains capable of causing extensive mortalities in adult salmonids were observed in the 1950's. There was concern that the operation of a plutonium plant complex at Hanford, Washington, might adversely influence disease epizootiology; however, no such effect was indicated. Rather, the increased virulence was attributed to environmental modifications associated with hydroelectric development of the Columbia River.

Buchanan, R. E., and N. E. Gibbons, editors. 1974. Bergey's manual of determinative bacteriology, 8th ed. Williams & Wilkins, Co., Baltimore, Md. 1246 pp.

In this edition of the standard United States reference on bacterial taxonomy and identification, the taxonomic positions of many fish-pathogenic bacteria have been changed. The long, thin gram-negative gliding

bacteria formerly classified as myxobacteria are here referred to as flexibacteria.

Bullock, G. L. 1972. Studies on selected myxobacteria pathogenic for fishes and on bacterial gill disease in hatchery reared salmonids. U.S. Fish Wildl. Serv., Tech. Pap. 60. 30 pp.

Characteristics of 55 cultures of fish-pathogenic myxobacteria are described. The organisms can be serologically divided into several serotypes. Myxobacterial gill disease differs significantly from columnaris.

Bullock, G. L., D. A. Conroy, and S. F. Snieszko. 1971. Bacterial diseases of fishes. Book 2A (151 pp.) *in* S. F. Snieszko and H. R. Axelrod, eds. Diseases of fishes. T.F.H. Publications, Inc., Neptune City, N. J.

Includes a detailed description of columnaris and the recommended treatments.

Campbell, A. C., and J. A. Buswell. 1982. An investigation into the bacterial etiology of black patch necrosis in Dover sole, *Solea solea* L. J. Fish Dis. 5:495-508.

When samples of tissue from farmed Dover sole, some of which were identified as having black patch necrosis, were examined microbiologically, a long, thin, filamentous gram-negative organism, strongly resembling *Flexibacter columnaris*, was repeatedly isolated from diseased tissue but not from healthy tissue. The organism was highly pathogenic, producing 100% mortality in 96 h at 17.5 °C (± 2 °C) but was reisolated from all artificially infected fish.

Chen, C. R. L., H. Y. Chung, and G. H. Kuo. 1982. Studies on the pathogenicity of *Flexibacter columnaris*—I. Effect of dissolved oxygen and ammonia on the pathogenicity of *Flexibacter columnaris* to eel (*Anguilla japonica*). Comm. Agric. Plann. Dev. Ser. 8, Fish Dis. Res. 4:57-61.

Mortality rates of eels bathed in a suspension of *Flexibacter columnaris* for 1 h were determined after the fish were held in stagnant, aerated, or running water. Dissolved oxygen, ammonia, and pH were determined daily during the 9 days of the experiment. Mortality was lowest in running water and highest in stagnant water. It appeared that the 24-h mortality rate correlated inversely with dissolved oxygen. When dissolved oxygen was adequate, total mortality increased with increases in ammonia.

Conrad, J. F., R. A. Holt, and T. D. Kreps. 1975. Ozone disinfection of flowing water. Prog. Fish-Cult. 37:134-136.

A small experimental ozonator capable of producing 1 g ozone per hour to disinfect a water flow of 22.7 L/min was developed. Spring water at 13 °C and 21 °C was introduced into a 132-L reservoir and distributed into four tanks. Water flow to two tanks passed through a mixing chamber, where it was exposed to ozone. Tests demonstrated that ozone significantly reduced the numbers of viable *Flexibacter columnaris* cells in flowing water.

Davis, H. S. 1922. A new bacterial disease of fresh-water fishes. U.S. Bur. Fish. Bull. 38:261-280.

This is the first description of columnaris disease. Species affected, causative agent, transmission, and treatment methods are described. Although the author gave an accurate microscopic description of the causative bacterium, he was unable to isolate it.

Davis, H. S. 1953. Columnaris disease. Pages 265-273 *in* H. S. Davis, ed. Culture and diseases of game fishes. University of California Press, Berkeley.

An excellent description of the signs in fish infected with the pathogen, by the discoverer of the disease. Control measures are also mentioned.

Endo, T., K. Ogishima, H. Hayasaka, S. Kaneko, and S. Ohshima. 1973. Application of oxolinic acid as a chemotherapeutic agent against infectious diseases in fishes—I. Antibacterial activity, chemotherapeutic effects and pharmacokinetics of oxolinic acid in fishes. Bull. Jpn. Soc. Sci. Fish. 39:165-171.

Oxolinic acid used as a 1-ppm bath for 24 h was nontoxic to various fishes, and was very effective in control of columnaris and other diseases.

Fijan, N. 1968. The survival of *Chondrococcus columnaris* in waters of different quality. Bull. Off. Int. Epizoot. 69(7-8):1159-1166.

Chondrococcus [*Flexibacter*] *columnaris* survived for 16 days in hard water or water rich in organic matter. However, survival was reduced to less than a day in water with a pH of 6.0.

Fijan, N. N., and P. R. Voorhees. 1969. Drug sensitivity of *Chondrococcus columnaris*. Vet. Arh. 39(9-10):259-267.

Results of in vitro sensitivity tests of *Chondrococcus* [*Flexibacter*] *columnaris* to 28 antibiotics, 5 nitrofurans, 11 sulfonamides, 20 disinfectants, and 16 other chemicals.

Fujihara, M. P., and R. E. Nakatani. 1971. Antibody production and immune responses of rainbow trout and coho salmon to *Chondrococcus columnaris*. J. Fish. Res. Board Can. 28:1253-1258.

Active immunity against *Chondroccus* (*Flexibacter*) *columnaris* was established in 3-month-old coho salmon by oral vaccination. After being vaccinated, salmon showed serum agglutinin titers up to 1:640. However, rainbow trout immunized by parenteral injection had titers up to 1:5,120.

Fujihara, M. P., P. A. Olson, and R. E. Nakatani. 1965. Antibody production and immune response of fish to *Chondrococcus columnaris*. Pages 194-196 *in* Hanford Biol. Res. Annu. Rep. for 1964, U.S.A.E.C. Res. Dev. Rep.

Rainbow trout exposed to natural infections as fingerlings may become immune as yearlings and thereafter be active carriers of the disease organism.

Fujihara, M. P., and R. L. Tramel. 1968. Columnaris exposure and antibody production in seaward and upstream migrant sockeye salmon. Pac. Northwest Lab. Annu. Rep. for 1967 to the U.S.A.E.C. Div. Biol. Med. Biol. Sci. 1:9.16-9.21.

Juvenile sockeye salmon developed antibodies on their seaward migration and again as adults on their return. The authors suggested that many spawning adults are immune.

Garnjobst, L. 1945. *Cytophaga columnaris* (Davis) in pure culture: a myxobacterium pathogenic to fish. J. Bacteriol. 44:113-128.

A detailed, illustrated description of the columnaris bacterium.

Hine, P. M., and N. C. Boustead. 1974. A guide to disease in eel farms. Fish Res. Div., Minist. Agric. Fish., N.Z. Occas. Publ. 6. 28 pp.

Known diseases in New Zealand eel farms are outlined, sites or signs of the organisms are illustrated, and treatments are suggested. Different sections cover microbial and parasitic infections, dietary imbalance, and implementation of treatment. Includes a glossary.

Holt, R. A., J. E. Sanders, J. L. Zinn, J. L. Fryer, and K. S. Pilcher. 1975. Relation of water temperature to *Flexibacter columnaris* infection in steelhead trout (*Salmo gairdneri*) coho (*Oncorhynchus kisutch*), and chinook (*O. tshawytscha*) salmon. J. Fish. Res. Board Can. 32:1553-1559.

Experimental fish were subjected to eight temperatures from 3.9 to 23.9 °C by increments of 2.8 Celsius degrees. The effects of water temperature on experimental infection with *Flexibacter columnaris* were very similar in the three species of salmonids. The lowest temperature at which deaths were observed was 12.2 °C, and mortality increased progressively as temperature increased from 12.2 to 20.5 °C. At 9.4 °C and below, conditions were unfavorable for progress of the infection.

Jee, L. K., and J. A. Plumb. 1981. Effects of organic load on potassium permanganate as a treatment for *Flexibacter columnaris*. Trans. Am. Fish. Soc. 110:86-89.

The therapeutic efficacy of potassium permanganate was evaluated in fathead minnows (*Pimephales promelas*) infected with *Flexibacter columnaris* in organically enriched pond water and in organically depleted tap water. The chemical was bactericidal in tap water but only temporarily reduced *F. columnaris* in pond water; its efficacy in pond water was enhanced by satisfying the demand of organic matter for it. The most effective therapeutic concentration for fathead minnows infected with *F. columnaris* was 4 mg/L plus the organic demand.

Liewes, E. W., R. H. Van Dam, M. G. Vos-Maas, R. Bootsma. 1982. Presence of antigen sensitized leukocytes in carp (*Cyprinus carpio* L.) following bath immunization against *Flexibacter columnaris*. Vet. Immunol. Immunopathol. 3:603-609.

Bath immunization of common carp resulted in protection of fish at natural challenge. Stimulation of leukocytes derived from the thymus, spleen, anterior kidney, and mid-kidney of fish immunized with *Flexibacter columnaris* bacterin revealed the presence of antigen-sensitized cells in all lymphoid tissues except the anterior kidney. After 28 days a response was obtained in leukocyte cultures of the thymus and spleen.

Maas, M. G., and R. Bootsma. 1982. Uptake of bacterial antigens in the spleen of carp (*Cyprinus carpio* L.). Dev. Compl. Immunol. Suppl. 2:47-52.

Common carp were injected with bacterins of *Flexibacter columnaris* and *Aeromonas salmonicida* (the causal agent of carp erythrodermatitis), and others were immersed in *F. columnaris* bacterins. The uptake and processing of bacterial antigens in carp spleen are described, as determined by using the indirect fluorescent antibody technique. After injection, as well as after immersion of the fish, *F. columnaris* antigen was recovered in the ellipsoids of the spleen, apparently trapped by sheath cells. The significance of these findings is discussed with regard to possible immunization of carp.

McCarthy, D. H. 1975. Columnaris disease. J. Inst. Fish. Manage. 6(2):44-47.

A description of columnaris disease in the British Isles.

Meyer, F. P. 1970. Seasonal fluctuations in the incidence of disease on fish farms. Pages 21-29 *in* S. F. Snieszko, ed. A symposium on diseases of fishes and shellfishes. Am. Fish. Soc. Spec. Publ. 5.

Case histories during a 5-year period showed that flexibacterial infections among pond fishes were most common in April during spawning, and again in late summer when pond conditions were poor and oxygen levels low.

Morita, R. Y. 1975. Psychrophilic bacteria. Bacteriol. Rev. 39:144-167.

Psychrophilic fish-pathogenic myxobacteria autolyzed rapidly at temperatures above their maximum, which is above 15 °C. They release powerful proteolytic enzymes.

Morrison, C., J. Cornick, G. Shum, and B. Zwicker. 1981. Microbiology and histopathology of 'saddleback' diseases of underyearling Atlantic salmon, *Salmo salar* L. J. Fish Dis. 4:243-258.

Heavy mortalities of underyearling Atlantic salmon parr occurred at the Mactaquac Fish Culture Station in New Brunswick as a result of "saddleback" disease. The organism causing this lesion was isolated and identified as *Flexibacter columnaris*. It was transmitted to other salmon parr, where it produced the typical saddle lesion and from which it was reisolated. The condition was more readily transmitted at 20 °C than at 15 °C. Transmission required entrance to the dermis through a break in the epidermal surface.

Ordal, E. J., and R. R. Rucker. 1944. Pathogenic myxobacteria. Soc. Exp. Biol. Med. Proc. 56:15-18.

Description of *Chondrococcus* [*Flexibacter*] *columnaris* and its life cycle and pathogenicity.

Ourth, D. D., and E. A. Wilson. 1982. Bactericidal serum response of the channel catfish against gram-negative bacteria. Dev. Comp. Immunol. 6:579-583.

Bactericidal activity of fresh catfish serum against six gram-positive and eight gram-negative bacteria was compared. Activity was nil against the gram-positive forms, but ranged from 3 to 100% against gram-negative bacteria. The activity was greatest against *Salmonella paratyphi*, *Pseudomonas fluorescens*, and *P. putida*, and least against *Aeromonas salmonicida* and *Flexibacter columnaris*.

Pacha, R. E. 1961. Columnaris disease in fishes of the Columbia River Basin. Ph.D. Thesis. University of Washington, Seattle. 322 pp.

A comprehensive study of the epizootiology of columnaris disease among Columbia River fishes and of characteristics of the causative bacterium.

Pacha, R. E., and E. J. Ordal. 1962. Columnaris disease in Columbia River salmon. Page 20 *in* Bacteriological proceedings 1962, abstracts of the 62nd annual meeting, American Society for Microbiology, Kansas City, Missouri, 6-10 May 1962.

Description of virulence studies carried out with different strains of *Flexibacter columnaris*.

Pacha, R. E., and E. J. Ordal. 1970. Myxobacterial diseases of salmonids. Pages 243-257 *in* S. F. Snieszko, ed. A symposium on diseases of fishes and shellfishes. Am. Fish. Soc. Spec. Publ. 5.

A complete description of myxobacterial diseases of salmonids in northwestern United States. The formation of fruiting bodies and microcysts was also described.

Phelps, R. P., J. A. Plumb, and C. W. Harris. 1977. Control of external bacterial infections of bluegills with potassium permanganate. Prog. Fish-Cult. 39:142-143.

A concentration of 3 mg potassium permanganate per liter, applied for 24 h, was recommended for the treatment of skin lesions associated with *Flexibacter columnaris* and *Aeromonas hydrophila* in adult bluegills.

Poston, T. M., D. A. Neitzel, C. S. Abernethy, and D. W. Carlile. 1985. Effects of suspended volcanic ash and thermal shock on susceptibility of juvenile salmonids to disease. Pages 359-374 *in* R. C. Bahner and D. J. Hansen, eds. Aquatic toxicology and hazardous assessment: eighth symposium, American Society for Testing and Materials, Battelle Memorial Institute, Columbus, Ohio, 15-17 April 1984. A.S.T.M. Spec. Tech. Publ. 891.

Susceptibility of salmonids to *Flexibacter columnaris* was used to assess sublethal stress after exposures to two environmental stressors—thermal shock and suspended volcanic ash. The susceptibility of juvenile rainbow trout to disease was increased after exposure of the fish to 0.3 and 11.5 g/L suspended volcanic ash. The response was dose-dependent. Exposure of juvenile chinook salmon to thermal shock did not result in increased susceptibility to disease. In several tests, susceptibility to disease was less in thermally exposed fish than in control fish.

Pyle, S. W., and E. B. Shotts, Jr. 1980. A new approach for differentiating flexibacteria isolated from coldwater and warmwater fish. Can. J. Fish. Aquat. Sci. 37:1040-1042.

Seventeen strains of flexibacteria associated with fish disease were evaluated with a standardized miniaturized system that allows performance of 20 biochemical tests. Isolates from warmwater fish, which use arabinose, melibiose, and sucrose, were differentiated from isolates from coldwater fish, which do not. Isolates from coldwater fish may be further divided into subgroups on the basis of gelatin hydrolysis. The standardized miniaturized system seems to provide the first biochemical differentiation of this group of poorly defined organisms.

Pyle, S. W., and E. B. Shotts, Jr. 1981. DNA homology studies of selected flexibacteria associated with fish disease. Can. J. Fish. Aquat. Sci. 38:146-151.

Seventeen strains of gliding bacteria (flexibacteria) associated with fish disease were evaluated by using DNA homology techniques. The data supported an earlier report that isolates from warmwater fish may be considered as different from isolates of coldwater fish. DNA analysis indicated that at least four of the warmwater isolates studied were genetically identical and that one other warmwater isolate was sufficiently unrelated to these four organisms to indicate a strain, or possibly a species difference, as judged by the results of work with enteric bacteria. On the basis of a selected reference organism, three groups of isolates within the coldwater forms were related to differing degrees. A strain of *Flexibacter columnaris*, included as an unlabeled reference, demonstrated a low degree of relatedness to the labeled reference strains.

Rogers, W. A. 1971. Principal diseases of catfish: how to identify and fight them. Fish Farming Ind. 2:20-26.

Potassium permanganate was added to pond water at 2 ppm for indefinite periods to control columnaris.

Ross, A. J. 1972. *In vitro* studies with nifurpirinol (P-7138) and bacterial fish pathogens. Prog. Fish-Cult. 34:18-20.

Furanace (nifurpirinol) inhibited test strains of *Flexibacter columnaris* and *Cytophaga psychrophila* at concentrations of 0.78 and 3.1 ppm, respectively.

Sanders, J. E., R. A. Holt, and J. L. Fryer. 1976. Serological comparison of *Flexibacter columnaris* isolates using rabbit and rainbow trout (*Salmo gairdneri*) antisera. J. Fish. Res. Board Can. 33:1386-1388.

Bacterial agglutination tests with antisera from rabbit and rainbow trout revealed common antigen or antigens in each of the three isolates. Assays with adsorbed antisera from both species indicated that three isolates belong to two serological groups, each with its own unique antigen(s). These similarities between antisera of rainbow trout and rabbit indicate that the serotypes of *Flexibacter columnaris*, as defined by rabbit antisera, are similar to those recognized in salmonids. This observation suggests that these distantly related vertebrates both responded to the same surface antigens of the *F. columnaris* isolates.

Schachte, J. H., Jr., and E. C. Mora. 1973. Production of agglutinating antibodies in the channel catfish (*Ictalurus punctatus*) against *Chondrococcus columnaris*. J. Fish. Res. Board Can. 30:116-118.

Fingerling channel catfish were injected subcutaneously and intramuscularly with *Chondrococcus* [*Flexibacter*] *columnaris* antigen to test for the production of agglutinating antibodies. Both routes of injection produced a mean titer of 1:4,337 in test fish.

Shieh, H. S. 1980. Studies on the nutrition of a fish pathogen *Flexibacter columnaris*. Microbios Lett. 13(51-52):129-134.

A synthetic medium was developed for *Flexibacter* and the nutritional requirements for the organism were examined. This medium was capable of supporting good growth of the bacterium.

Shiraki, K., F. Miyamoto, T. Sato, I. Sonezaki, and K. Yano. 1970. Studies on a new chemotherapeutic agent nifurprazine (HB-115) against fish infectious diseases. Fish Pathol. 4:130-137.

Nifurprazine inhibits *Flexibacter columnaris* in vitro at concentrations of 0.01 to 0.04 ppm.

Spangenberg, R. 1975. Orientierende Untersuchungen über das Vorkommen von Myxobakterien bei der Kiemennekrose des Karpfens. Z. Binnenfisch. DDR 22:121-127.

Excellent description and illustrations showing columnaris disease in common carp (*Cyprinus carpio*) in Germany.

Sugimoto, N., S. Kashiwagi, T. Matsuda. 1981. Pathogenetic relation between columnaris disease in cultured eel *Anguilla japonica* and the formula feeds. Bull. Jpn. Soc. Sci. Fish. 47:719-726.

The relation between columnaris disease in cultured eel and granules of fish meal in the formula feeds was investigated. Infection and mortality were increased by the addition of formula feeds to the experimental aquarium.

Wakabayashi, H., K. Kira, and S. Egusa. 1970. Studies on columnaris eels—I. Characteristics and pathogenicity of *Chondrococcus columnaris* isolated from pond-cultured eels. Bull. Jpn. Soc. Sci. Fish. 36:147-155.

A description of columnaris disease in pond-cultured eels in Japan. Formation of fruiting bodies and microcysts was observed on Borg's medium.

Wedemeyer, G. A. 1974. Stress as a predisposing factor in fish diseases. U.S. Fish Wildl. Serv., Fish Dis. Leafl. 38. 8 pp.

Crowding and handling of fish at 15 °C or above are stress factors that contribute to outbreaks of columnaris.

Williams, I. V. 1973. Tests with nifurpirinol (P7138) to control prespawning mortalities of Fraser River

sockeye. Int. Pac. Salmon Fish. Comm. Prog. Rep. 28. 30 pp.

Nifurpirinol used as a bath at 1 to 3 ppm was helpful but not completely effective in control of mortalities attributed to *Flexibacter columnaris* in prespawning Fraser River sockeye salmon.

Wood, J. W. 1974. Diseases of Pacific salmon, their prevention and treatment. Wash. Dep. Fish., Olympia. n.p.

Diagnosis and control methods for columnaris are described.

Note: Use of trade names does not imply U.S. Government endorsement of commercial products.

U.S. Government Printing Office: 1986—676-205/40018