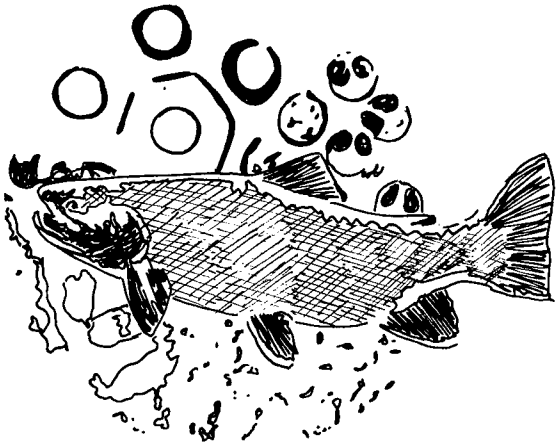
Infectious Diseases of Cultured Fishes: Current Perspectives



Developments *Discoveries*

Persistent Problems

UNITED STATES DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Fish and Wildlife Leaflet 5
Washington, D.C. • 1986

FISH AND WILDLIFE LEAFLETS

This series (formerly the *Wildlife Leaflet* series) primarily provides summaries of technical information for nontechnical readers. Each leaflet deals with a single subject such as a topic of current interest, inventory and survey results, management techniques, and fish or wildlife diseases. See inside back cover for a list of recent publications in this series.

Copies of this publication may be obtained from the Publications Unit, U.S. Fish and Wildlife Service, Matomic Building, Room 547, Washington, DC 20240 or may be purchased from National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161.

Library of Congress Cataloging-in-Publication Data

Bullock, G. L.
Infectious diseases of cultured fishes.

(Fish and wildlife leaflet ; 5)
Supt. of Docs. no.: I 49.13/5:5
1. Fishes—Diseases. I. Wolf, Ken. II. Title.
III. Series.
SH171.B85 1986 639.3 86-600049

Infectious Diseases of Cultured Fishes: Current Perspectives

By

G. L. Bullock and Ken Wolf

U.S. Fish and Wildlife Service
National Fish Health Research Laboratory
Box 700
Kearneysville, West Virginia 25430

Introduction

The U.S. Fish and Wildlife Service (FWS) has been a pioneer in research on fish health; its activities date back some 60 years to about 1925, when the work was carried out essentially by only one person, Dr. H. S. Davis. By the 1940's the number had increased to several investigators, notably Dr. S. F. Snieszko, Director of the Eastern Fish Disease Laboratory, Leetown, West Virginia, and his associate Dr. J. S. Gutsell; and Dr. Robert R. Rucker, Director of the Western Fish Disease Laboratory, Seattle, Washington. For more than 25 years, Drs. Snieszko and Rucker led the research at these laboratories and made significant contributions that advanced the techniques of diagnosis and disease control—principally for salmon and trout. They gradually recruited additional researchers and provided specialized training to many qualified persons from the United States and abroad.

In 1960, the Fish Farming Experimental Station was established at Stuttgart, Arkansas, where disease diagnosis, research, and extension services were the major activities.

Today, infectious disease research continues at the same three facilities, though two have different names: the National Fish Health Research Laboratory, and the Seattle National Fishery Research Laboratory. Additionally over the years, some State agencies and universities established their own laboratories dealing with fish health research. Many diagnosticians and other researchers at these laboratories received training at FWS laboratories.

The purpose of this overview of essentially the past three decades is twofold: to document the significant advances that have been made in the detection, diagnosis, and control of infections that historically have posed serious problems in fish husbandry, and to offer highlights of information on several diseases newly recognized within the period. Lest a too-optimistic impression be made, we emphasize certain diseases that continue to cause losses in spite of diligent research.

Fish Diseases of Long Standing

Early in the development of intensive fish husbandry, infectious diseases were an important cause of mortality and at times determined the success or failure of the operation. Early research focused on determining the etiological agent of the disease and on developing practical treatments. Continuing research has provided a better understanding of the gross and histopathological tissue changes, has improved diagnostic and detection methods, has developed new treatment procedures, and (for some of the diseases) has developed methods of immunization. The following are some examples of diseases historically associated with intensive fish culture.

Furunculosis

Furunculosis is a systemic bacterial infection caused by the gram-negative nonmotile rod-shaped bacterium *Aeromonas salmonicida*. The disease was named for the furuncle-like lesion seen in chronic infections (Fig. 1). Furunculosis was discovered first in Germany (in 1904) and apparently was brought to the United States with introductions of brown trout (*Salmo trutta*). It was soon recognized as a serious disease that killed thousands of

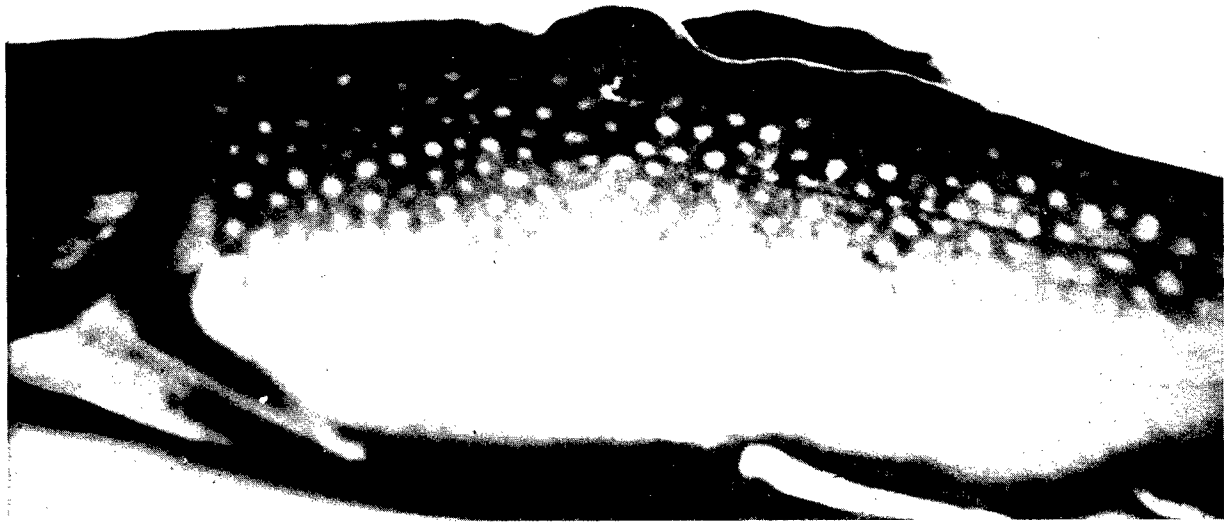


Fig. 1. Adult brook trout (*Salvelinus fontinalis*), showing furuncle typical of lesions seen in chronic furunculosis infections. (*From Bullock et al. 1971; reprinted with permission of T.F.H. Publications.*)

fish each year. Research on furunculosis has been carried out for more than 70 years, but only during the last 25 years has there been a marked increase in progress toward diagnosis and control.

Until the late 1960's, furunculosis was believed to occur only in salmonids in fresh water. However, it is now known that variants of *A. salmonicida*, differing only slightly from strains causing furunculosis in salmonids, cause goldfish ulcer disease (Fig. 2), carp erythrodermatitis (Fig. 3), trout ulcer disease, and infections in saltwater fishes. Therefore, in contrast to the scientific perception only a few years ago, *A. salmonicida* is now recognized as a cosmopolitan pathogen.

Years ago the diagnosis of furunculosis required that the causative organism be isolated and identified—a procedure that required as long as 4 days. More rapid diagnostic procedures were needed so that treatment could be started early in a disease outbreak. Research completed in the last 6 years at FWS and other laboratories now makes it possible to diagnose furunculosis within 1 h. Specifically, the furunculosis bacterium is identified in infected tissue by using a fluorescent antibody test. When reacted with specific antiserum that has been conjugated with fluorescein isothiocyanate, cells of *A. salmonicida* have an apple-green fluorescence when viewed by fluorescence microscopy. The development of an enzyme-linked immunosorbent assay test for furunculosis enables the detection of small numbers of *A. salmonicida* cells in carrier fish. Detection of carriers is essential to the recognition of sources of infection and to the prevention of spread of the disease.

During the 1940's, before drug therapy was developed, losses from furunculosis were severe. Although many antibacterial products are effective in treating the disease, the U.S. Food and Drug Administration requires that drugs be registered for legal use with food fish. The FWS began the registration process in the early 1960's, and the first drug, sulfamerazine, was approved 3 years later. The second drug, the antibiotic oxytetracycline (Terramycin), was registered in 1970, and the third, a potentiated sulfonamide (Romet), was registered in 1984.

Even though effective chemotherapy for furunculosis has been developed, the best health management practice consists of preventing infections; vaccination is widely used for that purpose. Vaccines for bacterial fish diseases were first produced commercially and marketed in the early 1970's. Recently completed FWS research demonstrated that proteins produced by *A. salmonicida* in culture media confer protection against furunculosis. On the basis on these results, a commercial vaccine is expected to be licensed.

The examples discussed above are but a few of the advances made in the diagnosis and treatment of furunculosis. In current and future research, the use of genetic engineering techniques should lead to a better understanding of furunculosis and its causative agent.

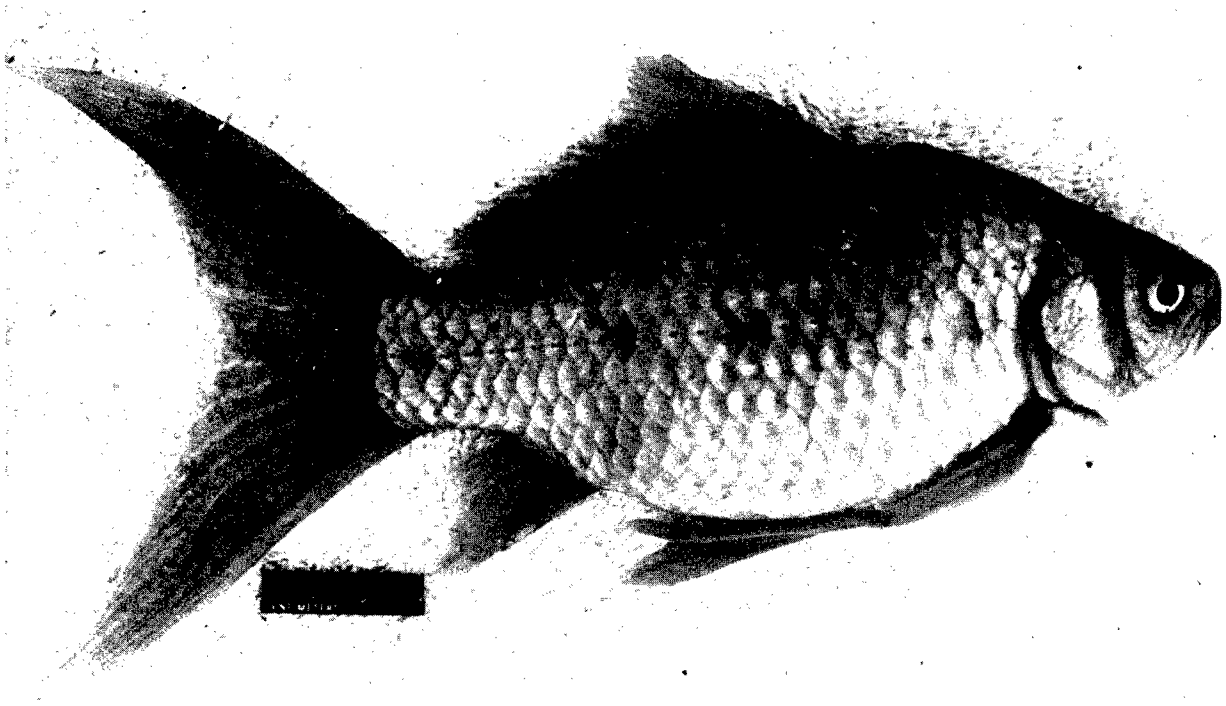


Fig. 2. Adult goldfish (*Carassius auratus*) with open lesion characteristic of goldfish ulcer disease. (*FWS photo.*)

Fig. 3. Common carp (*Cyprinus carpio*) showing typical lesions of carp erythrodermatitis. (*From Bullock et al. 1971; reprinted with permission of T.F.H. Publications.*)

Motile Aeromonas Septicemia

The term motile aeromonas septicemia (MAS) is used to describe motile aeromonad infections of warmwater and some coldwater fishes. In the past the infections were called hemorrhagic septicemia, rubella, red pest, or infectious dropsy. The etiological agent is *Aeromonas hydrophila*; however, the bacterium has been previously named *A. liquefaciens*, *A. punctata*, and *Pseudomonas punctata*.

Motile aeromonads can, under conditions of stress that are poorly understood, cause serious mortality among propagated warmwater fishes—the common carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*), and baitfishes. The organisms are ubiquitous in freshwater environments, commonly encountered as secondary invaders, and readily cultured on simple bacteriologic media. As a consequence, they have sometimes been mistakenly considered the cause of several diseases. As an example, an aeromonad was thought to cause carp dropsy, a disease that was described as occurring in two forms—an ascites form and an ulcerative form. Aeromonads were commonly found in fish with either form, but modern research has shown that the ascites form is caused by a rhabdovirus, and it is now known as spring viremia of carp. The cause of the ulcerative form is an atypical *Aeromonas salmonicida*, and that form is now termed carp erythrodermatitis.

Research on MAS has resulted in effective diagnostic procedures, better understanding of serological properties of the bacterium, and effective treatment methods. Because the causative bacterium is a normal inhabitant of the aquatic environment, fish are constantly exposed to infection. Disease outbreaks are common when fish are under stress from crowding, low oxygen, high temperatures, etc., and significant losses from such outbreaks occur annually. Treatment is limited to the addition of antibacterials to the feed; in the United States, only oxytetracycline is now registered for legal use. The increasing appearance of oxytetracycline-resistant strains of *A. hydrophila* will reduce the efficacy of this drug and additional antibacterials will have to be registered.

Efforts to develop a practical vaccine for prevention of MAS have not been successful, mainly because of the great serological diversity among *A. hydrophila* strains. Although fish immunized by immersion in an *A. hydrophila* vaccine are protected from challenge with the immunizing strain, they are not protected against serologically different strains.

Future research on MAS will probably be concentrated on the registration of additional antibacterials and on the development of a polyvalent vaccine. Continuing research on the nature of the virulence and of the protective factors of the bacterium should lead to the discovery of more fractions that will provide protection against the most commonly encountered strains of *A. hydrophila*.

Bacterial Kidney Disease

Bacterial kidney disease (BKD) is a chronic systemic infection of salmon and trout (Fig. 4) caused by the gram-positive rod-shaped bacterium *Renibacterium salmoninarum*. Although BKD causes serious mortality in both trout and salmon, it is now most severe in Atlantic salmon (*Salmo salar*) and chinook salmon (*Oncorhynchus tshawytscha*)—particularly spring chinook salmon. The causative bacterium grows slowly; its isolation in the laboratory often requires 2 to 3 weeks. The disease, too, characteristically progresses slowly; clinical signs usually require several months to develop. It is difficult to control BKD because the bacterium grows intracellularly—

Fig. 4. Effect of advanced renibacterial kidney disease in yearling brook trout. *Upper* specimen shows normal and uniformly dark kidneys (indicated within arrows). *Lower* specimen shows kidneys grossly swollen and abscessed due to bacterial infection. (*Photo courtesy of G. Camenish.*)

a feature that reduces the efficacy of therapeutic antibiotics. Also, the bacterium is transmitted inside eggs, and thus is passed from one generation to another.

Major research efforts to control BKD in chinook salmon resulted in the development of diagnostic and detection procedures, antibiotic treatment of brood stock and eggs to prevent transmission, and modification of diets to reduce disease incidence.

The fluorescent antibody technique allows diagnosis of BKD within 10 min, and the enzyme-linked immunosorbent assay test provides a means of detecting carriers of *R. salmoninarum*. The mating of brood stock free of BKD reduces the incidence of the disease.

Recent experiments involving the injection of adult salmon and the treating of eggs with erythromycin hold promise for reducing egg transmission of the disease. In addition, the experimental supplementation of diets with iodine and fluorine reduced the prevalence of BKD from 95% to 3–5%.

Although progress has been made in reducing losses from BKD, fully effective control of the infection will probably require additional years of research.

Vibriosis

Vibriosis is a systemic bacterial disease of estuarine and marine fishes caused by gram-negative motile rods of the genus *Vibrio*. The species most often isolated is *Vibrio anguillarum*, but *V. ordalii* causes epizootics in cultured salmon in the Pacific Northwest. The external and internal pathology of vibriosis is inseparable from that caused by other systemic gram-negative infections, such as furunculosis.

Vibriosis is probably the most serious bacterial disease of marine fish husbandry. Virtually all species are susceptible, but mortalities are most severe in eels cultured in Japan and Pacific salmon cultured along America's West Coast. Because *Vibrio* occurs naturally in seawater, the bacterium cannot be avoided. Outbreaks can be treated with oxytetracycline, but the usual result is an only temporary reduction of mortality. The most significant advance in controlling vibriosis has been the development of a vaccine for use in salmon. If salmon hatched and raised for several months in fresh water are immunized at least 2 weeks before transfer to seawater pens, they are protected from vibriosis.

The initial research on the West Coast involved the testing of an oral vibrio vaccine. However, the first vaccine that was licensed was applied by a hyperosmotic technique. Young salmon were exposed to a 4–5% solution of sodium chloride that dehydrated them and then were exposed to the vaccine solution. It was later learned that immunization can be effected more simply and better without sodium chloride pretreatment—the salmon are vaccinated by either direct immersion or spraying.

The use of the vibrio vaccine has reduced losses of pen-reared salmon.

Bacterial Gill Disease

Bacterial gill disease (BGD) is a serious respiratory infection of young salmon and trout that is triggered by a combination of as-yet-undefined environmental stressors and one or more kinds of bacteria. Because the gills in fishes serve the same respiratory function as lungs in mammals, BGD is analogous to pneumonia. The disease was first described in brook trout (*Salvelinus fontinalis*) in Vermont in 1926 and became more prevalent as salmon and trout husbandry intensified. Although hundreds of thousands of juvenile salmon and trout die of BGD each year, many aspects of the disease remain a mystery. Infected fish do not feed, are lethargic, and typically align themselves with the current to reduce the energy demands of respiration. Microscopic examination of gills shows swollen tissue and an abundance of filamentous gram-negative bacteria (Fig. 5). When BGD reaches this advanced stage, thousands of fish may die in a single day.

Researchers in the FWS and other agencies have shown that stressors such as crowding, low oxygen, and elevated levels of un-ionized ammonia contribute to outbreaks of BGD. If stressors are removed, the fish recover spontaneously. All attempts to reproduce BGD experimentally, by using bacteria isolated from diseased gills, have failed. Japanese investigators have described a *Flavobacterium* that morphologically resembles the filamentous forms seen in BGD. Although the bacterium is able to colonize gill surfaces, it has not consistently produced experimental BGD infections.

Unfortunately, little progress has been made during the past 30 years in identifying the factors responsible for BGD. Although the disease can be treated and sometimes prevented by exposing fish to low concentrations of external disinfectants, many fish die before the outbreak can be brought under control. A concentrated research effort is needed to identify the factors that lead to outbreaks and the bacteria that are involved.

Infectious Pancreatic Necrosis

Infectious pancreatic necrosis (IPN) is a common and highly contagious viral infection that typically runs an

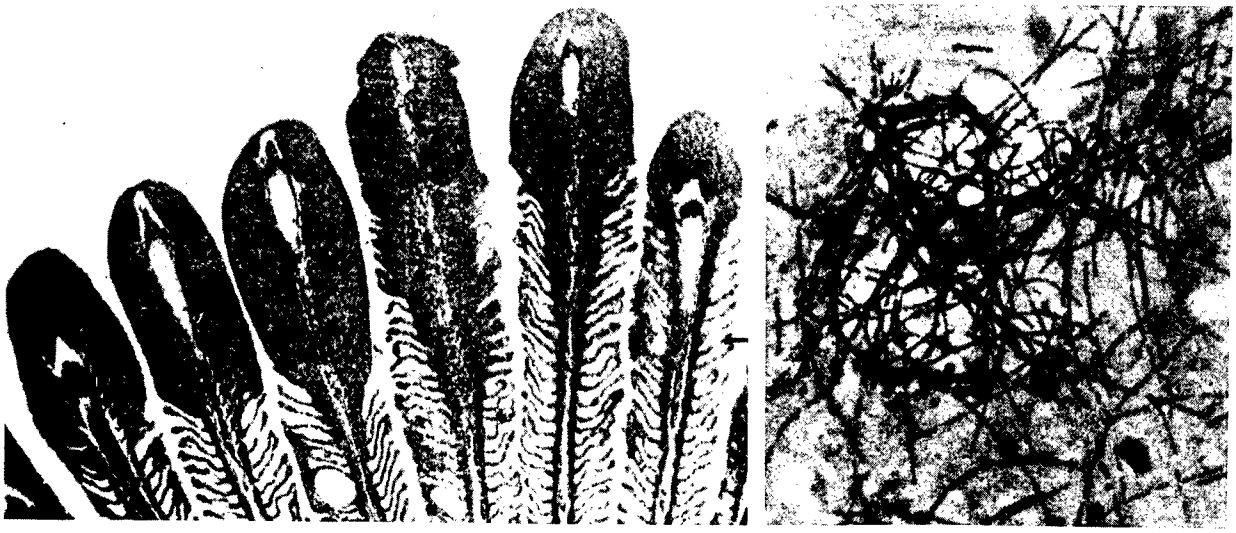


Fig. 5. Advanced bacterial gill disease characterized by swollen fused lamellae (*left*, ×25) and masses of long, thin bacteria (*right*, ×680). (*From Davis 1946.*)

acute course in the fry of some species of trout and results in mortality. The causal agent is the most widely distributed of the viruses that cause problems in salmonids.

The IPN virus (Fig. 6) was the first of the fish viruses to be isolated. That isolation was made at a FWS laboratory; a full report of the work was published 25 years ago. In earlier work it had been postulated that a virus caused acute fry mortality, and indicated a likelihood that the infection was carried by adults. Virological examinations showed that the agent was present with eggs and sperm and was thus passed from parent to offspring by vertical transmission.

During the first few years after its discovery, many aspects of IPN and its causal virus were regarded (in retrospect) much too simplistically. The initial findings in the eastern United States were followed by isolations at a FWS laboratory in the western United States. It was found later in Europe and Japan, and still later in South America. Its occurrence in these foreign lands was a result of vertically transmitted virus in fertilized eggs from the United States and other sources. Although IPN virus in nonsalmonid species was generally not associated with clinical disease and mortality, it was in time isolated from representatives of diverse families of fishes from both freshwater and marine environments. Research has shown that the very nature of IPN virus is complex, and that different serological strains vary in virulence. Moreover, some isolants from nonsalmonid fishes, and still others from mollusks and crustaceans, proved to be pathogenic for trout. Inasmuch as all the isolants have a bisegmented double-stranded genome of ribonucleic acid, they have been referred to as the family Birnaviridae.

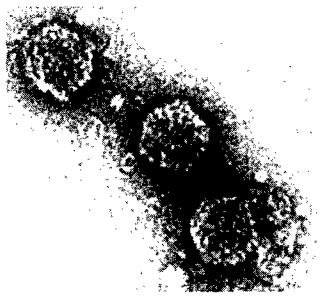


Fig. 6. Negatively stained virions of infectious pancreatic necrosis virus. (*From Ahne and Wolf 1980; reprinted with permission of Gustav Fischer Verlag, Stuttgart.*)

Early in the history of IPN, efforts were made by the FWS to achieve some measure of control. To that end, candidate brood stock populations of trout in hatcheries that had a virus-free water supply were examined virologically. Nondestructive methods were used and stocks of virus-free fish were identified for select propagation. Other work by the FWS showed that the testing of washings of body cavities and feces of adults, and of their progeny, enabled the identification and selection of virus-free parents from desirable stocks of fish that nevertheless harbored carriers. That concept is currently being applied by the FWS to reduce mortality from other viral disease—infectious hematopoietic necrosis—on the West Coast.

Control of IPN and other fish viral diseases by vaccination is a goal that has been sought but not yet achieved.

Virus strains of low or no virulence have been tested, but none yet found have conferred protection. Perhaps in frustration, some hatchery operators choose to tolerate IPN virus and its attendant losses. These operators compensate for losses by initiating each year's production with egg numbers that are twice (or more) their needs, to ensure that enough young will survive to meet the production goals.

Diagnostic needs for serological identification of IPN virus began with the preparation of an antiserum against the initial isolant. That antiserum proved capable of only partial neutralization of some later isolants. Accordingly, FWS personnel prepared an antiserum that was based first on five particular isolants that showed some degree of serological difference. Later work indicated the need for two additional isolants. The resulting FWS polyvalent anti-IPN virus serum represents a seven-strain product that is distributed to diagnostic laboratories, where it has thus far neutralized all strains encountered.

The present problems with IPN virus and related IPN-like viruses concern the need to develop a means of identifying specific strains. That objective is proving to be elusive because the various viruses fall into different groups, depending on methods used to evaluate them.

The IPN virus is the "oldest" of the known fish viruses; it is the most durable in its biophysical attributes and is thus far the most widely distributed. Eradication does not seem to be possible; therefore any future review of the infectious diseases of fishes is almost certain to include IPN and IPN-like viruses.

Infectious Hematopoietic Necrosis

Infectious hematopoietic necrosis, or IHN as it is commonly called, is an acute viral disease of young trout and salmon that typically causes significant mortality.

More than 30 years ago, FWS personnel in the Pacific Northwest described a suspect viral disease that killed young sockeye salmon (*Oncorhynchus nerka*), and just a few years later they described a viral disease of young chinook salmon. Their reports predated the general use of fish tissue culture, and for nearly a decade the disease of young salmon of the two species were considered as separate problems. The situation changed when a FWS virologist headed a group that isolated a rhabdovirus (Fig. 7) from acutely diseased young rainbow trout (*Salmo gairdneri*) and sockeye salmon and described the pathology clinically as IHN. The scientists noted that IHN resembled the so-called sockeye and chinook viral diseases—a concept that was soon substantiated by electron microscopy and serology. The IHN virus was traced to adult carriers that shed virus at spawning and thus ensured its continuity.

The disease grew in importance in FWS research, for it was found to be a problem from Alaska to California. Among young chinook salmon in California, mortality from IHN could be reduced by incubating eggs and fry in slightly heated water; other control measures were lacking, however, until researchers found that the virus was killed when fertilized eggs from infected trout were disinfected with certain iodine products. That method is still advocated but it is not foolproof: some virus remains within the egg—apparently carried there by the entering spermatozoan. A related recent finding by FWS researchers is that spermatozoa very rapidly adsorb IHN virus.

The production of salmon along the West Coast and in Alaska is still plagued with IHN virus and its attendant mortality. Although studies necessary for the development of a practical vaccine are under way, such a product is not yet available. However, an alternative approach has led to significant progress in the battle against the loss of young fish. Multiple virological testing that enables identification and specific culling of carrier brood stock has been used to derive virus-free trout. The

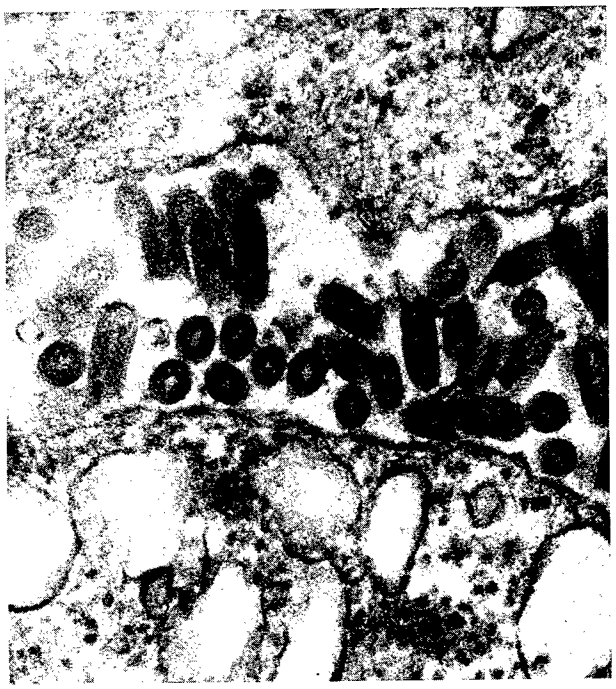


Fig. 7. Thin section of a cell showing longitudinal and cross-sections of the bullet-shaped rhabdovirus that causes infectious hematopoietic necrosis. (*Photomicrograph courtesy of R. W. Darlington.*)

approach is most practical for use with captive populations; migrant Pacific salmon present special problems because they are not conveniently available for repeated testing. As a means of partly circumventing these problems, Pacific salmon are mated in the hatchery as individual pairs, and samples of the sex products are inoculated into tissue cultures for evidence of virus. During the week required for that determination, the eggs of each pairing are held in separate incubators; all egg lots that show evidence of virus are then destroyed. This approach has not eliminated all virus, but it has dramatically reduced losses to the disease.

For the future, more rapid and more sensitive methods of detecting the virus should improve the efficiency of this brood stock selection program. Application of nucleic acid probes is one such method—and it provides an answer in a matter of a few hours. A nucleic acid probe, coupled with an effective vaccine and possibly with anti-viral drugs, should reduce the problem of IHN to a tolerable minimum. Like the human influenza virus, the fish virus will probably always be present to attack susceptible hosts.

Salmonid Whirling Disease

Whirling disease is a chronic infection of trout and (to a lesser extent) salmon caused by a protozoan parasite that attacks cartilage. The organism causes abnormal swimming behavior if organs of equilibrium are involved, and deformities and death result if the infection is severe.

In the United States, salmonid whirling disease was first encountered in the mid-1950's when the telltale spores of *Myxosoma cerebralis* were found in abnormally whirling and deformed trout in Pennsylvania and Nevada. In both states, circumstantial evidence implicated a European origin, where the popular rainbow trout can be raised more economically than in the United States but where whirling disease has been common for more than 80 years. Somehow, spores of *M. cerebralis* were released from cartilaginous crypts in infected consumable trout from Europe and introduced into trout hatcheries in the United States. The introduction appears to be permanent, for whirling disease has spread to certain waters in California and to nine additional eastern states.

After an exotic pathogenic protozoan was discovered, the FWS undertook long-term research on the disease. Results of early studies showed that all salmonids were susceptible, that heavy infection of young fish caused mortality, and that certain physical and chemical treatments killed the assumed causal spore.

A key feature of whirling disease is that in all its history in Europe and North America, no one had ever been able to infect trout with spores of *M. cerebralis*. In effect, the parasite's life-cycle was an 80-year-old enigma; however, a tantalizing clue lay in a finding of Russian workers that spores of *M. cerebralis* had to be "aged" in an aquatic environment for 3 or more months before they produced infectivity. That fact was used by all later investigators, but the riddle of the life cycle was eventually solved in the United States.

As a result of efforts of the FWS, methods were developed for releasing and concentrating *M. cerebralis* spores and thereby providing a sensitive method for detecting whirling disease infection. Rabbits were immunized with spores and the resulting antiserum was used in a serological method of identification. That develop-

Fig. 8. The two phases of the whirling disease organism. (*Top*) Stained spores of *Myxosoma cerebralis* are small disk-like bodies with twin pear-shaped polar capsules at the anterior end. This spore stage develops in the host fish. (*Bottom*) Phase contrast micrograph of the triactinomyxon phase that develops in the tubificid worm host. Scale bars are in micrometers. (*FWS photos.*)

ment proved to be critically important in testing later life cycle findings.

New findings by FWS personnel expanded on the Russian work and showed that mere "aging" of spores in the aquatic environment was not capable of generating the infectivity of whirling disease. Instead, small red aquatic worms known as tubificids had to be involved. More recent work showed that the spores of *M. cerebralis* infected the worm. The parasite then increased in number—a common phenomenon among parasites—and underwent a dramatic change in shape from a small disk-like spore to a larger three-tailed grapple-shaped organism known as a triactinomyxon (Fig. 8).

Triactinomyxons have long been known as parasites of worms but, in the present example, just as its alter ego *M. cerebralis* could not be used to infect trout, this triactinomyxon could not be used to infect worms. Research, in effect, solved two biological mysteries and showed that whirling disease was but half of a life cycle and that the other half took place in a tiny worm that thrives in the rich aquatic soils of busy trout hatcheries (Fig. 9).

Most fish relish the taste of worms and when trout—particularly the small and vulnerable fry or young trout—eat the infected tubificid worms, whirling disease is contracted. Triactinomyxons are also released to the environment in feces, and trout can also be infected by the waterborne organism when it is either ingested or becomes lodged among the gill plates.

The biology of whirling disease, which has now been revealed, resembles that of another protozoan, the malaria organism, which uses a mosquito as an alternate host to man and monkeys. The whirling disease discoveries explain the inability of *M. cerebralis* spores to infect fish and the ability of ultraviolet light, filters, and strong chemicals to reduce or eliminate infectivity.

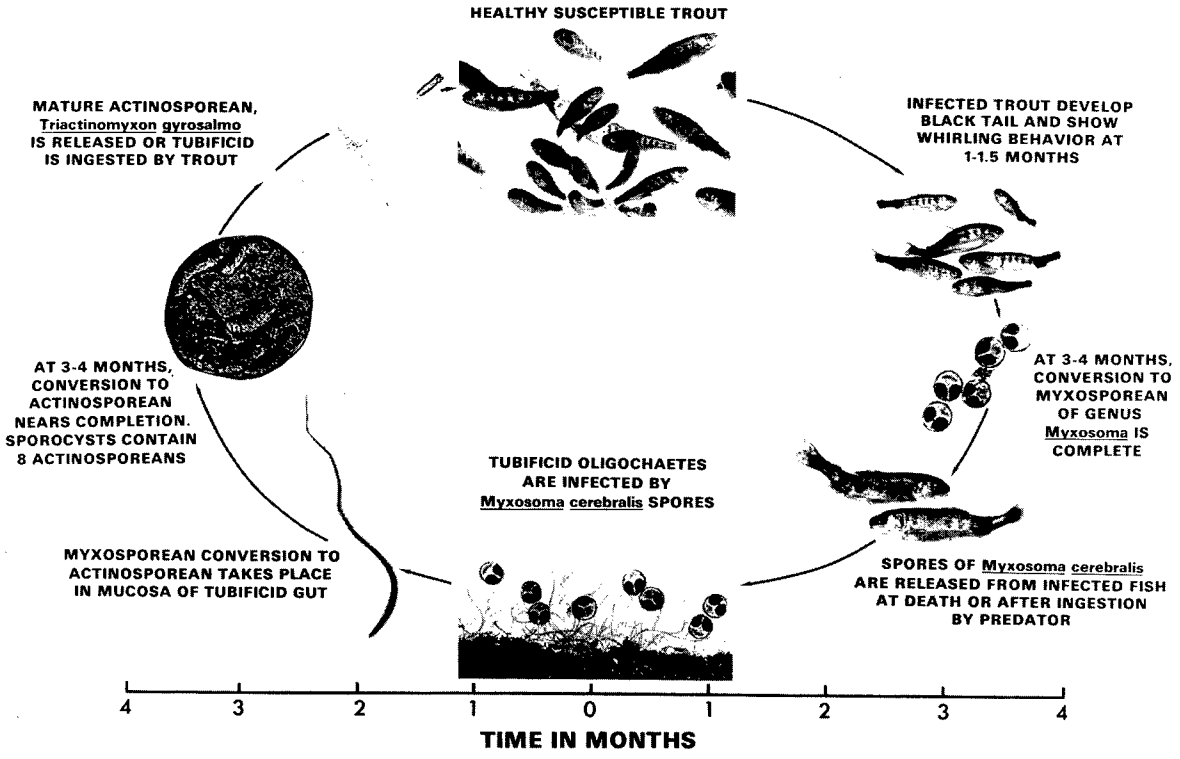


Fig. 9. Two-phase life cycle of the whirling disease organism. *Clockwise from bottom center*: Spores of the myxosporean phase (*Myxosoma cerebralis*) released at death, or after cannibalization of infected fish, are ingested by small red tubificid worms. Within the worm gut, alternation to the actinosporean phase begins and is completed in 3 to 4 months, when large sporocysts mature. The actinosporean phase, termed *Triactinomyxon gyrosalmo*, initiates whirling disease when trout eat the infected tubificid worms or eat or aspirate waterborne triactinomyxons. Within the fish, alternation to the myxosporean phase begins and is completed in 3 to 4 months, when spores of *M. cerebralis*, found in all cases of whirling disease are present. (*From Wolf and Markiw 1984; reprinted with permission of the American Association for the Advancement of Science.*)

On the negative side, the new discoveries emphasize the formidability of the problem of trying to stamp out whirling disease in hatcheries, and especially in natural environments.

Treatment of whirling disease has not been effective. Where the infectivity cannot be avoided, the only recourse is to propagate the more resistant brown trout or coho salmon (*Oncorhynchus kisutch*). The future holds promise for the development of a vaccine that will reduce the incidence of infection. In the meantime, existing knowledge is sufficient to prevent further spread.

Fish Diseases Described Since 1950

As the field of fish pathology continues to expand, more researchers and diagnosticians are recruited and new diseases are discovered and described. There are several reasons for the emergence of these new diseases. Fish that are latent carriers of disease transport causal agents to new geographic areas. The spread of whirling disease and enteric redmouth disease and the introduction of the Asian tapeworm are examples of diseases being spread by carrier fish. New diseases also occur when pathogens adapt to new hosts. The discovery that goldfish ulcer disease is caused by a variant of *Aeromonas salmonicida* might be an example of such an adaptation. In some instances, as in channel catfish virus disease and enteric redmouth disease, the origin of the pathogen is unknown. We have chosen the following diseases as examples of those discovered since 1950.

Enteric Redmouth Disease

Enteric redmouth disease (ERM) is a systemic bacteremia that principally affects rainbow trout. The disease was first described by a FWS researcher in 1950 in Idaho's Snake River Valley, where there is a concentration of Federal, State, and commercial rainbow trout hatcheries.

The disease is characterized by hemorrhage and inflammation in and around the mouth and gill covers. Once fish become infected, continuous chronic mortality is common, but losses become large if the fish are stressed. Although ERM was described first in rainbow trout, other salmonids are susceptible. Its geographic range now includes all areas where salmon and trout are grown, in the United States as well as in Canada, France, Great Britain, Italy, and West Germany. The increase in geographic range is due to the transportation of carrier fish and an increased awareness of the disease by fish pathologists.

Enteric redmouth disease is the first fish disease for which a commercial vaccine was developed. Researchers of the FWS had shown that rainbow trout could be protected from ERM with an oral vaccine. However, commercial interests developed a vaccine that is given by simple bath exposure. Trout are immunized with the ERM vaccine when they weigh 1 to 3 g. Since the introduction and application of the product, losses from ERM have been significantly reduced.

Edwardsiella *infections*

Edwardsiella infections in fish are caused by gram-negative motile rod-shaped bacteria that belong to the group known as enterics. *Edwardsiella tarda* was isolated from large diseased channel catfish in 1973, and is the same bacterium that Japanese investigators isolated from eels in 1962 and named *Paracolobactrum anguillimortiferum*. A second species of the genus—*E. ictaluri*—was isolated from diseased channel catfish in 1976.

Edwardsiella tarda and *E. ictaluri* are both major pathogens of catfishes, but during the past several years, losses due to *E. ictaluri* have far exceeded those due to *E. tarda*. Catfish infected with *E. tarda* have gas-filled lesions in deep muscle tissue and abscesses in internal organs, whereas those infected with *E. ictaluri* have small skin lesions, exophthalmia, pale gills, and small pale lesions on the frontal bone of the head.

Because *E. ictaluri* has been reported from channel catfish and one aquarium species, fish could be the source or reservoir of the bacterium. In contrast, *E. tarda* has been isolated from many species of cold-blooded and warm-blooded animals; consequently the specific source and reservoir are unknown.

Outbreaks of *E. tarda* or *E. ictaluri* are treated with oxytetracycline (Terramycin) for 10-14 days at the rate of 50 mg per kilogram of fish per day. The potentiated sulfonamide Romet is effective for controlling *E. ictaluri* when given for 5 days at 50 mg/kg per day. Preliminary information suggests that both *Edwardsiella* diseases can be controlled by immunization.

Channel Catfish Virus Disease

Channel catfish virus disease (CCVD) is an acute infection of fry and fingerlings. It was first described in 1968, but, because it is found in channel catfish with secondary bacterial diseases, it was probably not recog-

nized until the virus was isolated (Fig. 10). Channel catfish with CCVD swim convulsively, sink to the bottom, respire weakly, and die. The virus is probably carried by fish that survive CCVD outbreaks and eventually transmit the infection to their offspring. The disease occurs in the Southern States and in intensive culture operations in California, Colorado, Kansas, Nebraska, and Oklahoma. Disease outbreaks occur primarily in summer, but the intensity varies from year to year.

Unlike the virus of IPN, which can be isolated from adult carriers, that of CCVD can be isolated only during disease outbreaks. There has been active research toward developing procedures for isolating CCVD from carrier brood stock, to prevent further spread. However, no method has yet been developed for detecting carriers, and finding such a method remains a major research goal.

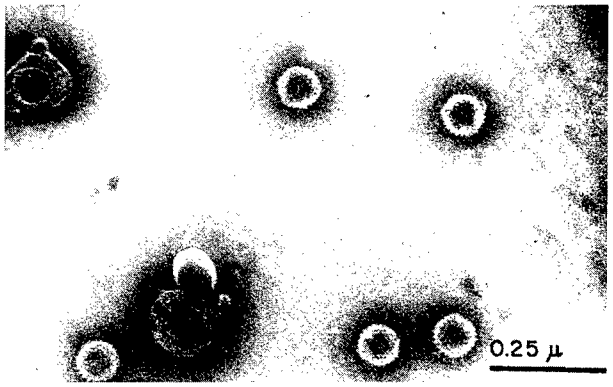


Fig. 10. Negatively stained channel catfish herpesvirus. Four capsids without envelopes are shown at *right* and two enveloped virions are at *left*. (*From Wolf and Darlington 1971; reprinted with permission of the American Society for Microbiology.*)

Golden Shiner Virus

The golden shiner virus (GSV) is a reovirus and a sometimes virulent pathogen of the commercial bait minnow *Notemigonus crysoleucas*, a species that is extensively propagated in North America's midsouth region.

The virus, a double encapsidated icosahedron having a genome of double-stranded RNA, was first encountered in 1977 by investigators at Auburn (Alabama) University. That isolant was the first representative of the reovirus family to be isolated from a fish. The work, reported in 1979, served to reinforce growing evidence that the viruses found in fishes belong to the same families as those that infect birds and mammals.

Golden shiner virus differs from the usual fish pathogens in that its known virulence is not directed to the young of the species but to the adults, in which it produces readily visible hemorrhages in the dorsal musculature. In vitro, the virus also behaves somewhat differently in that its cytopathic effect is transient—areas of vacuolation, syncytia, and necrosis are commonly overgrown by seemingly normal cells.

The golden shiner is the only known host to GSV, but other cyprinid fishes could be susceptible. However, when two species of propagated cyprinids—grass carp (*Ctenopharyngodon idella*) and fathead minnow (*Pimephales promelas*)—were inoculated with GSV, neither showed signs of disease. (Strangely, the fathead minnow is the source of the fathead minnow cell line, the preferred cell for growing GSV in vitro.)

Compared with virulent viruses such as infectious hematopoietic necrosis virus, GSV is only mildly pathogenic and is not often encountered; it is, however, the only virus thus far known from the species. Fortunately it is not considered to be among the viruses that cause significant mortality. That situation might persist, or greater significance might be discovered.

Epitheliocystis

Epitheliocystis is a chronis infection of fish epithelial cells of skin or gills, or both. The causal organism is considered to be a *Chlamydia* present in the enlarged epithelial cells.

The story of epitheliocystis in the United States involves features of role-reversal and historical replay. The role reversal describes the change that has occurred in the way epitheliocystis has been perceived: it was initially regarded as a benign condition—really a curiosity among fish diseases—but later showed the ugly facet of being able to kill. The historical replay involves the description of epitheliocystis in the United States: the same disease was described in Europe as mucophilosis, many years ago.

The North American chapter on epitheliocystis began in 1969 when young bluegills (*Lepomis macrochirus*) were found with large cyst-like cells in the epithelium of the gills and skin. Originally thought to be of protozoal or viral origin, the cysts were later found to harbor *Chlamydia*—obligate intracellular organisms whose relatives in man produce such diseases as trachoma and the so-called parrot fever. The organisms fit somewhere between pathogenic bacteria and viruses; one of their redeeming features is that they, like bacteria, are sensitive to broad-spectrum antibiotics.

Epitheliocystis in the freshwater bluegill seemed to be a benign condition, and it was so considered a year later when it was found in estuarine striped bass (*Morone saxatilis*) and white perch (*M. americanus*) along the eastern coast of the United States. The next reports concerned one of the marine mullets (*Liza ramada*) and the sea bream (*Sparus aurata*) in Israel, but more important was the fact that in that country epitheliocystis was considered to cause significant mortality. That mortality was attributed to the well-recognized propensity of epitheliocystis cells to stimulate proliferation of otherwise normal neighboring cells. When heavy infection of gill epithelium was involved, the result was massive proliferation and attendant reduction in respiratory function; the victims gradually suffocated.

Hungarian scientists recognized similarities between epitheliocystis and mucophilosis, a disease of common carp. Mucophilosis of common carp has been recognized in Europe for 65 years but its etiology was incorrectly attributed first to a primitive fungus and then to an alga. The Hungarian workers found no evidence of fungus; instead the peculiar cyst-like cells contained abundant chlamydial cells. In effect, the Hungarian work unified mucophilosis—a serious disease of young fish—with epitheliocystis.

In the United States, epitheliocystis (nee mucophilosis) has been found in aquarium species, in channel catfish, and in salmonids—notably chinook salmon and steelhead trout. More important, in young salmonids mortality sometimes claims one-third of the afflicted fish, and the incidence can approach 100%. Until the actual involvement was demonstrated, the condition was informally termed "drop-out disease" because of the inexorable attrition that occurred.

The former curiosity has thus shown its repulsive aspect. The next round in the battle will probably feature the application of antibiotics; however, the principal contender—chloramphenicol—is costly and will be difficult and expensive to register for legal use in the United States.

Proliferative Kidney Disease

Proliferative kidney disease (PKD) is a chronic and debilitating infection, principally of rainbow trout—but also of other salmonids and possibly nonsalmonids as well. The presumed causal agent is a protozoan that is abnormally present in the typically swollen kidneys—the major manifestation of the disease. Concern about PKD stems from the fact that incidences in a hatchery can approach 100% and, although mortality is usually low (10 to 20%), stress such as handling, hauling, low dissolved oxygen, or non-PKD health problems can precipitate major mortality. The presence of PKD seems to predispose fish to unusual vulnerability to other debilitating factors.

The disease is diagnosed by demonstrating the presence of the peculiar protozoan in kidney and other tissues. That organism, termed PKX, is tacitly assumed to be the etiologic agent; however, it has yet to be isolated and cultured and even its life cycle remains to be determined.

Although PKD occurs in several countries of Europe and the United Kingdom, the name of the condition—now universally accepted—was coined only 10 years ago. Nevertheless, descriptions in German literature suggest that what is now termed PKD was recognized as far back as the early 1970's. A shorter but somewhat similar history of the disease occurs in literature from the United States. The first published report, which appeared in the early 1980's, described outbreaks in Idaho's Snake River Valley, an area noted for the commercial production of nearly 30 million pounds of rainbow trout annually. More recently, PKD has been found in Pacific salmon in a hatchery in California. Moreover, reexamination of histologic sections in storage since 1965 has shown that the presumed PKX organism was present in California at least 20 years ago.

After the recent outbreaks in the United States, research was undertaken by university staffs in California and Idaho and by the FWS. The objectives are to transmit the disease experimentally, to isolate the PKX organism, and to solve its life cycle. Thereafter, it is to be hoped that immunological and more effective control measures can be developed.

Experimental transmission of PKD has been achieved in Europe, the United Kingdom, and in California, but only by intraperitoneal injection of homogenized infected kidney tissue. Since the disease is not known in the eastern United States, a stock or source of infected fish was needed for research at the National Fish Health Research Laboratory. Two shipments were made from Idaho, and kidneys of the fish were prepared and inoculated into healthy fingerling rainbow trout. Transmission of PKD was not achieved—even though the temperatures at which test fish were held were favorable for incubation (12 and 15°C; 54 and 59°F). The postulated cause of failure was the fact that the shipment of living fish required icing; low temperature is unfavorable for the protozoan and might have killed or inactivated it.

The disease is especially troublesome under hatchery conditions of intensive husbandry, and although antibacterial and antiprotozoal drugs have been tested, nothing has brought PKD under control. The most effective measure is avoidance—preventing the introduction to new areas.

In the United States, at least, PKD is a qualified "new disease of fish." In that respect it can be compared with several of mankind's "new diseases"—legionnaire's disease, toxic shock syndrome, and acquired immune deficiency syndrome (AIDS). Controlling these new or newly recognized diseases represents challenges to research—and research on PKD is only in an early stage of development.

Supplemental Reading

For additional information on specific diseases, the following references are suggested.

Ahne, W., and K. Wolf. 1980. Viral diseases of fishes. (Viruserkrankheiten der Fische.) Pages 56–105 *in* H. H. Reichenback-Klinke, ed. Krankheiten und Schadigungen der Fische. Gustav Fischer Verlag, Stuttgart. [In German]

Amend, D. F. 1974. Infectious hematopoietic necrosis (IHN) virus disease. U.S. Fish Wildl. Serv., Fish Dis. Leafl. 39. 6 pp.

Bullock, G. L. 1977. Vibriosis in fish. U.S. Fish Wildl. Serv., Fish Dis. Leafl. 50. 11 pp.

Bullock, G. L. 1980. Bacterial kidney disease of salmonid fishes caused by *Renibacterium salmoninarum*. U.S. Fish Wildl. Serv., Fish Dis. Leafl. 60. 10 pp.

Bullock, G. L. 1984. Enteric redmouth disease of salmonids. U.S. Fish Wildl. Serv., Fish Dis. Leafl. 67. 14 pp.

Bullock, G. L., R. C. Cipriano, and S. F. Snieszko. 1983. Furunculosis and other diseases caused by *Aeromonas salmonicida*. U.S. Fish Wildl. Serv., Fish Dis. Leafl. 66. 29 pp.

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.

Cipriano, R. C., G. L. Bullock, and S. W. Pyle. 1984. *Aeromonas hydrophila* and motile aeromonad septicemias of fish. U.S. Fish Wildl. Serv., Fish Dis. Leafl. 68. 23 pp.

Clifton-Hadley, R. S., D. Bucke, and R. H. Richards. 1984. Proliferative kidney disease of salmonid fish: a review. J. Fish Dis. 7(5):363–377.

Davis, H. S. 1946. Care and diseases of trout. U.S. Fish Wildl. Serv., Res. Rep. 12. 98 pp.

McAllister, P. E. 1983. Infectious pancreatic necrosis (IPN) of salmonid fishes. U.S. Fish. Wildl. Serv., Fish Dis. Leafl. 65. 12 pp.

Paperna, I., and A. P. Alves de Matos. 1984. The developmental cycle of epitheliocystis in carp, *Cyprinus carpio* L. J. Fish Dis. 7(2):137–147.

Plumb, J. A. 1977. Channel catfish virus disease. U.S. Fish Wildl. Serv., Fish Dis. Leafl. 52. 8 pp.

Plumb, J. A., P. R. Bowser, J. M. Grizzle, and A. J. Mitchell. 1979. Fish viruses: a double-stranded RNA icosahedral virus from a North American cyprinid. J. Fish. Res. Board Can. 36(11):1390–1394.

Rogers, W. A. 1983. Edwardsiellosis in fishes. Pages 153–159 *in* D. P. Anderson, M. Dorson, and Ph. Dubourget, eds. Antigens of fish pathogens. Fondation Marcel Merieux, Lyon, France.

Snieszko, S. F. 1981. Bacterial gill disease of freshwater fishes. U.S. Fish Wildl. Serv., Fish Dis. Leafl. 62. 11 pp.

Wolf, K., and R. W. Darlington. 1971. Channel catfish virus: a new herpesvirus of ictalurid fish. J. Virol. 8(4):525–533.

Wolf, K., and M. E. Markiw. 1984. Biology contravenes taxonomy in the myxozoa: new discoveries show alternation of invertebrate and vertebrate hosts. Science 225(4669):1449–1452.

Wolf, K., and M. E. Markiw. 1985. Salmonid whirling disease. U.S. Fish Wildl. Serv., Fish Dis. Leafl. 69. 13 pp.

Bullock, G. L. and Ken Wolf. 1986. **Infectious Diseases of Cultured Fishes: Current Perspectives.** Fish Wildl. Leafl. 5. 13 pp.

Infectious diseases that are important in contemporary husbandry of North American sport and resource fishes are presented in a two-part overview. Advances and persistent obstacles to progress are emphasized. Major consideration is given to diseases of historical importance—furunculosis, motile *Aeromonas* septicemia, bacterial kidney disease, vibriosis, bacterial gill disease, infectious pancreatic necrosis, infectious hematopoietic necrosis and salmonid whirling disease. A second category consists of important diseases that were discovered since 1950—enteric redmouth disease, edwardsiellosis, channel catfish virus disease, golden shiner virus disease, epitheliocystis and proliferative kidney disease. Key references are cited for each problem disease.

Key words: Bacteria, virus, parasite, disease, detection, control, and diagnosis.

Bullock, G. L. and Ken Wolf. 1986. **Infectious Diseases of Cultured Fishes: Current Perspectives.** Fish Wildl. Leafl. 5. 13 pp.

Infectious diseases that are important in contemporary husbandry of North American sport and resource fishes are presented in a two-part overview. Advances and persistent obstacles to progress are emphasized. Major consideration is given to diseases of historical importance—furunculosis, motile *Aeromonas* septicemia, bacterial kidney disease, vibriosis, bacterial gill disease, infectious pancreatic necrosis, infectious hematopoietic necrosis and salmonid whirling disease. A second category consists of important diseases that were discovered since 1950—enteric redmouth disease, edwardsiellosis, channel catfish virus disease, golden shiner virus disease, epitheliocystis and proliferative kidney disease. Key references are cited for each problem disease.

Key words: Bacteria, virus, parasite, disease, detection, control, and diagnosis.

Bullock, G. L. and Ken Wolf. 1986. **Infectious Diseases of Cultured Fishes: Current Perspectives.** Fish Wildl. Leafl. 5. 13 pp.

Infectious diseases that are important in contemporary husbandry of North American sport and resource fishes are presented in a two-part overview. Advances and persistent obstacles to progress are emphasized. Major consideration is given to diseases of historical importance—furunculosis, motile *Aeromonas* septicemia, bacterial kidney disease, vibriosis, bacterial gill disease, infectious pancreatic necrosis, infectious hematopoietic necrosis and salmonid whirling disease. A second category consists of important diseases that were discovered since 1950—enteric redmouth disease, edwardsiellosis, channel catfish virus disease, golden shiner virus disease, epitheliocystis and proliferative kidney disease. Key references are cited for each problem disease.

Key words: Bacteria, virus, parasite, disease, detection, control, and diagnosis.

☆ **U.S. Government Printing Office: 1986—676-205/40014**

A list of recent *Wildlife Leaflets* follows.

513. Guide for Collecting and Seeding Native Forbs for Wildlife in Douglas-fir Clearcuts, by Dan L. Campbell and Larry E. Johnson. 1981. 13 pp.
413. Distribution of Animal Damage in Southwestern Oregon Forests, by James Evans, Dan L. Campbell, Gerald D. Lindsey, Victor G. Barnes, Jr., and R. Michael Anthony. 1981. 12 pp.
515. Senegal's Trade in Cage Birds, 1979–81, by Philippe Ruelle and Richard L. Bruggers. 1983. 11 pp.

A list of recent *Fish and Wildlife Leaflets* follows.

1. Acid Rain: Effects on Fish and Wildlife, by Kathleen Stecher Mayer, Ell-Piret Multer, and R. Kent Schreiber. 1985. 8 pp.
2. Interpretation of Criteria Commonly Used to Determine Lead Poisoning Problem Areas, by Milton Friend. 1985. 4 pp.
3. Kenai River Salmon...A Unique Resource in South-central Alaska, by Carl V. Burger, David B. Wangaard, and Richard L. Wilmot. 1985. 14 pp.
4. Monitoring Fish and Wildlife for Environmental Contaminants: The National Contaminant Biomonitoring Program, by Joel Jacknow. 1986. 15 pp.

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering the wisest use of our land and water resources, protecting our fish and wildlife, preserving the environmental and cultural values of our national parks and historical places, and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to assure that their development is in the best interests of all our people. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

UNITED STATES
DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
OFFICE OF INFORMATION TRANSFER
1025 PENNOCK PLACE, SUITE 212
FORT COLLINS, COLORADO 80524

THIRD-CLASS MAIL
POSTAGE AND FEES PAID
U.S. DEPARTMENT OF THE INTERIOR
PERMIT No. G-77

NOTE: Mailing lists are computerized. Please return address label with change of address.