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Fish Health Management

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Introduction

Maintaining the health of hatchery fish requires a program of good management and continuous attention to detail. In its most basic sense, disease can be defined as a deviation of the body from its normal or healthy state, and disease in fish can be caused by infectious agents or by noninfectious conditions. Thus, optimal fish health is best achieved by rearing fish in a good environment, with good nutrition, with a minimum of stress, and isolated from sources of infectious agents. Whenever possible, prevention of infectious disease by avoidance of contact between the host fish and a pathogen should be a critical goal. This is best achieved with a pathogen-free water supply, the use of certified pathogen-free stocks, and strict attention to sanitation.

The principal reasons for control of infectious diseases in hatcheries are to prevent losses in production; to prevent the introduction of pathogens to new facilities when eggs, fry, or broodstock are moved; to prevent the spread of disease to wild fish via the hatchery effluent or when hatchery fish are released or stocked out; and to prevent the amplification of pathogens already endemic in a watershed. Although only a few fish pathogens cause human health problems (i.e., are zoonotic agents), certain hatchery practices can create a hazard when toxic compounds are used or if fish are contaminated with drugs or chemicals when they are released or sold as food. If a disease outbreak should occur, a serious attempt should be made to eradicate the infectious agent from the facility or to change the conditions that led to disease, rather than to rely upon frequent treatments to control future outbreaks.

Since the first edition of this book in 1982, some very significant changes have occurred in the field of fish health. These include an improved fish health infrastructure for the diagnosis of fish diseases, the discovery of important new fish pathogens, expansion of the host and geographic ranges for some of the known pathogens, increased restrictions on the use of drugs and chemicals in aquaculture, the application of molecular methods for improved diagnosis of diseases, elucidation of important epizootiological features of fish diseases, creation of new regulations to prevent the spread of important diseases, and the development of novel methods for control of some diseases, including the commercial availability of effective vaccines. These topics are included in this new version of the chapter.

Disease Characteristics

Factors Leading to Disease

Conceptually, it is useful to think of disease or health as a set of interactions among the host, the pathogen, and the environment. Host factors include the species of fish, size or age, strain or stock, immune status, and general physiological condition. For the pathogen, factors include the number of infectious organisms present, the strain of the pathogen, and its virulence. The difference between health and disease typically depends on the balance between the pathogen and the host, and that balance is greatly influenced by environmental factors such as temperature and water chemistry. For example, an adverse environmental factor such as high temperature may speed the replication of a pathogen and cause stress on the host resulting in reduced resistance.

Stress plays a major role in the susceptibility of fish to infectious disease. Wild fish are typically in equilibrium with the pathogens that are endemic within a geographic area under normal conditions, and natural outbreaks are rarely observed unless major environmental changes occur. However, fish in intensive culture are frequently affected by adverse environmental conditions (e.g., temperature or water quality) and by management practices (e.g., crowding or handling) that can impose significant stress on the defense mechanisms of most fishes. Whereas some pathogens of fish are highly virulent and cause disease even in a well-managed facility, many diseases are enhanced by stress. For example, although several species of opportunistic bacterial pathogens are present in many hatchery water supplies, disease typically will not occur unless environmental quality or the defense systems of the fish have deteriorated. Prevention of these diseases can best be done through good hatchery management. Environmental stresses and asso-

ciated disease problems are minimized by high water quality, low rearing densities, and excellent nutrition.

Two other types of disease are important to fish culturists in addition to those caused by pathogenic organisms. The first is nutritional in origin and is usually caused by inadequate diet or poorly stored feeds. The second is caused by environmental factors, including bad hatchery practices and poor water quality. Both of these classes of disease can result in significant losses if severe enough or uncorrected for a prolonged period. While these diseases are covered in other chapters of this book, it is important to remember that such noninfectious diseases may act synergistically with infectious agents to produce exceptionally high losses.

Disease-Causing Organisms

Organisms that cause diseases in fish include a large number of viruses, bacteria, fungi, protozoans, and metazoans. Generally, they can be categorized as either pathogens or parasites, although the distinction is not always clear. Viruses are submicroscopic agents that are dependent upon living cells for their replication and often have highly specific requirements for a particular host and even for certain tissues within that host. Viruses are always maintained in a host and are found free in nature only in a nonreplicating state. Bacteria may also be obligate pathogens if they cannot live outside a host; however, many bacterial fish pathogens are found free-living in nature and only affect fish as opportunists. Protozoans and metazoans are considered parasites and can reside either inside or on the surface of the host. Pathogens or parasites do not always cause disease in fish, but may be present in a subclinical or carrier state.

Disease Recognition

Only a few fish diseases produce behaviors or clinical signs that are pathognomonic (specific to a given disease). Nevertheless, careful observation of signs exhibited by the fish will often allow one to restrict the cause of the disease to a particular type (or group) of causative agent(s).

Some obvious changes in behavior of fish suffering from disease include (1) loss of appetite; (2) abnormal distribution in a pond or raceway, such as swimming at the surface, along the tank sides, or in slack water, or crowding at the head or tail screens; (3) flashing, scraping on the bottom or sides of the rearing unit, darting, whirling, twisting, or loss of equilibrium; and (4) weakness, loss of vitality, and loss of ability to withstand stresses during handling, grading, seining, loading, or transportation.

In addition to changes in behavior, disease may produce physical signs such as lesions that can be seen by the unaided eye. Such gross

signs may be external, internal, or both. Gross external signs of disease include darkening or discolored areas on the body; eroded areas, ulcers, or sores on the body, head, or fins; cysts, tumors, or swelling on the body or gills; exophthalmia (popeye); and hemorrhages, especially around the head and at the base of fins. Gross internal signs of disease are changes in the color of organs or tissues (pale liver or kidney or congested organs); hemorrhages in organs or tissues; changes in the texture or size of organs or tissues; accumulation of fluid in body cavities; and cysts, tumors, or lesions.

Besides changes in behavior and gross physical signs, information such as the disease history of the facility, geographic area, species and age of fish, water temperature, and time of year can assist in the formation of a presumptive diagnosis. If a serious disease problem is suspected, a fish health professional should be contacted immediately for assistance in identifying the causative agent and in helping to develop an appropriate strategy to reduce potential losses.

Defense Mechanisms of Fish

As with all living organisms, fish stay healthy only if they prevent excessive growth of microorganisms on their external surfaces and invasion of their tissues by pathogenic agents. Invasion of microorganisms is inhibited by physical barriers, and replication within the host is held in check by natural and acquired immune mechanisms.

Barriers

Physical barriers are an important first line of defense. Fish eggs are protected by the structurally tough and chemically resistant chorion. However, during oogenesis, the egg may become infected or contaminated with certain viruses or bacteria present in the female. Once hatched, the fry are then likely to have become infected. Transmission of infectious agents from adult to progeny via the egg is termed vertical transmission.

Fishes are protected from injury and invasion of disease agents by the external barriers of mucus, scales, and skin. For example, the skin protects against microbial colonization by continuously producing and sloughing mucus, which allows infectious agents only temporary residence on the host. Mucus also may contain nonspecific antimicrobial substances, such as natural antibodies, lysozyme, or other antimicrobial factors, as well as specific antibodies produced in response to prior infection or vaccination.

Gill tissue also contains mucus cells that can serve the same purpose as those in the skin. However, irritants may cause accumulation of mu-

cus on the gill tissue and lead to asphyxiation. This is an example of a defense mechanism that can work against the host.

Immune Mechanisms

The immune system of the fish can be divided into nonspecific defense mechanisms (also termed natural immunity), which are effective against a broad range of organisms, and specific defense mechanisms, which are usually acquired upon recovery from infection with a given pathogen or following vaccination. In addition, both nonspecific and specific defense mechanisms may be innate (present continuously) or induced by infection or vaccination. One of the primary natural defense mechanisms is the inflammatory response of the vascular system, which is triggered by invasion of pathogenic agents and other irritants. Dilation of capillaries increases the supply of humoral and cellular agents at the focus of infection. The inflammatory response proceeds to dilute, localize, destroy, and remove the agent that stimulated the response.

Fish, like most animals, also have an important defense mechanism in the form of fixed and wandering phagocytes in the lymphatic and circulatory systems. Phagocytes are cells capable of ingesting bacteria, foreign particles, and infected cells. Fish also have natural killer cells, natural antibodies, and other innate humoral and cell-mediated defenses against infectious disease that are intrinsic to the species and individual. An example is the production of interferon in response to viral infection. This induced response is nonspecific and increases resistance to other virus infections, as well as to the agent that stimulated it. These types of defenses may account for some portion of the differences in the natural resistance of various species and strains of fish to certain diseases. Some of these differences have been shown to have a genetic basis as resistance to a pathogen may differ between families of the same species of fish and the trait shows significant heritability.

Fish have a relatively well-developed specific immune system and are able to produce both circulating antibodies (humoral response) and a cell-mediated response to antigenic stimuli. These humoral and cellular arms of the immune system are required to respond in differing degrees for various viral, bacterial, protozoan, and fungal pathogens. The fish's immune system shows proliferation of specific cells involved in the immune response and an immunological memory to a limited degree. These specific, adaptive immune responses are also stimulated by vaccines that are commercially available and that can be used to protect fish against certain diseases. The immune response of cold-blooded animals depends heavily upon environmental temperature. Lowering of the water temperature below a fish's optimum usually reduces or de-

lays the onset of the immune response. Other environmental factors that stress the fish, as well as various physiological states (e.g., spawning) can reduce the magnitude or efficacy of the immune response. Age is also an important factor. The fish's specific immune system develops after hatching and, depending upon species and temperature, may not be fully functional until the fish is several months of age.

Genetic Resistance

Significant heritabilities have been shown for resistance to various fish diseases, and, under controlled environmental conditions, some increased resistance to a disease can be expected from a managed breeding program. However, selection for resistance to several pathogens can be difficult, except perhaps for closely related diseases. Intentionally or unintentionally, specific disease resistance has been increased at hatcheries by the continued use of survivors of outbreaks as broodstock. Potential exists for genetic selection and breeding to increase the survival of propagated fishes, but certain risks must be anticipated in any major breeding program that may be counter to programs designed to maintain the genetic composition of wild stocks. In addition, changes in gene frequencies resulting from selection for disease resistance may cause changes in the frequencies of other genes that are unrelated to disease resistance leading to a concomitant loss in some other desired trait (e.g., growth or fecundity). Thus, managers always run the risk of altering the fitness of their stock in unwanted ways during selective breeding programs.

In any natural population, individual fish may be found that are more or less resistant to the common diseases. In a population of wild fish, this natural genetic variation among individuals is important to ensure that not all fish in the population are equally susceptible during changes in the environment or in the levels or types of pathogens present. Natural recombination within the breeding population assures that these variations are maintained. In the absence of important needs to the contrary, any artificial propagation program should ensure that a similar genetic variability is maintained within the population as its lack may put the entire population at risk.

Diseases of Fish

The first edition of this book had photographs and relatively detailed descriptions of many of the major infectious diseases of hatchery fish that were known at the time. It also included recommendations for treatment, sometimes using drugs or chemicals that were not approved for

use in food fish. Because the field of fish health was relatively new, there were few books or reference materials that could be consulted by the hatchery manager. Also, the fish health infrastructure available to assist hatchery managers was poor in many areas. In the nearly 20 years following publication of the first edition, the amount of information on fish diseases has expanded exponentially and many excellent references have become available that describe the clinical signs, diagnosis, and treatment of fish diseases. Furthermore, the list of diseases affecting cultured fish has continually grown as new species are reared or as established species are cultured in new geographic areas. Finally, the network of fish health professionals has improved greatly, and several organizations have published books containing standard methods for the diagnosis of fish diseases. For these reasons, this new edition will only briefly describe some of the most significant diseases of the fish species cultured in North America.

The material in the following sections has been developed from information contained in references listed at the end of this chapter. For further information, these references should be consulted. It is assumed that a progressive hatchery manager will have a library containing many of these references and an established relationship with a fish health professional who can provide expert advice in the diagnosis and treatment of fish diseases.

Viral Diseases of Fish

Among the infectious diseases of fish, those caused by viruses are important because they can result in explosive, uncontrollable losses and are often the subject of state, federal, and international regulations. Because viral diseases of fish are untreatable and there are few approved vaccines for their prevention, avoidance of the pathogen remains the major control option available. This is best accomplished by rearing fish in a virus-free water supply, using only certified virus-free stocks, paying strict attention to sanitation, and having sound quarantine practices. While requiring effort, it is still easier to avoid viral pathogens than to deal with their effects or to try to eradicate them from a facility once introduced. For hatcheries having open water supplies and that are located in an endemic area, viral diseases can present insurmountable problems. For example, until methods were developed for avoidance of infectious hematopoietic necrosis virus (IHNV), sockeye salmon could not be propagated successfully in Alaskan hatcheries having open water supplies because the virus was present in wild, spawning adults in nearly all populations tested.

The host specificity of fish viruses can vary widely. Some have a broad range of species in which they replicate, while other viruses have a very narrow host range that may include only certain tissues within a single host. Similarly, there may be significant genetic differences among strains of fish viruses within certain virus groups resulting in substantial differences in virulence or in reactivity with serological reagents. Many of these different strains occur within a defined geographical area making it important to restrict viruses to locations in which they are endemic.

The diagnosis of viral diseases has traditionally relied upon growth of the virus in cell culture and identification by various serological methods including neutralization, fluorescent antibody, and enzyme-linked immunosorbent assays. These methods are time-consuming, relatively labor intensive, and require specialized training and equipment. In recent years, the techniques of molecular biology have produced new diagnostic tools having great speed and precision. Among the new approaches are the use of monoclonal antibodies, DNA probes, and the polymerase chain reaction (PCR) for identification of a viral pathogen. However, many of the required certification examinations still rely upon cell culture methods because they are considered validated or benchmarked tests. In some cases, nonlethal methods have been developed that typically rely upon detection of neutralizing antibodies in a population of fish and, more recently, the use of PCR to detect viral genomes in nonlethal samples such as blood or mucus.

While a diagnosis of a viral disease is relatively straightforward, the management implications are not. Because fish viral diseases can be caused by strains of the causative agent having differences in host specificity, virulence, serological reactions, or growth characteristics, it may be necessary to examine the causative agent further before a recommendation can be made as to the disposition of the stock. For example, fish harboring a low level of IHNV should not be moved to other watersheds, even those containing fish infected with IHNV, unless it has been shown that an identical strain of the virus is present. Similarly, the emergence or introduction of a different strain of virus at a hatchery should be treated with caution.

During the time since the first edition of this book, the number of identified fish viruses has increased very substantially and only a few will be discussed below. Other viral diseases may be important locally, and the conscientious fish hatchery manager will become familiar with those diseases at risk of being found among the species cultured in the geographic area in which the facility is located. Many of the following viral diseases are listed by the Office International des Epizooties as being either "Notifiable" or as "Other Significant Diseases" of finfish, while a few others are included that are of interest to aquaculture.

Rhabdoviral Diseases

The rhabdoviruses that infect finfish generally cause viremia with resulting hemorrhage and widespread necrosis of major organ systems, which may result in very high losses. These viruses have a relatively broad host specificity and are among the oldest and best studied viral pathogens of fish. The diseases caused by these viruses typically show both a size and temperature dependence.

Viral Hemorrhagic Septicemia

Viral hemorrhagic septicemia (VHS) is best known as an infectious disease of rainbow trout, brown trout, grayling, whitefish, northern pike, and turbot. The disease is caused by viral hemorrhagic septicemia virus (VHSV, synonym: Egtved virus). Formerly thought to be confined to western Europe, in the last decade, VHSV has been isolated from an increasing number of free-living marine fish species in the North Pacific Ocean, the North Atlantic Ocean, the North Sea, and the Baltic Sea.

Typically, isolates of VHSV from marine fish are not distinguishable from normal freshwater isolates by serological means; however, isolates of VHSV from the Pacific Ocean are genetically distinguishable from the European isolates. There appear to be several distinct genetic lineages among the European isolates. In contrast to the European freshwater strain, isolates from wild marine fish species in both North America and Europe typically induce low mortality in infection trials with rainbow trout fry. Several European marine isolates are pathogenic to turbot fry, and isolates from marine species in the Pacific are highly pathogenic to Pacific herring.

Among rainbow trout infected with the European freshwater strains, infection is often lethal due to the impairment of the osmotic balance and occurs within a clinical context of edema and hemorrhages. Virus multiplication in endothelial cells of blood capillaries, leukocytes, hematopoietic tissues, and nephron cells underlies the clinical signs.

The reservoirs of VHSV are clinically infected fish and covert carriers among cultured, feral, or wild fish in the marine environment and in freshwater. Virus is shed in the feces, urine, and sexual fluids, whereas kidney, spleen, heart, liver, and digestive tract are the sites in which virus is most abundant. Several factors influence susceptibility to VHSV. Among each fish species, there is individual variability in susceptibility, and the age of the fish appears to be of some importance—the younger the fish, the higher the susceptibility. In susceptible fish stocks, however, overt infection is seen in all sizes of fish.

Water temperature is an important environmental factor. Disease generally occurs at temperatures between 4 and 14°C (39.2 and 57.2°F). Low

water temperatures (1–5°C [33.8–41.0°F]) generally result in an extended course with low daily mortality, but high total mortality. At high water temperatures (15–18°C [59.0–64.4°F]), the disease generally takes a short course with an initial acute mortality, but a more modest accumulated mortality. VHS outbreaks occur during all seasons, but are most common in spring when water temperatures are rising or fluctuating.

Control methods for VHS rely upon avoidance of the virus through official health surveillance plans coupled with control measures, such as eradication. Effective use of these measures has resulted in the eradication of the disease from several parts of Europe. At present, effective vaccines are in the experimental stage as are genetic approaches using selection for disease-resistant stocks.

Infectious Hematopoietic Necrosis

Infectious hematopoietic necrosis (IHN) is an important infectious disease of rainbow or steelhead trout; Pacific salmon, including chinook, sockeye, chum, cherry (also known as amago and yamame), and coho salmon; and Atlantic salmon. Historically, the geographical range of IHN was limited to the western parts of North America, but the disease, caused by a rhabdovirus (IHNV), has spread to continental Europe and the Far East via the importation of infected fish and eggs. IHN has become a cause for concern because of its clinical and economic consequences in trout and salmon farming and its effects on wild stocks. Infection is often lethal due to the impairment of osmotic balance and occurs within a clinical context of edema and hemorrhages. Virus multiplication in endothelial cells of blood capillaries, hematopoietic tissues, and nephron cells underlies the clinical signs. High levels of virus are shed from infected juvenile fish. Older fish are increasingly resistant to infection, but adult fish at spawning may shed virus in sexual products. Survivors of IHNV infection demonstrate a strong protective immunity with the synthesis of circulating antibodies to the virus and, in certain individuals, a covert carrier state. Variations in the virulence of IHNV strains have been recorded during both natural cases of disease and in experimental infections.

The reservoirs of IHNV are clinically infected fish and covert carriers among cultured, feral, or wild fish. Virus is shed via feces, urine, sexual fluids, and external mucus, whereas kidney, spleen, encephalon, and the digestive tract are the sites in which virus is most abundant during the course of overt infection. The transmission of IHNV between fish is primarily horizontal; however, cases of vertical or "egg-associated" transmission have been recorded. Horizontal transmission is typically by direct exposure, but invertebrate vectors have been proposed to play a role in some cases. Egg-associated transmission is significantly reduced by the now common practice of surface disinfection of eggs

with an iodophor solution; but egg-associated transmission is the only mechanism accounting for the appearance of IHN in new geographical locations among alevins originating from eggs that were incubated and hatched in virus-free water. Once IHNV is established in a farmed stock or in a watershed, the disease may reside among carrier fish.

Among individuals of each fish species, there is a high degree of variation in susceptibility to IHNV. The age of the fish appears to be extremely important—the younger the fish, the more susceptible to disease. As with VHSV, good overall fish health condition seems to decrease the susceptibility to overt IHN, while handling and other types of stress frequently cause subclinical infections to become overt.

The most prominent environmental factor affecting IHN is water temperature. Clinical disease occurs between 8°C and 15°C (46.4°F and 59.0°F) under natural conditions.

Control methods for IHN currently rely on avoidance of exposure to the virus through the implementation of strict control policies and sound hygiene practices. The thorough disinfection of fertilized eggs and the incubation of eggs and rearing of fry and alevins in virus-free water supplies are critical for preventing the occurrence of IHNV in a defined fish production site. At present, vaccination is at an experimental stage; however, several new vaccine preparations have shown substantial promise in both laboratory and field trials.

Spring Viremia of Carp

Spring viremia of carp (SVC) is a rhabdoviral disease of several carp and other cyprinid fish species. Overt infections have been recognized in common carp, grass carp, silver carp, bighead carp, crucian carp, goldfish, tench, and sheatfish. The common carp is the most susceptible of these species and is the principal host. The geographical range of SVC includes countries of the European continent that experience low water temperatures during winter, although the disease may be present in Asia and other regions where carp are traditionally reared or have been introduced.

As with other rhabdoviruses of fish, infection by SVC virus (SVCV) can be lethal due to the impairment of water balance that occurs in a clinical context of edema and hemorrhages. Virus multiplication, especially in endothelial cells of blood capillaries, hematopoietic tissue, and nephron cells, underlies the clinical signs.

Overcoming SVCV infection results in a strong protective immunity associated with the presence of circulating antibodies detectable by methods such as virus neutralization, immunofluorescence, or enzyme-linked immunosorbent assay. In certain individuals, this health status also results in a covert virus carrier state. Differences in virulence of virus strains have been recorded during natural cases of disease and experimental infections. A related virus, the northern pike fry rhabdovirus, shares

antigens that cross-react in some serological tests, but the two agents can be distinguished by serum neutralization tests and by molecular methods.

The reservoirs of SVCV are clinically infected fish and covert virus carriers from cultured, feral, or wild fish. Virulent virus is shed in the feces, urine, gill and skin mucus, and from the exudate of skin blisters or edematous scale pockets. However, liver, kidney, spleen, gill, and encephalon are the organs in which SVCV is most abundant during the course of overt infection.

The principal mode of transmission for SVCV is horizontal from fish to fish, but vertical or egg-associated transmission cannot be ruled out. Animate vectors and fomites are also involved in transmission of SVCV. Among animate vectors, the parasitic copepod *Argulus follaceus* and the leech *Piscicola piscicola* are able to transfer SVCV from diseased to healthy fish. Once SVCV is established in pond stock or pond farm stock, it may be very difficult to eradicate without destroying all kinds of life on the production site.

There is a high variability in the degree of susceptibility to SVC among individuals of the same fish species. Apart from the physiological state of the fish, age or age-related status of innate immunity appears to be extremely important: the younger the fish, the higher the susceptibility to overt disease, but even adult broodfish can be susceptible to infection. Water temperature is a critical environmental factor. In yearling or older fish, overt infection is not often observed above 17°C (62.6°F), whereas fry can be affected at temperatures as high as 22–23°C (71.6–73.4°F). The implementation of hygiene measures and control policies are the only control methods currently feasible. Vaccination is still mostly at the experimental stage.

Herpesviral Diseases

Fish herpesviruses usually have a rather narrow host specificity. The diseases caused by herpesviruses vary from external, fluid-filled blisters, to systemic infections affecting several organ systems and the production of tumors on the body of the fish. Some of these herpesvirus diseases are described below.

Channel Catfish Virus Disease

Channel catfish virus disease (CCVD) is caused by a herpesvirus designated Ictalurid herpesvirus 1, but the commonly used name is channel catfish virus (CCV). Channel catfish virus affects channel catfish in the United States. Channel catfish virus disease is of importance because of its clinical and economic consequences in channel catfish farming where

it causes high mortality rates in populations of fry and juvenile catfish in the summer months. Diseased fish demonstrate ascites, exophthalmia, and hemorrhage in fins and musculature. Histologically, the most remarkable damage occurs in the kidney with extensive necrosis of renal tubules and interstitial tissue.

In survivors, CCVD results in a strong protective immunity, the synthesis of circulating antibodies to the virus, and a covert latent carrier state. During this latent carrier state, the virus is undetectable by traditional culture or antigen detecting means, even when adults are immunosuppressed during spawning. Some variation in the virulence of CCV strains has been recorded during natural outbreaks of disease and has been demonstrated experimentally. Additionally, molecular data indicate genetic variation among isolates of the virus.

Reservoirs of CCV are clinically infected fish and covert carriers. Infectious CCV can be detected in the water from tanks of experimentally infected fish, but the route of shedding has not been determined. The sites where the virus is most abundant during the course of overt infection are posterior kidney, skin, gill, spleen, and intestine, respectively, in decreasing magnitude. The transmission of CCV is horizontal and vertical. Animate vectors and fomites could also act in CCV transmission. Vertical transmission is thought to be common, but the mechanism of vertical transmission is not known as infectious virus has not been detected on the skin or in the sexual products of spawning adults. Once CCVD occurs in a fish population, it becomes established among carrier fish.

Channel catfish and blue catfish are the only species naturally infected with CCV, and variations in susceptibility to CCV have been recorded depending on fish strain. The age of the fish is extremely important for overt infection. Although experimental data suggest that older fish are susceptible to natural outbreaks of acute CCVD, the disease occurs almost exclusively in fish that are less than 1 year of age and generally less than 4 months of age. Water temperature is the critical environmental factor. The mortality rate is high above 27°C (80.6°F), but readily decreases and ceases below 18°C (64.4°F).

Control methods currently rely on maintaining relatively low stocking densities and avoiding stressful handling of young fish during the summer months. Also, control policies and hygiene practices have been used, where practical, in catfish husbandry. The incubation of eggs and rearing of fry and juveniles in facilities separated from carrier populations are critical for preventing the occurrence of CCVD in a CCV-free fish production site. Because the virus is only detected during active outbreaks, defining CCV-free status has been done largely from historical data or by identifying populations that are seronegative to the virus.

Recent use of PCR and DNA probes to detect latent CCV genomic DNA suggests that CCV is present in many populations that have no history of the disease. Vaccination, although experimentally promising, is not in use at this time.

Salmonid Herpesviral Diseases

Two herpesviruses of salmonids are currently recognized. These are salmonid herpesvirus 1 (formerly termed *Herpesvirus salmonis*) and salmonid herpesvirus 2 (also known as *Oncorhynchus masou* virus [OMV]). Salmonid herpesvirus 1 was originally isolated from rainbow trout broodstock in Washington State. Subsequently, steelhead trout in California were also found to be carriers of the virus. However, the virus has been shown to be of relatively low pathogenicity for salmonids and is now regarded as being of limited concern.

In contrast, *Oncorhynchus masou* virus disease (OMVD) is an oncogenic and ulcerative condition among salmonid fish in Japan and probably in the coastal rivers of eastern Asia that harbor Pacific salmon. In Japan, the causative agent has been given a variety of names, but is now termed *Oncorhynchus masou* virus or salmonid herpesvirus 2. Fish species that are susceptible to OMV include kokanee salmon, cherry salmon (also known as masu salmon), chum salmon, coho salmon, and rainbow trout. On the basis of antigenic studies conducted with neutralizing polyclonal rabbit antisera, OMV can be easily distinguished from salmonid herpesvirus 1.

Clinically, the initial infection by OMV appears as a systemic and frequently lethal infection that is associated with edema and hemorrhages. Virus multiplication in endothelial cells of blood capillaries, hematopoietic tissue, and hepatocytes underlies the clinical signs. Four months after this first clinical condition, a varying number of surviving fish exhibit epitheliomas occurring mainly around the mouth (upper and lower jaw) and, to a lesser extent, on the caudal fin, opercula, and body surface. These neoplastic growths may persist for up to 1 year postinfection. In the case of coho salmon, 1-year-old infected fish, in particular, show ulcers on the skin, white spots on the liver, and tumors around the mouth parts or body surface. In rainbow trout, the diseased fish exhibit almost no external signs, although some fish manifest ulcerative lesions on the skin. Internally, intestinal hemorrhages and white spots on the liver are observed.

Following the septicemic phase, an immune response takes place that results in the synthesis of neutralizing antibodies to OMV. A carrier state frequently occurs, which leads to virus shedding via the sexual products at the time of spawning.

The reservoirs of OMV are clinically infected fish and covert carriers among groups of cultured, feral, or wild fish. Infectious virus is shed via

feces, urine, sexual products, and probably skin mucus; whereas the kidney, spleen, liver, and tumors are the sites where virus is the most abundant during the course of overt infection. The transmission of OMV is horizontal and possibly egg-associated. Disinfection of the eggs just after fertilization or at the eyed stage is effective in preventing OMV infection. OMV disease has not been reported in alevins originating from disinfected eggs that had been incubated and hatched in virus-free water.

Salmonids are the only fish species susceptible to OMV. The age of the fish is critical. One-month-old alevins are the most susceptible to infection. The main environmental factor favoring OMV infection is low water temperature (below 14°C [57.2°F]).

Control methods currently rely on the implementation of avoidance and hygiene practices in the operating of salmonid husbandry. The thorough disinfection of fertilized eggs and the incubation of these eggs and rearing of fry and alevins in premises completely separated from those harboring virus carriers and free from contact with fomites are the key measures needed to decrease contamination of OMV in a defined fish production site.

Cyprinid Herpesvirus Disease

The disease caused by cyprinid herpesvirus (CHV, formerly *Herpesvirus cyprini*) has been known as carp pox or *epithelioma papulosum* and is most often seen as benign, raised, white to gray, hyperplastic papillomas on the body surface. The lesions are smooth and may eventually cover large areas in fish that are stressed. Koi (ornamental strain of common carp) and common carp are the most often affected; however, the disease has been reported from other cyprinid species. The disease probably has a worldwide distribution wherever cyprinids are native or have been introduced. It is common in Europe and Asia and has been reported from areas of the United States where cyprinids are commercially reared. In addition to monetary losses from the unsightly lesions, the disease can produce mortality in juvenile cyprinids. The causative virus is a typical herpesvirus and can be detected by isolation in carp cell lines. Serum neutralization tests and molecular probes have been used to confirm many features of the life cycle of the virus.

Cyprinid herpesvirus is probably shed from the external lesions to affect juvenile fish in the rearing water. Infection of juvenile carp can result in a distended abdomen, darkening, exophthalmia, hemorrhages, and high mortality. Fish that recover often become carriers and will show external lesions much later in life. The induction of these lesions, like cold sores in humans, is thought to be stress related. At lower temperatures (15°C [59.0°F]), the carp immune system is less effective and virus replication can lead to production of the characteristic lesions. However, at warmer temperatures when conditions are favorable for the host (20–

30°C [68.0–86.0°F]) and the fish immune system is fully operational, the lesions will regress and disappear.

There is no control method other than avoidance. Carp to be shipped into an area where the disease has not been reported should come from stocks that have been carefully screened and that have no history of the disease. The long incubation period and latency of the virus make pathogen-free certification difficult.

More recently, an important emerging viral disease of cyprinids has caused mass mortality of koi carp in Israel and the eastern United States. The principal external signs of the disease were pale and irregularly colored gills. Microscopically, the gills showed severe hyperplasia and necrosis of the epithelial cells. A new herpesvirus was isolated from diseased fish using a koi carp cell line. Experimental infections produced high mortality in koi, and the virus was reisolated from several tissues of the dead fish. This new herpesvirus of cyprinids can be differentiated from *Herpesvirus cyprini* by serological means.

Birnaviral Diseases

The aquatic birnaviruses are small, icosahedral viruses belonging to the family Birnaviridae and have a genome composed of two segments of double-stranded RNA. Birnaviruses have been isolated from a very large number of fish, crustacean, and mollusk species in both freshwater and marine environments. Serological and genetic testing has revealed that there are very substantial differences among the various isolates. Within this collection are viruses that seem to cause little mortality within the hosts that have been experimentally infected, as well as viruses that cause very high mortality in important species for aquaculture.

Infectious Pancreatic Necrosis

Infectious pancreatic necrosis (IPN) is a highly contagious viral disease of salmonids. The disease, caused by infectious pancreatic necrosis virus (IPNV) most characteristically occurs in fry of rainbow trout, brook trout, brown trout, Atlantic salmon, and several Pacific salmon species. Susceptibility generally decreases with age, except for Atlantic salmon smolts, which can suffer from the disease after transfer to seawater. The first sign of an outbreak is frequently a sudden and, usually, progressive increase in daily mortality, particularly in the faster growing individuals. Clinical signs include darkening, a pronounced distended abdomen, and a spiral swimming motion. Cumulative mortalities may vary from less than 10% to more than 90% depending on the virus strain, size and species of host, and environmental conditions.

The disease is transmitted both horizontally through the water route

and vertically via the egg. Surface disinfection of eggs is not entirely effective in preventing vertical transmission.

The disease has a wide geographical distribution, occurring in most, if not all, major salmonid-farming countries of North and South America, Europe, and Asia.

Different isolates of IPNV display wide antigenic diversity and marked differences in degrees of virulence. Other birnaviruses showing serological relatedness to IPNV have been reported to cause diseases in some farmed marine fish species, such as yellowtail, turbot, and halibut; subclinical covert infections have been detected in a wide range of estuarine and freshwater fish species, such as loach, northern pike, and numerous other species in the families Anguillidae, Atherinidae, Bothidae, Carangidae, Catostomidae, Cichlidae, Clupeidae, Cobitidae, Cyprinidae, Esocidae, Percichthyidae, Percidae, Poeciliidae, Sciaenidae, and Soleidae.

Control methods currently rely on the implementation of control policies and of hygiene practices in salmonid husbandry, through the avoidance of the introduction of fertilized eggs originating from IPNV-carrier broodstock, and through the use of a protected water supply (e.g., spring or well) where the ingress of fish, particularly virus carriers, can be prevented. In outbreaks, reducing the population density may help to reduce the overall mortality. No treatment is available. A recombinant DNA vaccine is commercially available in Norway to protect Atlantic salmon, and other vaccines are in development and appear promising.

Orthomyxoviral Diseases

A good example of an emerging viral disease is found among the orthomyxoviruses. Until the 1980s, the only observation of a disease thought to be caused by a member of this group of viruses was recorded among eels. Later, increasing losses among Atlantic salmon in Norway resulted in the discovery of an important pathogen that has now been identified in salmonids from several countries.

Infectious Salmon Anemia

Infectious salmon anemia (ISA) is a disease of Atlantic salmon caused by an orthomyxovirus. It has, so far, been reported to occur in Norway, the United Kingdom, Canada, and the United States. Atlantic salmon is the only susceptible fish species known to develop the disease, but the ISA virus (ISAV) may survive and replicate in brown trout (also known as sea trout), rainbow trout, and Atlantic herring, which could act as carriers of the virus. Clinically, the initial infection appears as a systemic and lethal condition that is characterized by anemia, ascites, congestion,

and darkening and enlargement of the liver and spleen, as well as peritoneal petechiae (small, inflamed red lesions). Hemorrhages in the eyes may also be seen. Hepatocellular degeneration and necrosis, tubular necrosis, and hemorrhages in the kidneys are consistent histopathological findings in typical outbreaks. The infection is mainly observed in fish held in seawater or in fish exposed to seawater, but indications of disease outbreaks in fish held in freshwater have also been reported. The infection spreads slowly and the virus is considered to be of low virulence, although high mortality can ensue in some outbreaks of disease.

The reservoirs of ISAV are not known with certainty, but spread of the disease has occurred from the movement of subclinically infected Atlantic salmon smolts from farm to farm and from the effluent of slaughterhouses where organic material (especially blood and processing water) from ISA-infected fish has been discharged directly into seawater without further treatment. The presence of natural reservoirs of ISAV among marine fish species is supported by the isolation of genetically distinct strains of the virus from Atlantic salmon cultured in sea cages in different geographic areas. Few environmental factors have been identified that can be directly linked to outbreaks of the disease. In a latent carrier population, various stress factors, such as treatment against salmon lice, cestodes, or infectious diseases may be followed by disease outbreaks some 2–3 weeks later.

The incidence of ISA may be greatly reduced by implementation of general regulatory measures regarding the movement of fish, mandatory health control, and slaughterhouse and transport regulations, as well as specific measures including restrictions on affected, suspected, and neighboring farms; epizootiological studies; enforced sanitary slaughtering; generation segregation ("all in/all out"); and disinfection of offal and wastewater from fish slaughterhouses and fish processing plants. Development of an effective vaccine will be a critical step toward the eventual control of ISA in marine aquaculture and a killed viral vaccine shows significant promise in field trials.

Iridoviral Diseases

Another good example of emerging diseases of finfish is seen in the increasing number of iridoviruses isolated from outbreaks of disease among fish and amphibians on several continents. For many years, the only iridoviral diseases of significance among finfish were lymphocystis and viral erythrocytic necrosis (VEN). These diseases caused external warty growths (lymphocystis) among many freshwater and marine species or an erythrocytic anemia (VEN) among many marine species. In recent

years, severe losses have been associated with a group of iridoviruses that are related to the amphibian pathogens that are members of the *Ranavirus* genus of the family Iridoviridae*.*

Systemic Freshwater Ranaviruses

Epizootic hematopoietic necrosis (EHN) is a systemic iridovirus (ranavirus) infection of redfin perch (also known as Eurasian perch), rainbow trout, sheatfish, and catfish. The disease is caused by one of a group of closely related iridoviruses: epizootic hematopoietic necrosis virus (EHNV), European sheatfish virus (ESV), and European catfish virus (ECV). The geographical range of EHNV is currently restricted to Australia. The ECV and ESV agents have only been detected among fish in Europe. In North America, a virus has been isolated from largemouth bass that appears similar to these agents suggesting this group of viruses from fish and amphibians is distributed worldwide. Although closely related and inducing similar diseases in their respective hosts, these viruses can be distinguished from each other by molecular techniques.

Epizootic hematopoietic necrosis is generally lethal in redfin perch, while rainbow trout are relatively resistant and only a small proportion of individuals become infected. Infections with ESV and ECV can induce high morbidity and mortality in their catfish hosts. The largemouth bass virus (LMBV) has been associated with large natural mortalities among bass in the southern United States. The disease caused by these systemic iridoviruses is characterized by necrosis in the liver, spleen, hematopoietic tissue of the kidney, and other tissues. Experimental exposure studies have shown that rainbow trout can be infected by ECV and ESV without showing morbidity and mortality, while infections of largemouth bass with LMBV produce darkening, spiral swimming, abdominal distention, listlessness, and high mortality.

The viruses can be identified by immunofluorescence, enzyme-linked immunosorbent assay, or immunoelectron microscopy. The viruses share several antigens with each other and at least one antigen in common with the amphibian iridoviruses from North America (Frog Virus 3) and Australia (Bohle Iridovirus). Polyclonal antibodies against EHNV detect all of these viruses; however, nucleotide sequence analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Western blotting, and PCR assays can differentiate, to varying degrees, among the viruses.

The factors modulating the susceptibility of fish to systemic iridovirus infection are poorly understood. Clinical outbreaks due to EHNV are associated with poor water quality. In rainbow trout, infection occurs naturally at water temperatures from 11°C to 17°C (51.8°F to

62.6°F) and experimentally from 8°C to 21°C (46.4°F to 69.8°F). Disease in redfin perch does not occur at temperatures below 12°C (53.6°F). The following fish species were found to be susceptible to EHNV following bath exposure: redfin perch, rainbow trout, Macquarie perch, western mosquitofish, silver perch, and mountain galaxias. Both juvenile and adult redfin perch may be affected in outbreaks, but juveniles may be more susceptible to the disease. EHNV has now been detected in diseased rainbow trout ranging from hatchery fry to table-sized fish, although mortalities are most often seen in 0+ fish up to 125-mm fork length.

White Sturgeon Iridoviral Disease

The white sturgeon iridoviral disease (WSIVD) is a significant cause of mortality among farm-raised juvenile white sturgeon in North America and among Russian sturgeon in Europe. Lake sturgeon have been experimentally infected. White sturgeon iridovirus (WSIV) was originally described among hatchery-raised white sturgeon in North America; the source of WSIV was assumed to originate from adult wild white sturgeon broodstock collected from the Sacramento River in California and the Columbia River and its tributaries in Oregon, Washington, and Idaho. The virus also has been detected among wild juvenile white sturgeon collected from the lower Columbia River, and the virus is potentially endemic in wild white sturgeon populations throughout the Pacific Northwest of North America. An iridovirus similar to WSIV has been identified in Russian sturgeon from Northern Europe where it may be endemic among cultured populations of several species of sturgeon.

The WSIV infects tissues of the skin, gills, and upper alimentary tract. Infections of the oral mucosa and olfactory organ epithelium are presumed causes of the cessation of feeding that leads to a progressive emaciation or starvation of the fish—the principal external sign of the disease. Cumulative mortality of up to 95% has been reported among groups of infected fish in the hatchery and secondary infections with external protozoa or bacteria often contribute to the overall mortality. Infected fish with moderate to severe emaciation began dying 2–3 weeks following exposure to the virus at water temperatures of 17–19°C (62.6–66.2°F). Hemorrhages on the abdomen and the ventral scutes may be present, but these are not specific for WSIVD. There are no specific internal signs of infection as the virus does not invade systemically. Viral infection is evident on microscopic observation of stained tissue sections from infected fish. The integument and, particularly, the skin may show focal or diffuse hyperplasia with characteristic amphophilic to basophilic enlarged Malpighian cells. The cells are filled with virus particles as demonstrated by electron microscopy.

The modes of transmission of WSIV are not completely understood,

but horizontal transmission via water has been demonstrated in the hatchery and experimentally in the laboratory. There is strong circumstantial evidence that the virus is transmitted vertically from adult broodstock, but the virus has never been isolated or observed in adult fish.

There appears to be little antigenic relationship of WSIV to the systemic iridoviral agents represented by EHNV or the red sea bream iridovirus from Asia. The larger size and inner membrane structure of the WSIV virion, host cell line specificity, type of cytopathic effect, and the location of the target host cells (epitheliotropic and not systemic) distinguish the agent from other groups of fish iridoviruses.

Control methods currently rely on avoidance of the agent where possible. Because there are currently no methods for detecting the virus in adult broodstock, quarantine and testing of juveniles suffering mortality are the principal means to detect WSIV in young fish.

Lymphocystis

Lymphocystis disease, although rarely lethal, is of interest because of its wide range of occurrence among many propagated and free-ranging fish species. Many marine and freshwater fish species are susceptible, but the disease has not been reported among salmonids. Among freshwater fishes, walleyes and most centrarchids are susceptible.

Lymphocystis is a chronic viral disease causing generally granular, wart-like or nodular tissue lesions composed of greatly enlarged host cells and their covering membrane. Cells of infected tissue may attain a size of a millimeter or more and resemble a spattering of sand-like granules or, when larger, a raspberry-like appearance.

The causative agent of the disease is an iridovirus maintained in susceptible host fishes. Healthy fish may be exposed when infected cells burst and the virus particles are released. This release of virus can occur intermittently through the duration of infection or can be massive upon death and decomposition of infected fish. Lymphocystis lesions are persistent and commonly remain for several months; some may continue for a year or more.

No method of treatment is known and avoidance remains the only control strategy. Fish with the disease should be removed from the population to control the spread of the infection.

Bacterial Diseases of Fish

Bacterial diseases of fish have been recognized for many years, although new examples are continually being discovered as new species of fish are placed into aquaculture for the first time or as traditional species are cultured in new geographic areas. Today, perhaps more than 100 bacte-

rial species have been isolated from diseased fish and many of these have been demonstrated to be pathogens. In a few cases, these bacterial species can infect humans and are termed zoonotic agents.

The diagnosis of bacterial diseases has traditionally been accomplished by light microscopy and by culture of the bacteria on appropriate artificial media followed by identification using biochemical or serological testing. More recently, techniques of molecular biology (e.g., PCR) have provided rapid and sensitive alternatives.

While many bacterial diseases of fish are treatable with drugs or chemicals, the widespread use of these compounds has become of increasing concern (see the section on drugs and chemicals) and substantial restrictions are now being enforced in North America and Europe. These restrictions have significantly limited the numbers of compounds available and the specific conditions under which they can be legally used. In addition, withdrawal times for approved uses in food fish (or fish that may enter the human food chain) must be respected. Fortunately, several efficacious vaccines are now commercially available to prevent some of the most important bacterial diseases of fish and others are expected in the near future.

As stated earlier, the resistance of fish to infectious diseases is significantly reduced by stress; stress is often necessary for disease to occur from opportunistic bacterial pathogens that are commonly present in the aquatic environment. For these diseases, hatchery management practices are the difference between health and disease. The following are examples of some bacterial diseases of cultured fish. The list is not inclusive, and the conscientious hatchery manager will seek advice from a fish health professional to become familiar with bacterial diseases affecting the species of fish cultured in manger's geographic area and the approved methods for their prevention or treatment.

Flavobacterial Diseases

Several important diseases are caused by long, thin (0.5×10 mm), gram-negative, rod-shaped bacterial pathogens of the genus *Flavobacterium*. While the taxonomy of this group has undergone significant revision in the last decade, the diseases that these bacteria cause have been recognized for many years. Most flavobacterial species are common in the environment and become opportunistic pathogens when susceptible fish are present and the conditions are suitable. The nature of the disease caused by flavobacteria depends upon the location in the host in which they are growing. Some are external, causing gill diseases or external lesions, while others can result in bacteremia and systemic disease. Because these bacteria are common inhabitants in soil and water, avoid-

ance of these diseases is difficult, even with a pathogen-free water supply. Perhaps more than any other, these diseases are best prevented by good hatchery management.

Bacterial Gill Disease

Bacterial gill disease is a common disease of cultured trout and salmon and can result in explosive losses; it also is an occasional disease of warmwater and coolwater fish reared in ponds. Sudden lack of appetite, lethargy, flared opercula, and gathering near the surface of the water are typical signs of fish infected with bacterial gill disease. Gills show proliferation of the epithelium that may result in clubbing and fusing of lamellae or even entire filaments. The disease is greatly exacerbated by stress and by adverse water conditions, especially high ammonia levels, where mortalities can rise rapidly to 50% or greater per day. Microscopic examination of affected gill tissue reveals long, thin bacteria arranged in thick patches over the epithelium. Necrotic gill tissue may be grayish-white, and many of the filaments may be completely eroded. The causative agent is typically one of a large number of the flavobacteria, especially *Flavobacterium branchiophilum*, that are common in the soil and water and can be isolated from diseased fish.

A combination of large numbers of bacteria and gill epithelial proliferation differentiates bacterial gill disease from other gill problems caused by nutritional or environmental conditions (nutritional gill disease or environmental gill disease). Crowding, abrasive silt in the water supply, and dusty starter diets are important factors that contribute to outbreaks of the disease and may be a necessary predisposing cause in some outbreaks. Water temperatures above 12°C (53.6°F) are favorable for the bacteria. Yearling and older fish are less susceptible than fry, but outbreaks can be acute in all ages of fish.

Bacterial gill disease is exacerbated by stress and seldom is a problem among warmwater fish, particularly those being reared in earthen ponds. It occasionally becomes a problem when young channel catfish, largemouth bass, bluegills, or redear sunfish are held in crowded conditions in tanks or troughs for extended periods. After the problem is under control, the fish population should be thinned or the water flow increased. Unless the management practice that precipitated the outbreak is corrected, bacterial gill disease will reappear.

Columnaris

The causative agent of columnaris is *Flavobacterium columnare*. The agent is a long, thin, gram-negative bacterium that moves in a creeping or flexing action and that has a peculiar habit of stacking up to form distinctive columns, hence the name "columnaris."

Columnaris most commonly involves external infections, but can occur as an internal systemic infection with no visible external signs. Externally, the disease starts as small, grayish lesions on the body, gills, or fins. The lesions rapidly increase in size and become irregular in shape. As the lesions get larger, the underlying musculature can be exposed. The margins of the lesions and, occasionally, the centers may have a yellowish color due to large aggregations of the bacteria. Frequently, the most lethal infections involve mouth and jaws. In Pacific salmon and warmwater fish, particularly catfish, lesions may be confined to the gills. Lesions on the gills are characterized by yellowish-brown necrotic tissue beginning at the tip of the filaments and progressing toward the base.

Columnaris disease usually is associated with some kind of stress condition such as high water temperature, low oxygen concentration, crowding, and handling. Under appropriate conditions, columnaris may take an explosive course and cause catastrophic losses in 1–2 d after the first appearance of the disease. Therefore, it is incumbent upon the fish culturist to maintain the best possible environmental conditions for the fish and to minimize any stress conditions.

Although columnaris disease attacks practically all species of freshwater fish, catfish are particularly susceptible. In warmwater fish, most outbreaks of columnaris occur when the water temperature is above 18–20°C (64.4–68.0°F), but the disease can occur at any time of the year. Columnaris disease is common in salmonids held at water temperatures above 15°C (59.0°F). Progress of the disease usually is faster at the higher temperatures.

Flavobacteria are common inhabitants of soil and water and commonly are found on the surface of fishes, particularly on the gills. The stress of crowding, handling, spawning, or holding fish at above-normal temperatures, as well as the stress of external injury, facilitates the transmission and eruption of columnaris disease. A disease resembling columnaris occurs in the marine environment and is caused by *Flavobacterium maritimus*.

Presumptive diagnosis of columnaris is accomplished best by microscopic examination of wet mounts of scrapings from lesions and detection of many long slender bacteria that move by flexing or creeping movements and form "haystacks" or "columns."

Preventative measures include maintenance of optimum water temperatures for salmonids, reduced handling during warm weather, maintenance of the best possible environmental conditions, and avoidance of overcrowding fish.

Bacterial Coldwater Disease

Bacterial coldwater disease (BCWD) or low temperature disease is one of the more common diseases of young salmonids where it produces low to moderate mortality. The disease typically manifests itself in the form of external lesions on the dorsal surface or on the caudal peduncle, hence the name "peduncle disease"; however, the bacteria frequently become systemic where they can invade the vertebral column and central nervous system leading to darkening and spinal deformities that can mimic IHN or whirling disease. As its name implies, the disease occurs at low water temperatures in the range of 4–10°C (39.2–50.0°F). Affected fish become darkened, and lesions may develop on the back near the dorsal fin, on the caudal peduncle, or on the isthmus anterior to the pectoral fins. The caudal fin may be completely destroyed. A lesion usually starts on the caudal peduncle behind the adipose fin, where it causes inflammation, swelling, and gradual erosion. The disease progresses posteriorly and the caudal fin may be eroded.

Coldwater disease is caused by *Flavobacterium psychrophilum.* The bacterium is water borne and can be transmitted from carrier fish in the water supply. The bacterium can also be transferred vertically from adult to progeny via the egg. The disease sometimes occurs without external signs and may be due to the bacterium being acquired by vertical transmission. Crowded conditions stimulate a disease outbreak, but are not necessary for the disease to appear. While all salmonids (and a few nonsalmonids) are thought to be susceptible, coho and chum salmon are the most vulnerable and, in sac fry of these species, the yolk sac may be ruptured. Typically observed in young fish during the spring when water temperatures are low, larger fish may be affected if conditions are favorable for the disease. Efforts to develop a vaccine are underway.

Gram-Negative Septicemias

Many members of the *Enterobacteriaceae* (enterics), *Vibrionaceae* (vibrios and aeromonads), and *Pseudomonadaceae* (pseudomonads) cause disease in fish. Different bacterial species within these families grow optimally at very different temperatures and typically cause disease in hosts that are cultured at those temperatures. The diseases are typically gram-negative septicemias with hemorrhagic lesions or liquifactive necrosis of organs. They can result in high losses. The first commercially licensed fish vaccines were developed as formalin-killed bacterial cultures (bacterins) to control some of these diseases.

Furunculosis

Furunculosis was traditionally known as a septicemic disease of salmonids reared in freshwater, but significant losses now occur among salmonids cultured in marine net-pens. The causative agent of furunculosis is *Aeromonas salmonicida*, and virtually all trout and salmon hatcheries with open water supplies are probably exposed to the bacterium at one time or another. Furunculosis has been reported in most salmonids of all ages. Among salmonids, brook trout, brown trout, rainbow trout, and Atlantic salmon are most often affected. While furunculosis is endemic in many hatcheries, severe outbreaks are now less common due to advances in fish culture, drug therapy, and use of vaccines.

The disease is characterized by a generalized bacteremia with focal necrotic lesions in the muscle, often seen as swellings under the skin that are not true "furuncles." The swollen skin lesions are filled with pink fluid containing blood, and necrotic tissue may have a purple or iridescent blue color. These lesions are especially apparent in chronic infections, but similar lesions may occur from other diseases caused by gram-negative bacteria. Hemorrhaged and frayed fins also are common. Internally, diseased fish may exhibit petechiae in the lining of the body cavity and especially on the visceral fat. The pericardium usually is filled with bloody fluid and is inflamed. The spleen, normally dark red in color, often will be a bright cherry-red and swollen. The lower intestine often is highly inflamed and a bloody discharge can be manually pressed from the vent.

The incidence of furunculosis generally follows the seasonal temperature pattern with most outbreaks occurring in the warmer months. When conditions are optimal for the disease, it occurs as an acute form in which death results from massive bacteremia before gross lesions can develop. In such outbreaks, only a few clinically sick fish may be seen at any one time in spite of the high death rate. Acute cases of furunculosis have incubation periods of 2–4 d with few apparent signs. Chronic cases usually occur at temperatures below 14°C (57.2°F) and may have an incubation period of one to several weeks, depending upon the water temperature. Latent cases may develop during low temperature periods and flare up with greater severity, displaying many typical signs, when water temperatures rise.

Fish exposed to furunculosis may become carriers of the disease and nonsalmonids in the water supply should be considered likely reservoirs of infection. Furunculosis may break out in virtually any freshwater fish population, including warmwater species, if conditions such as high temperature and low dissolved oxygen favor the pathogen.

Sanitation provides the most important long-range control of furun-

culosis. If a population of fish at a hatchery is free of furunculosis and if the water supply does not contain fish that harbor the pathogen, strict sanitation measures should be used to prevent the introduction of the disease via incoming eggs or fish. Eggs received at a hatchery should be disinfected upon arrival with iodophors used as recommended. Maintenance of favorable environmental conditions for the fish is of prime importance in preventing furunculosis outbreaks. Proper water temperatures, adequate dissolved oxygen, efficient waste removal, and avoidance of overcrowding must be observed.

A presumptive diagnosis of furunculosis takes into consideration the frequency of outbreaks in a certain area, presence of typical lesions, and the occurrence of short gram-negative rods in the lesions, kidneys, spleen, and blood. A confirmatory diagnosis can be made only after *Aeromonas salmonicida* has been identified as the predominant organism isolated.

Drugs are effective only in the treatment of outbreaks, and drug resistance is a common problem. Recurrences of furunculosis are likely as long as *A. salmonicida* is present in the hatchery system and environmental conditions are suitable. In recent years, effective vaccines have been licensed for control of furunculosis. While these vaccines are most effective when delivered by injection in an adjuvant, among Atlantic salmon farms, the vaccines provide important protection and are highly cost-effective.

In addition to salmonids, an atypical form of furunculosis has been reported in many warmwater species, mainly ornamental fish, including carp, Koi, goldfish, and eel. The disease often produces hemorrhages on the body surface that can lead to deep ulcers of the skin and musculature. This atypical form of furunculosis is caused by a different subspecies of the organism and the disease is known as erythrodermatitis of carp, carp furunculosis, ulcer disease, or atypical furunculosis.

Enteric Redmouth

Enteric redmouth disease (ERM) refers to an infection of rainbow trout and Atlantic salmon by the gram-negative enteric bacterium *Yersinia ruckeri*. *Yersinia ruckeri* produces a systemic infection that results in marked inflammation and erosion of the jaws and palate. Trout with ERM typically become sluggish and dark in color and show inflammation of the mouth, opercula, isthmus, and base of fins. Reddening occurs in body fat and in the posterior part of the intestine. The stomach may become filled with a colorless watery liquid and the intestine with a yellow fluid. This disease often produces sustained low-level mortality, but can cause large losses. Large-scale outbreaks occur if chronically infected fish are stressed during hauling, exposed to low dissolved oxygen, or exposed to other poor environmental conditions.

The disease has been reported in rainbow, steelhead, and cutthroat

trout, and coho, chinook, and Atlantic salmon. Evidence suggests that the spread of the disease is associated with the movement of infected fish, and fish-to-fish contact provides transfer of the bacterium to healthy trout. Because spread of the disease can be linked with fish movements, the best control is avoidance of the pathogen. Fish and eggs should be obtained only from sources known to be free of ERM. This can be accomplished by strict sanitary procedures and avoidance of carrier fish.

While drug therapy has been used in the past to control ERM, the emergence of drug resistance strains of *Y. ruckeri* is a significant problem. Highly effective vaccines are now commercially available for the control of ERM. These are efficiently administered by immersion vaccination of fry and fingerlings providing cost-effective protection for fish in waters containing the bacterium. These bacterins produce long-term immunity and should be viewed as the recommended method for control.

Motile Aeromonas Septicemia

Motile aeromonas septicemia (MAS) is a ubiquitous disease of many freshwater fish species and is typically caused by one of several, gram-negative, motile bacteria that are members of the genus *Aeromonas.* Some of the species frequently isolated in outbreaks are *A. hydrophila, A. sobria,* and *A. caviae.* Occasionally, various species of pseudomonads, especially *Pseudomonas fluorescens,* can cause an indistinguishable form of the disease. A definitive diagnosis of MAS can be made only if the causative agent is isolated and identified because the signs of MAS can be easily confused with several other diseases.

When present, the most common signs of MAS are superficial circular or irregular grayish-red ulcerations with inflammation and erosion in and around the mouth as in ERM. Fish may have a distended abdomen filled with a slightly opaque or bloody fluid (dropsy) or exophthalmia if fluid accumulates behind the eyeball. Other fish, minnows in particular, may have furuncles like those in furunculosis, which may erupt to the surface, producing deep necrotic craters. Fins also may be inflamed.

In addition to the presence of fluid in the abdominal cavity, the kidney may be swollen and soft and the liver may become pale or greenish. Petechiae may be present in the peritoneum and musculature. The lower intestine and vent often are swollen and inflamed and may contain bloody contents or discharge. The intestine usually is free of food, but may be filled with yellow mucus.

Motile aeromonas septicemia occasionally takes an acute form in warmwater fish, and severe losses can occur even though fish show few, if any, clinical signs of the disease. In general, most outbreaks in warmwater fish are in the spring and summer, but the disease may occur at any time

of year. Temperature is very important. Largemouth bass and channel catfish are susceptible particularly during spawning and during the summer if stressed by handling, crowding, or low oxygen concentrations. Aquarium fish can develop the disease at any time of the year. Among salmonids, rainbow trout seem to be the most susceptible and outbreaks are associated with handling stress and crowding of fish. Fish and frogs that recover from the disease usually become carriers and may contaminate water supplies if not removed. The disease has been identified throughout the world and apparently infects virtually any species of freshwater fish under conditions favoring the bacteria.

Observation of strict sanitary practices and the elimination of possible carrier fish from the water supply are extremely important to the control of MAS at trout and salmon hatcheries. For warmwater fish, everything possible should be done to avoid stressing the fish during warm weather. Because of the many types of bacteria associated with MAS, it is unlikely that a vaccine will be developed in the near future. Also, due to the common occurrence of drug resistance, therapeutic drug treatments cannot be assumed to be effective.

Enteric Septicemia of Catfish

Enteric septicemia of catfish (ESC) or edwardsiellosis is caused by the bacterium *Edwardsiella ictaluri*. It is one of the most important infectious disease problems in the commercial catfish industry in the southern United States. Most reported cases of ESC occur among channel catfish, but the bacterium has been isolated from white catfish, yellow bullhead, black bullhead, and brown bullhead. Enteric septicemia of catfish has also been reported from *Clarias batrachus* in Thailand and from several ornamental species, and the susceptibility of other species, including salmonids, has been shown experimentally. *Edwardsiella ictaluri* should not be confused with *E. tarda*, another member of the same genus that is frequently found in aquatic animals and is responsible for opportunistic infections in fish and mammals, including humans.

Acute outbreaks of ESC occur within a limited temperature range, from 18 to 28°C (64.4 to 82.4°F). Low-level mortality due to ESC can occur in carrier populations outside of this temperature range. This results in seasonal fluctuations, spring and autumn being the critical periods. Other environmental factors (water quality, organic compounds, stocking density, and stress factors) are likely to modulate the virulence of the agent In spite of these characteristics, *E. ictaluri* is generally considered to be a true obligate pathogen.

Clinical signs may differ according to the fish species. Two clinical forms of ESC have been described. The infection seems to begin most often in the olfactory sacs through the nasal route and to progress slowly

upward generating granulomatous inflammation in brain tissues. This chronic meningoencephaIitis can account for behavioral manifestations with alternating listlessness and chaotic swimming. In late stages of this disease manifestation, "hole in the head" is observed—an ulceration developing from the inside through the dorsocranial part of the head exposing the brain. Although general infection can result from such chronic forms, it is more often associated with acute septicemia following infection through the enteric route. It has been demonstrated that *E. ictaluri* can cross the intestinal mucosa very easily. Then, as in many other bacteremias, skin petechial hemorrhages are observed around the mouth, on the throat, and at the base of the fins. Multifocal distinct 2-mm diameter, raised, hemorragic cutaneous lesions that progress to depigmented ulcers also occur. Anemia, moderate gill inflammation and exophthalmia are common signs. Internally, hemorrhages and necrotic foci are scattered in the liver and other internal organs. Hemorrhagic enteritis, systemic edema, accumulation of ascitic fluid in the body cavity, and enlargement of the spleen are nonspecific signs. Histological examination reveals a systemic infection of all organs and skeletal muscles with diffuse granulomatosis.

Fish that recover from ESC generally remain infected, even though protective immunity and circulating antibodies can be clearly evidenced. *Edwardsiella ictaluri* has been detected in the kidney of such fish well over 4 months after exposure, suggesting that carrier fish act as the natural reservoir for the organism. It is believed that shedding with feces is the main means of dissemination into the environment. The pathogen persistence and the common practice of continual partial harvest and stocking within a production pond have contributed to the success of this pathogen and the prevalence of ESC in the industry. Moreover, the agent can survive in pond sediments for an extended period of time, and this may be another important factor in disease recurrence in given areas.

Enteric septicemia of catfish may be controlled through chemotherapy or prophylactic measures. The most common antimicrobial treatments are oral application of potentiated sulfonamide (sulfadimethoxine-ormethoprim) or oxytetracycline, but plasmid-mediated resistance to these antibiotics has developed recently and many producers are now focusing on alternative methods to reduce losses. This relies on management to reduce stress in fish and on vaccination, which has shown promise and remains an active area of research.

A related enteric organism, *Edwardsiella tarda*, also causes mild to severe disease in catfish and among salmonids at warmer temperatures. In catfish, the disease progresses from small cutaneous lesions to large abscesses filled with necrotic tissue, a condition termed "emphysema-

tous putrefactive disease" which indicates the foul smell occurring in fish affected by the disease. In addition to the potential to cause disease in many fish species reared in warm water, the agent is capable of infecting humans. Control methods for *E. tarda* are similar to those used for *E. ictaluri*. Because *E. tarda* is not an obligate pathogen and is relatively common in the environment, it is difficult to eliminate the infection from open ponds.

Vibriosis

Vibriosis is a systemic disease of marine, estuarine, and (occasionally) freshwater fishes. It is known also under the names of red pest, red boil, red plague, or salt water furunculosis. Many species of vibrios have been isolated from diseased fish; however, the most important include *Vibrio anguillarum, V. ordalii, V. salmonicida, V. damsela,* and *V. vulnificus.* Depending upon the causative species, vibriosis can occur any time of year, even in water temperatures as low as 4°C (39.2°F; coldwater vibriosis). However, it is most prevalent in the temperate zones during the warmer summer months, and outbreaks can be expected when water temperatures reach 15°C (59.0°F).

Signs of the disease usually do not become evident until the fish have been in salt water for 2 weeks or more under crowded conditions. Diminished feeding activity is one of the first noticeable signs. Lethargic fish gather around the edges of holding units; others swim in erratic, spinning patterns. Diseased fish have hemorrhages around the bases of their pectoral and anal fins or bloody discharge from the vent. When a fish is opened for necropsy, diffuse pinpoint hemorrhages of the intestinal wall and liver may be evident. The spleen frequently is enlarged and may be two to three times its normal size.

Vibriosis is worldwide in its distribution and virtually all species of marine and estuarine fishes are susceptible. Among salmonids, pink salmon and chum salmon are the most susceptible, but serious outbreaks have occurred in coho salmon, rainbow trout, and Atlantic salmon. Stresses associated with handling, low oxygen, and elevated temperature can predispose fish to vibriosis. Vibrios are ubiquitous in marine and brackish waters and infections probably are waterborne and may be spread by contact. Salmonids usually die within a week after exposure. Fish of all ages are susceptible.

Diagnosis of vibriosis requires isolation of a slightly curved, gram-negative, motile, rod-shaped bacterium on salt-containing medium. The organism may be identified by serological or biochemical reactions. There is no reliable presumptive diagnosis of vibriosis because of its similarity to other gram-negative septicemias.

Prevention of vibriosis depends on good sanitation, no crowding, and minimal handling stress. While drug therapy has been used in the past to control vibriosis, drug resistance is a problem. Highly effective vaccines are now commercially available for the control of vibriosis and were the first commercially licensed vaccines for fish. These vaccines are efficiently administered by immersion vaccination and result in significant economic benefit. These bacterins produce long-term protection and should be viewed as the recommended method for control.

Bacterial Kidney Disease

Bacterial kidney disease (BKD) is a chronic insidious infection of salmonid fish. The disease is slow to develop, but once established, it may be difficult to control and virtually impossible to cure. The causative agent of BKD, *Renibacterium salmoninarum,* is a small, nonmotile, nonacid-fast, gram-positive diplobacillus.

The course of kidney disease is that of a chronic bacteremia. Once the pathogen enters the fish, either from vertical transmission through the egg or from contact with other infected fish in the water supply, the bacteria multiply slowly within macrophages and other cells. Foci of infection develop in the kidney and in other organs such as the liver, spleen, and heart. White cellular debris collects in blisters, and ulcers that develop in these organs are seen easily. Lesions developing in the posterior kidney are easiest to spot and may reach a centimeter or more in diameter. Some lesions extend into the musculature and result in externally visible blisters under the skin. If the disease has reached the stage in which gross lesions are apparent, therapeutic treatment has little effect.

Bacterial kidney disease has been found in many species of salmonids in North America. A tendency toward seasonal periodicity has been noted, but the incidence varies at different hatcheries. Brook trout and chinook, chum, pink, and sockeye salmon are highly susceptible. Infected or carrier fish are considered to be sources of infection. Experimentally, from 1 to 3 months will elapse before mortality begins, depending upon the route of administration. Vertical transmission via the egg has been proven and is an important source of infection. Such infections within the egg are impossible to eliminate via iodophor treatment of eggs.

Historically, diagnosis of BKD has been based on the demonstration of small, gram-positive diplobacilli in infected tissues. More recently, serological procedures such as fluorescent antibody techniques and enzyme-linked immunosorbent assays have become widely used and are considered more reliable. Molecular methods such as the PCR assay have been published and will probably become standard methods in the future.

Control methods for BKD rely upon reducing hatchery conditions

that exacerbate disease and in reducing the infection levels among broodstock. Complete avoidance of *R. salmoninarum* has proven difficult because the bacterium is present at subclinical levels in many stocks of salmonids and is transmitted *intra ovum*. Thus, only the most highly sensitive detection methods have been successful at identifying and avoiding infected broodstock (broodstock segregation). Drug treatment is typically only effective in delaying mortality and not in eliminating infections. Among hatcheries rearing salmonids, low-level infections should be anticipated; however, should a serious outbreak occur, strict quarantine and eradication measures are recommended. The development of vaccines against BKD remains an active area of research.

Piscirickettsiosis

Piscirickettsiosis is a disease of salmonids caused by *Piscirickettsia salmonis,* a gram-negative, highly fastidious, intracellular bacterial pathogen of fish. Thus far, the disease has been described from Chile, Ireland, Norway, and both the west and east coasts of North America. *Piscirickettsia salmonis* has been detected in coho salmon, chinook salmon, sakura salmon, rainbow trout, pink salmon, and Atlantic salmon. Coho salmon are believed to be most susceptible.

The first evidence of disease may be the appearance of small white lesions or shallow hemorrhagic ulcers on the skin. Affected fish appear dark and lethargic, hanging at the net sides. The major gross pathological changes are gill pallor, peritonitis, ascites, an enlarged spleen, a swollen gray kidney, and a liver with large pale necrotic lesions. The mechanisms of transmission are not completely understood. The disease has been primarily reported in marine fish farms, but has also been observed in freshwater facilities. Horizontal transmission in salt water and freshwater has been demonstrated. Transmission by vectors remains a consideration, and the role of vertical transmission is presently under investigation.

The identification of *P. salmonis* is based on isolation of the causative agent in cell culture with subsequent testing for characteristics of rickettsiae. *P. salmonis* occurs in cytoplasmic vacuoles in the host cell. The identity of *P. salmonis* isolated in cell culture or observed in smears from diseased tissue may be confirmed by means of the fluorescent antibody test using type-specific antiserum. Molecular diagnostic methods using the PCR have been developed.

The implementation of hygienic measures and sound management policies are the only control methods currently available. Eggs may be disinfected as part of good hatchery practice, and elimination of infected stocks may be of some use unless the fish are continually exposed to

carriers present in the marine environment. Although antibacterial treatment provides some benefit, it is not entirely effective as a means of controlling the disease, and the emergence of drug resistance has been reported to be an increasing problem. Intensive efforts are underway by various groups to develop an effective vaccine.

Mycobacteriosis

Mycobacteriosis of fish is typically a chronic, granulomatous disease that is caused by one of several freshwater or marine species of mycobacteria, including *Mycobacterium fortuitum, M. chelonae, or M. marinum.* The disease is important both from a fish health perspective and because these bacteria are zoonotic agents capable of causing disease in humans.

Mycobacteriosis is usually seen in fish reared in temperate conditions, and outbreaks have been reported in striped bass, sea bass, sea bream, cod, red drum, mackerel, tilapia, and salmonids. Clinical signs include lethargy, skin discoloration, popeye, and a distended abdomen. Internally, grayish to white nodular lesions are typically observed in the liver, spleen, or kidney. Mortality is typically chronic and unrelenting. Occasionally, the disease assumes an acute fulminating form with extensive necrosis of internal organs.

Diagnosis is by observation of acid-fast organisms in the lesions or by culture on special media. There is no effective therapy or vaccine for control of this disease. Removal of fish and complete disinfection of facilities are the only control options.

Streptococcal Infections

In the United States, *Streptococcus iniae* is responsible for significant losses in tilapia, striped bass, and other warmwater species. In freshwater species, it produces an acute disease characterized as meningitis (in trout) and meningoencephalitis (in tilapines). In the marine species red drum, infections are characterized as an acute to subacute disease with multiple necrotic lesions affecting the skin and internal organs. Bacteria have been found in both diseased and asymptomatic fish. *S. iniae* is considered a zoonotic agent capable of infecting humans, causing a localized form of cellulitis; cases have been identified in the United States and Canada.

The slow growth and similarity of *S. iniae* to other species of gram-positive cocci complicate identification by conventional methods, and PCR assays have been developed. Formalin-killed bacterins have been tested in the laboratory and in the field. Further research on vaccine development is ongoing.

Parasitic Diseases of Fish

Parasites probably cause more disease problems in fish culture than any other type of fish pathogen. It is common to find parasites of many taxonomic classes in or on wild fish, and fish reared under intensive conditions rarely are without some parasites. When present in small numbers, they usually produce no obvious damage and are of limited concern; however, under intensive rearing conditions, parasite numbers can build up rapidly, leading to problems. Here, vigilance is important. If problems can be anticipated and effective measures taken early, parasite losses may be moderated, but under adverse conditions, parasites, even normally benign ones, can result in high mortality.

Traditionally, parasites are identified or classified by microscopic observation of morphological features using fresh or fixed material. More recently, the application of molecular techniques has revealed differences among parasites with similar morphologies, as well as the identity of parasites previously thought to be entirely different organisms. These methods have also been used to develop more rapid methods for confirmation of a few parasitic diseases that formerly had to be diagnosed by more labor-intensive means, including histology.

While, literally, thousands of species within the many parasite genera affecting fish have been described, only a few examples of those appearing commonly and of major importance to fish husbandry are presented below. It should be remembered that almost all species of parasites can cause problems if allowed to reach very high levels, but, typically, significant losses due to parasites are usually a sign of inattention to good hatchery practices. Over time, hatchery managers should become familiar with the types of parasites that can present problems for the species being reared at a given location. In general, the same problems will occur routinely and can be anticipated. Because many parasites can be identified with the aid of a simple light microscope, the progressive hatchery manager will be proactive in examining fish in order to take appropriate action or to seek early assistance from a fish health professional.

Dinoflagellates

Dinoflagellates are protozoans that occur frequently in the aquatic environment. Some produce potent toxins that have caused mass mortalities among fish. While most are free living or parasites of invertebrates, several have been reported to cause disease in fish, including *Amyloodinium* and members of several related genera.

Amyloodinium is a serious parasite of fish living in warmwater marine and brackish waters. It occurs worldwide and has been associated

with large losses of both cultured and wild fishes. The disease caused by *Amyloodinium* is often referred to as marine velvet disease; the parasite affects the gills and skin causing a dusty or velvety appearance. Death occurs by suffocation in heavy infestations of the gills. Most species of marine fish are susceptible.

Diplomonads, Kinetoplastids, and Amoebas

The diplomonads, kinetoplastids, and amoebas are parasitic protozoans that produce external and internal infections of many vertebrates, including fish. The best known fish pathogens of the group include species of *Cryptobia, Hexamita, Ichthyobodo, Trypanosoma,* and several amoebas.

Hexamita is the only common flagellated protozoan found in the intestine of salmonids of all ages and occasionally of ornamental fish and grass carp. Infection can occur at all temperatures and results in poor growth and elevated mortality in small fish. All species of salmonids are susceptible to infection. Because there are no well-defined signs, a diagnosis of hexamitiasis must be made by microscopic examination of gut contents from the anterior portion of the intestine and pyloric caeca. The flagellates are minute, colorless, pear-shaped organisms that dart rapidly in every direction. Gross signs of infected fish may include nervous behavior, including swimming in a cork-screw pattern and a dark emaciated condition.

Species of *Ichthyobodo* (formerly *Costia*) are very small flagellated ectoparasites easily missed during routine microscopic examinations of gills and body scrapings. These protozoans are free-swimming, move by means of long flagella, and are about 5×12 µm in size. Two species, *Ichthyobodo pyriformis* and *I. necatrix,* are commonly seen and produce "blue slime" disease of fish. The characteristic blue slime or bluish sheen is caused by increased mucus production in response to irritation. Virtually all species of fish are susceptible at all water temperatures.

An early sign of an *Ichtyobodo* infection is a drop in appetite of the fish and a general listlessness. "Flashing" may be evident if the skin is infected, but only rarely if just the gills are involved. Signs of the disease sometimes are mistaken for bacterial gill disease. Heavily infected fish often develop a bluish slime over the entire body; however, fish less than 3 or 4 months old may die before this condition develops.

Ichtyobodo can be a serious problem on all species and sizes of warmwater fish, particularly channel catfish. This flagellate can cause problems any time of year, but is most common on warmwater fish from February to April in the Northern Hemisphere. Infections with the para-

site can be treated, but prevention through improved hygiene, adequate water flows, and continuous vigilance is typically a better choice.

Ciliates

Among the more that 7,000 species of ciliated protozoans are a few of the better-known fish pathogens. These include *Chilodonella, Ichthyophthirius, Trichodina,* and *Trichophyra.* Infections are typically external and have been successfully treated with a variety of chemicals. These parasites affect a wide range of fish species and typically do limited harm. When mortality occurs, it is usually a result of adverse conditions that stress the fish making them more susceptible to infection.

Ichthyophihirius multifilis, also termed white spot or "Ich," is a large ciliated protozoan exclusively parasitic on fish. It causes serious disease in catfish, but also is a common parasite of other warmwater fishes and can be a problem for ornamental fish and salmonids. Ich can be seen by the naked eye; when fully grown it may be as large as 1.0 mm in diameter and appears as gray-white grains of salt. Positive identification is based on the finding of a large, ciliated protozoan, with a horseshoe-shaped macronucleus, embedded in gills, skin, or fin tissue.

The feeding stages, or trophozoites, of Ich are found in the epithelium of the skin, fins, and gills. When mature, the adult parasites drop off the host and attach to the bottom or sides of the pond. Once encysted, they reproduce by multiple fission and, within 2 to several days, depending upon temperature, each adult may produce up to 1,000 ciliated tomites. Upon contact with the fish, the tomites penetrate the skin and begin to feed and grow into adults. At optimal temperatures of 20–23°C (68.0–73.4°F), the life cycle may take as few as 3–4 d. The cycle requires 2 weeks at 15°C (59.0°F), more than 5 weeks at 10°C (50.0°F), and months at lower temperatures.

Ich is known as "salt and pepper" and "white spot" disease by aquarists because of the gray-white specks that appear on the skin. However, in some species of warmwater fish, Ich is found almost exclusively on the gills. On rare occasions, Ich infections on catfish also may be restricted to the gills. In severe outbreaks, losses may precede the appearance of the mature parasites on the fish. Young fish exhibit considerable flashing off the bottom and often show erratic spurts of activity, jumping out of the water and thrashing about due to irritation caused by the parasites. Successful treatment of Ich depends upon the elimination of parasite stages that are free in the water and the prevention of reinfection. Tomites and adult parasites leaving the fish are, therefore, the target of therapeutic efforts.

The best control for Ich, as for any disease, is prevention. Hatchery water supplies always should be kept free of fish. If possible, any warmwater fish brought onto a hatchery should be quarantined for at least 1 week at 20°C (68.0°F) and coldwater fish for at least 2 weeks at 15°C (59.0°F) to determine if they are infested with Ich.

Ich is more difficult to treat because the encysted forms are resistant to treatment; only the free-swimming forms are vulnerable. Successful treatment usually is long and expensive. There are several pond treatments for either warmwater fish or salmonids that can be used successfully if started in time.

Species of *Chilodonella* are small, oval, colorless protozoans, 50–70 μm long, which may be found in vast numbers on the skin, fins, and gills of virtually all species of fish at all water temperatures. Under high magnification, faint bands of cilia can be seen over much of the organism. The parasite primarily affects weakened fish and is a significant problem for warmwater fish during the winter when lower temperatures suppress host resistance. Heavily infected fish are listless, produce excessive mucus, do not feed actively, and may flash. Mortality is often associated with heavy gill infestations.

Trichodinids are saucer-shaped protozoans with cilia around the margins of their body. These protozoans live on the skin, fins, and gills of fish and, when abundant, cause severe irritation and continual flashing. Salmon yearlings, if left untreated, develop a tattered appearance. Secondary bacterial infections may develop in untreated cases.

Species of Trichophrya sometimes are found on the gills of fish and can cause serious problems in catfish and occasionally in other warmwater species. They have rounded to pyramid-shaped bodies (30 × 50 mm) and are distinguished by food-catching tentacles in the adult stage. Live organisms have a characteristic yellowish-orange or yellowish-brown color that makes them very conspicuous when wet mounts of gill tissue are examined using a microscope. Affected fish gills are pale and clubbed and may be eroded. Infected fish will be listless, as if they were suffering from an oxygen deficiency.

Microsporidia

Microsopridia are intracellular protozoan parasites characterized, as their name implies, by the production of very small spores. Species within 11 genera affect a wide variety of fish worldwide; the most significant ones are *Pleistophora, Enterocytozoon, Glugea,* and *Loma.*

Several species of *Pleistophora* infect hatchery fish. *Pleistophora* spores are about the size of large bacteria, 3–6 mm long and somewhat bean

shaped. Severe infections have been reported in the gills of rainbow trout and in the ovaries of golden shiners. In golden shiners, the parasites infest up to about half of the ovary and significantly reduce the fecundity of broodstock populations.

The only known control for *Pleistophora* is prevention. Rainbow trout or their eggs should not be transferred from infected to uninfected hatcheries. Broodstocks known to be infected should be phased out and the rearing facilities disinfected. Because there are few stocks of golden shiners free of *Pleistophora ovariae,* proper management is the best answer to this problem. The severity of infections increases with age, so only 1-year-old broodstock should be used and all older fish destroyed.

Myxosporea

The family Myxosporea is a large and important group of parasites, most of which occur in fish. More than 1,250 species have been described from marine, brackish, and freshwater fish species worldwide. Many species of Myxosporea have a narrow host range, but others can affect several species of fish within a family (e.g., whirling disease in salmonids). An interesting feature of the myxosporeans is the presence of alternating hosts in the life cycle of several species that have been studied extensively. In addition to the fish, an invertebrate host (e.g., a tubificid worm in the case of whirling disease) is an essential part of the life cycle producing the actinospore that infects the fish. Important genera affecting fish include *Myxidium, Ceratomyxa, Sphaerospora, Chloromyxum, Parvicapsula, Myxobolus, Henneguya,* and *Kudoa.*

Whirling Disease

Myxobolus cerebralis is the causative agent of whirling disease, a serious condition of both wild and hatchery-reared salmonids. The disease was initially endemic in central and western Europe, but has been spread by movement of infected fish to several other countries including the United States. The obvious sign of tail chasing (whirling) becomes evident about 40–60 d after infection and is caused by erosion of the cranial cartilage, particularly around the organ of equilibrium, by the trophozoite stage of the parasite. In general, only trout less than a few months old when infected go on to exhibit whirling disease because cartilage becomes ossified after a few months making the fish more resistant to neural damage. However, older fish can become infected even though they show no clinical signs. Mortality has varied greatly among outbreaks, sometimes minor, sometimes devastating. The severity of disease among wild and

hatchery fish has been shown to be related to the dose received and the age of the fish at infection.

The complete life cycle of *M. cerebralis* has been established and involves an alternate host, the aquatic oligochaete worm *Tubifex tubifex*. Triactinomyxon spores released by the worm infect the fish skin where the sporoplasm leaves the spore, develops into the trophozoite, and migrates to the developing cartilage where it sporulates to form the typical myxospores observed in diagnostic examinations.

External signs alone are not adequate for diagnosis of *M. cerebralis* infections as the signs mimic those of several other conditions including BCWD and IHN. Traditionally, verification required labor-intensive identification of the myxospore stage in the cartilage of fish heads, a stage that does not appear until several months after infection. The myxospores are ovoidal (front view) or lenticular (in profile), and have two pyriform polar capsules containing filaments at the anterior end. The diagnosis of whirling disease and detection of *M. cerebralis* in both the fish and worm host has been aided greatly by the development of a PCR assay.

Because of the seriousness of whirling disease for both hatchery and wild fish, control and treatment measures must be rigorous. Infected fish should not be stocked and earthen rearing units should be converted to concrete. Hatcheries where whirling disease has occurred should be subjected to complete disinfection of facilities and equipment with high concentrations of such chemicals as sodium hypochlorite or calcium oxide. Allow the treated area to stand for 4 weeks, clean thoroughly, and repeat disinfection. New eggs or fry should be obtained from a known uncontaminated source and raised in spore-free ponds or raceways provided with a pathogen-free water supply.

Henneguya

Several species of *Henneguya* have been described from a wide variety of North American freshwater fishes. *Henneguya* infections are histozoic and localize in specific tissues. Infections may appear as white cysts within the gills, barbels, adipose fins, skin, gallbladder, connective tissue of the head, subcutaneous tissues, or sclera and muscles of the eye.

Spores of *Henneguya* grossly resemble spermatozoa; they possess two anterior polar capsules and an elongate posterior process that may or may not separate along the sutural plane. *Henneguya salminicola* has been found in cysts in the body or musculature of coho, pink, and chinook salmon. Chum salmon also are subject to infection.

In channel catfish, *Henneguya* infections are categorized with respect to the tissue parasitized and the site of spore formation. An intralamellar bronchial form develops cysts within gill lamellae. A cutaneous form causes large lesions or pustules within the subcutaneous layers and un-

derlying musculature of the skin; a granulomatous form causes large tumor-like lesions. An integumentary form causes white cysts on the external body surface. A gallbladder form develops within that organ and may obstruct the bile duct. An adipose fin form localizes solely within the tissue of that fin.

The interlamellar form of *Henneguya* develops spores within basal cells between gill lamellae. This form, in contrast to the intralamellar form, has caused large losses among very young channel catfish. Mortalities of 95% or more among fingerlings less than 2 weeks old have been reported. Loss of respiratory function accompanies acute infections. Fish exhibit signs of anoxia, swimming at the surface of ponds with flared gill opercula. Infected fish are unable to tolerate handling. Most attempts to treat with parasiticides have resulted in additional losses.

As with other myxosporean infections, prevention is the only control measure because no chemical treatment is effective. The disease has been spread from hatchery to hatchery with shipments of infected fingerlings. Confirmation of the interlamellar form in a catfish population may warrant destruction of the infected fish and disinfection of the rearing facilities involved.

Ceratomyxa

Ceratomyxa shasta is a serious myxosporean parasite of salmonids in western North America that causes severe losses of rainbow trout, steelhead, and chinook salmon. Heavy mortalities of adult salmon have occurred just before spawning, and outbreaks have been reported among some wild salmonid populations. Natural infections also have been found in cutthroat, brook, and brown trout, and in chum, coho, pink, sockeye, and Atlantic salmon. The natural life cycle is now known to involve the freshwater polychaete worm *Manayunkia speciosa*, which releases the actinosporean stage that infects fish.

The spores of *C. shasta* are elongated and found in great numbers in the gut and in cysts in the liver, kidney, spleen, and muscle of infected fish. The disease is contracted by adult salmon upon entering infected freshwater and by outmigrating juveniles passing through an area where the infective stage is present. The first signs of infection include lack of appetite, listlessness, and movement to slack water. The fish may darken, shed fecal casts, and the abdomen swell with ascites. Exophthalmia often occurs. The first internal changes appear as small, whitish, opaque areas in the tissue of the large intestine. As the disease progresses, the entire intestine becomes swollen and hemorrhagic. Mortality generally occurs at water temperatures greater than 10°C (50°F).

There is no known treatment for *C. shasta*; however, significant dif-

ferences have been shown in the susceptibility of various stocks of salmonids to infection. Water supplies known to harbor infected fish should not be used for hatchery purposes without pretreatment unless such resistant stocks are used. The diagnosis of *C. shasta* infections in salmonids relies upon the observation of the typical kidney bean shaped mature spores in wet mounts or histological sections. Recently, the development of a PCR assay has greatly facilitated the diagnosis of *C. shasta* infections, especially the pre-spore stages.

Proliferative Gill Disease

In farmed channel catfish alevins, a condition known as "hamburger gill" or proliferative gill disease causes large losses. The parasite *S. ictaluri*, once suggested to be a species of *Sphaerospora*, normally sporulates in the kidney; however, the presence of the pre-spore stages in the gill cause a strong inflammatory response, leading to necrosis of the gill cartilage and hyperplasia of the gill epithelium. When conditions favor the parasite, high mortality can occur from massive loss of gill function. Initial signs are usually loss of appetite and listlessness with increased susceptibility to low oxygen levels. As the infection proceeds, cyst-like lesions can be seen on the gills with the naked eye, giving the gill a characteristic appearance. The disease is most severe in catfish ponds at 16–20°C (60.8–68°F).

Proliferative Kidney Disease

A condition known as proliferative kidney disease (PKD) affects rainbow trout and steelhead, as well as chinook and coho salmon. Infection of fish by the pre-spore stages of the causative parasite, termed PKX, causes massive swelling of the kidney. The disease appears to be an inflammatory response to the parasite; it typically develops in the spring. Mortality is directly affected by water temperature and can be exceptionally high. Initial clinical signs are a swollen abdomen from the highly swollen kidney tissues. Infected fish appear to be more susceptible to secondary infections with bacterial or other pathogens in the water. The inflammatory response leads to a chronic granulomatous response and necrosis of kidney tubules; in severe infections, other organs may also become necrotic. Fish surviving the disease may become normal when water temperatures subside; these fish are then resistant to reinfection. The disease has now been shown to be caused by a *Tetracapsula* species having a bryozoan host. Because mature spores are rarely observed in rainbow trout, some have suggested this species is an abnormal host.

Monogenetic Trematodes

Monogenetic trematode parasites of fish can complete their life cycles on fish without involving other species of animals. Although most are too small to be seen by the naked eye, some species may reach 5 mm in length. The posterior organ of attachment, the "haptor," is used in identification of different genera and species. There often are marginal hooklets around the margin of the haptor and either zero, two, or four large anchor hooks.

Species of the family Gyrodactylidae generally are found on the body and fins of fish, rarely on the gills. These parasites move around freely. The members of this family give birth to live young similar in appearance to the adults. *Gyrodactylus* can be identified by the developing embryo inside the adult, as well as by their lack of eye spots. The haptor has 2 large anchor hooks and 16 marginal hooklets. Diagnosis is made from microscopic examination of wet mounts of fin tissue or skin scrapings. The parasites may occur in large numbers and cause skin irritation and lesions. Fish with large numbers of *Gyrodactylus* may appear listless, have frayed fins, and flash frequently. In ponds, they may gather in shallow water in dense schools.

Dactylogyrus is but one genus of several dactylogyrids commonly found on the gills of warmwater fish. These worms are particularly serious parasites of cyprinids. *Dactylogyrus,* a small gill parasite, can be identified by the presence of 4 eye spots, 1 pair of anchor hooks, and 16 marginal hooklets. No embryos will be found internally, as these worms lay eggs. These parasites feed on blood and can cause serious damage to the gills of warmwater fish when numerous. Clinical signs easily can be mistaken for those caused by an oxygen deficiency or other gill infections.

Cleidodiscus spp. is common on the gills of catfish and a variety of other warmwater fish species. Like *Dactylogyrus,* it has eye spots, but has four large anchor hooks and lays eggs, which may be seen within the adult worm. *Cleidodiscus* is found only on the gills where, when numerous, it causes respiratory problems by severely damaging the tissue. Signs of infection, therefore, are those of gill damage and may be similar to those seen when oxygen is low.

Digenetic Trematodes

Digenetic trematodes require one or more animal hosts, in addition to fish, to complete their life cycles. These parasites can be divided into two major groups: (1) those that live in fish as adults, producing eggs that leave the fish to continue the life cycle, and (2) those that penetrate

the skin of the fish and live in the fish as larvae, usually encysted in the tissue, until the fish is eaten by the final host.

Blood flukes *Sanguinicola davisi* live as adults in arterioles of the gill arches of salmonids and other fish species. These tiny worms lay eggs that become trapped in the capillary beds of the gills and other organs, where they develop into miracidia, which have a characteristic dark eye spot. When fully developed, the ciliated miracidia burst from the gill to be eaten by an operculate snail, the only intermediate host in the life cycle. Cercaria emerge from the snail and penetrate fish to complete the cycle. Control of blood flukes is difficult. It depends upon either continual treatment of infected water supplies to kill the cercaria or eradication of intermediate host snails. In most cases, however, blood flukes are debilitating, but not the cause of serious losses of fish. It is conceivable that large numbers of miracidia leaving the gill at one time could cause a significant loss of blood and damage to the gills. Eggs and developing miracidia also interfere with the circulation of blood in the gill capillaries and in the capillary beds of the kidney and liver.

Copepods

Most copepods in freshwater and salt water are an important part of the normal diet of fish. Certain species, however, are parasitic on fish and the sites of their attachment may become ulcerated and provide access for secondary infections by fungi and bacteria. Crowded hatchery rearing units provide ideal conditions for infestations by copepods because of the dense fish populations and rich environmental conditions.

Argulus spp. have been given the common name of fish lice because of their ability to creep about over the surface of the fish. On first glance, they look like a scale, but on closer examination, are seen to be saucer shaped and flattened against the side of the fish. They have jointed legs and two large sucking discs for attachment, which may give them the appearance of having large eyes. Argulids have an oral stylet that pierces the skin of the host fish. They then inject a cytolytic substance and feed on blood. If these organisms become abundant, even large fish may be killed.

Lernaea spp. are most commonly found on warmwater fish. However, one species, *L. elegans,* lacks host specificity and even attacks frogs and salamanders. Heavy infestations have caused massive mortality in carp and goldfish populations. The parasite penetrates beneath scales and causes a lesion at the point of attachment. The damage caused is associated with loss of blood and exposure to secondary infections by fungi, bacteria, and possibly viruses. *Lernaea* are long (5–22 mm) slender copepods that, when attached, give the appearance of a soft sticks with

two egg sacs attached at the distal ends. Actually, the head end is buried in the flesh. This end has large, horn-like appendages that aid in identification of the parasite.

Fungal Diseases of Fish

Fungi are common in the aquatic environment and are frequently encountered by all fishes at one time or another during their lives. Under cultural conditions, certain fungi can be particularly troublesome. Species of *Acklya, Aphanomyces, Branchiomyces, Leptomitus, Phoma, Pythium,* and *Saprolegnia* have been reported as pathogens. These fungi grow on many types of decaying organic matter and are widespread in nature. Fungi infesting fish or eggs generally are considered to be secondary invaders of tissues damaged by injury; but once they start growing, fungi may spread to healthy tissues and can cause death. Fungi often attack dead fish eggs and will typically spread to the adjacent live eggs, killing them.

Species of the family Saprolegniaceae affect both fish and fish eggs. In fish, *Saprolegnia parasitica* (or other species) is typically a secondary invader of tissues damaged by trauma, infectious disease, or poor environmental conditions. Conditions that suppress the fish's normal immune response (e.g., spawning in adult Pacific salmon or holding warmwater fish in cold water) also can render fish more susceptible to fungal infections. Winter saprolegniosis of catfish is a good example of the latter.

The presence of *Saprolegnia* infections on fish or fish eggs is noted by a white to off-white, cottony growth. This growth consists of a mass of hyphal filaments; these hold the sporangia containing flagellated zoospores that escape to begin infections on other fish or eggs. Unless control measures are taken, the expanding growth ultimately may cover the entire surface of the fish or every egg in the incubator.

Good sanitation and cleanliness are absolutely essential to effective control of fungi under intensive culture conditions. Mechanical and chemical methods have been used for the control of fungal infections on eggs. The mechanical method is used on both salmonid and catfish eggs and involves picking dead and infected eggs at frequent intervals during incubation. This, however, is time consuming, and some healthy eggs may be injured in the process. Chemical control of fungal infections on eggs can be achieved, and formalin is now approved for use to control *Saprolegnia* on eggs of all fish species.

Gill rot, a disease caused by fungi of the genus *Branchiomyces,* is a major threat to fish culture in Europe. Primarily a disease of cyprinids in warmer water, it has been found in trout, bass, northern pike, pumpkinseed, and guppies in the United States. Clinical signs associated with

branchio-mycosis include red-brown coloration of the gills with severe necrosis, fish gasping at the surface, and losses that can exceed 50% in a few days.

A presumptive diagnosis of branchiomycosis can be made by microscopic examination of gill tissue. The red-brown, nonseptate hyphae and round spores of the fungus are seen in surface scrapings or in capillaries and tissue of the gill lamellae. There is no control for branchiomycosis except destruction of infected fish and disinfection of facilities.

An important emerging fungal disease of fish is caused by species of the genus *Aphanomyces* that typically affect fish in marine or brackish waters. In Asia, a disease termed epizootic ulcerative syndrome or EUS is rapidly spreading and causing severe losses among species cultured in brackish water. Initially thought to be caused by viral or bacterial pathogens, the disease is characterized by deep ulcerative lesions that show an intense inflammatory response. In severe cases, internal organs are affected. Similar disease conditions in Australia, termed red-spot disease, and in Japan, termed mycotic granulomatosis, have been described and attributed to *A. invadens*. More recently, an ulcerative mycosis has been associated with losses of marine species in estuaries of the eastern United States, as well as the northwestern Atlantic Ocean.

Diagnosis of *Aphanomyces* infections can be accomplished by light microscopy of scrapings from ulcerative lesions, as well as by culture and the PCR using specific primer sets.

Drugs and Chemicals

Drugs, disinfectants, pesticides, diagnostic reagents, and vaccines are needed to ensure the health and productivity of cultured fish stocks. These regulated products must be used in a manner to avoid risks to public safety and animal health or potential loss of consumer trust. It is the responsibility of everyone using, prescribing, or recommending the use of regulated products to know which products legally can be used under federal, state, and local regulations.

Much of the material in this section was taken (in some places verbatim) from the Web site of the Aquaculture Network Information Center (AquaNIC). This important resource (see electronic references) sponsored by the Joint Subcommittee on Aquaculture should be consulted frequently for current information about the status of drugs and chemicals used in aquaculture because there is a high likelihood that the list of approved products will change frequently. This is due to the conflicting pressures to reduce the use of chemicals released into the environment or of animal drugs that may give rise to antibiotic resistance in human

pathogens and the substantial effort by many supporters of the aquaculture industry to increase the numbers of approved drugs and chemicals for fish.

Legal Framework

Various federal and state agencies are involved in regulating drugs, vaccines, pesticides, and other products used in aquaculture. In the United States, the Food and Drug Administration (FDA) is responsible for ensuring the safety, wholesomeness, and proper labeling of food products; ensuring the safety and effectiveness of human and animal drugs; and protecting consumers from economic fraud. The FDA's regulatory programs are intended to ensure compliance with existing laws, and enforcement activities include actions to correct and prevent violations, remove illegal products or goods from the market, and punish offenders. The testing of domestic and imported aquaculture products for drug and pesticide residues is part of these enforcement activities.

The FDA's Center for Veterinary Medicine (CVM) regulates the manufacture, distribution, and use of animal drugs. CVM is responsible for ensuring that drugs used in food-producing animals are safe and effective and that food products derived from treated animals are free from potentially harmful residues. CVM approves new animal drugs based on data provided by a sponsor (usually a drug company). To be approved, an animal drug must be effective for the claim on the label and safe when used as directed for (1) treated animals, (2) persons administering treatment, (3) the environment, including nontarget organisms, and (4) consumers. CVM establishes tolerances and withdrawal periods as needed for all drugs approved for use in food-producing animals. CVM has the authority to grant investigational new animal drug (INAD) exemptions so that data can be generated to support the approval of a new animal drug.

The Environmental Protection Agency (EPA) is responsible for registering or licensing all pesticides used in the United States under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The FIFRA requires that the EPA register pesticides for specific uses, provided that the use does not pose an unreasonable risk to human health or the environment. Any pesticide sold or distributed in the United States must be registered by the EPA. Products regarded as pesticides include algicides, disinfectants, fish toxicants, and herbicides.

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture regulates all veterinary biologics distributed in the United States. This includes vaccines, biologics, and test kits for

the diagnosis of disease. States may impose additional requirements on the use of veterinary biologics. For example, APHIS requires that state approval for distribution of products be obtained before APHIS authorizes field trials with experimental products or before a conditionally licensed product is marketed in the state.

The FDA and EPA have some areas of mutual regulatory responsibility. A memorandum of understanding provides guidance in the area of aquaculture, particularly as to which agency has jurisdiction over a particular substance for its intended use. The EPA has jurisdiction over disinfectants, sanitizers, and aquatic treatments used solely for the control of algae or bacterial slime and over any other aquatic treatments used solely for pest control that do not include claims for control of parasites or diseases of fish. The EPA or a delegated state regulatory agency also regulates the National Pollutant Discharge Elimination System (NPDES), which controls the discharge of drugs or pesticides into receiving waters. The FDA has jurisdiction over new animal drugs, including products intended to treat or prevent parasites or diseases of fish, anesthetize aquatic species, and alter the sex or regulate the reproduction of aquatic species. The FDA has taken the position that if a pesticide registered by the EPA for aquaculture or aquatic site use is being used properly (i.e., the labeled conditions in fact exist in the facility or site at the time the pesticide is used, and the compound is not misused under FIFRA), the FDA will not object to that proper use even though the pesticide may have a secondary therapeutic benefit.

State departments of agriculture or other designated state agencies may also register federally approved pesticides to permit their legal distribution and sale within a state or territory. States may have additional regulatory requirements, including the provision of further data or additional restrictions on use and licensing. Some states license or impose additional regulations on the use of certain veterinary biologics. Some states may not allow the use of specific products or may require their administration by licensed veterinarians. States also participate in the approval of field trials of veterinary biologics in their respective jurisdictions and in the experimental use of certain veterinary biologics. The use of a drug under an INAD exemption may require approval by a state agency to comply with any local, state, and regional EPA discharge regulations. Discharge approval is intended to ensure that the possible impacts of a discharge (effluent) containing a specific compound or its residues are addressed.

Legal Options for Obtaining Drugs

According to the Federal Food, Drug, and Cosmetic Act, a drug is defined as an article that is intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; an article (other than food) intended to affect the structure or any function of the body of man or other animals; or an article that is recognized in official drug compendia. A new animal drug is a drug intended for animals that is not generally recognized by qualified experts as safe and effective for the uses recommended on the label. At present, no drugs used in aquaculture are considered by FDA to be generally recognized as safe (GRAS) or effective (GRAE) for their proposed uses. For a compound to be classified as GRAS or GRAE, general recognition by experts must be supported by published scientific studies that meet strict FDA standards.

There are several options for legally obtaining and using drugs and chemicals. Use of drugs in a manner other than the options discussed below is subject to regulatory action by the FDA.

1. FDA-approved new animal drugs. Only a few new animal drugs are currently approved by the FDA for use in food-producing aquatic species. Each drug is approved for specific species, for specific disease conditions, and at specific dosages. Refer to Table 1 for a listing of these approved drugs and their approved uses. This list will change occasionally and current information should be obtained from the AquaNIC Web page or other sources.

2. Investigational new animal drugs. These drugs are used under an investigational new animal drug exemption. Investigational new animal drug exemptions are administered by the FDA CVM to allow for the purchase, shipment, and use of unapproved new animal drugs for collection of effectiveness and safety data that will support a decision on drug approval. Numerous requirements must be met for the establishment and maintenance of INADs. An INAD exemption is provided with the expectation that useful data will be generated and submitted to FDA CVM. The Food and Drug Administration authorizes specific conditions of use and monitors all drugs under each INAD exemption. Adequate drug accountability, data recording, and reporting are foremost among the requirements for INAD exemptions. Additional information concerning INAD exemptions may be found on the CVM Web site. It must be stressed that INAD exemptions are granted with the expectation that meaningful data will be generated to support a new animal drug approval and must not be seen as a method of circumventing current law. For a

TABLE 1.   FDA-approved new animal drugs.

Trade name	NADA number	Sponsor	Active drug	Use and species
Chorulon	140-927	Intervet, Inc.	Chorionic gonadotropin	Aid in improving spawning function in finfish.
Finquel (MS-222)	042-427	Argent Laboratories, Inc	Tricaine methanesulfonate	Temporary immobilization (anesthetic) for Ictaluridae, Salmonidae, Esocidae, and Percidae.
Formalin-F	137-687	Natchez Animal Supply Co.	Formalin	Control of external protozoa and monogenetic trematodes in trout, salmon, catfish, largemouth bass, bluegill. Control of fungi of the family Saprolegniacae on salmon, trout, and esocid eggs.
Paracide-F	140-831	Argent Laboratories, Inc.	Formalin	Control of external protozoa and monogenetic trematodes on trout, salmon, catfish, largemouth bass, and bluegill. Control of fungi of the family Saprolegniacae on salmon, trout, and esocid eggs.
Parasite-S	140-989	Western Chemical, Inc.	Formalin	Control of external protozoa and monogenetic trematodes on finfish. Control of fungi of the family Saprolegniacae on finfish eggs. Control of external protozoan parasites on cultured penaeid shrimp.
Romet 30	125-933	Alpharma, Inc.	Sulfadimethoxine and ormetoprim	Control of enteric septicemia in catfish. Control of furunculosis in salmonids.
Sulfamerazine in fish	033-950	Alpharma, Inc.	Sulfamerazine	Control of furunculosis in rainbow trout, brook trout, and brown trout. Comments: According to sponsor, this product is not presently being distributed.

TABLE 1.   (Continued.)

Terramycin for fish	038-439	Pfizer, Inc.	Oxytetracycline	Control of bacterial hemorrhagic septicemia and pseudomonas disease in catfish. Control of gaffkemia in lobster. Control of ulcer disease, furunculosis, bacterial hemorrhagic septicemia, and pseudomonas disease in salmonids. Marking of skeletal tissue in Pacific salmon.
Tricaine-S	200-226	Western Chemical Inc.	Tricaine methanesulfonate	Temporary immobilization (anesthetic) for Ictaluridae, Salmonidae, Esocidae, and Percidae.

listing of current INAD applications, consult the Web page of the National Aquaculture Drug Coordinator (see electronic references).

3. Unapproved new animal drugs of low regulatory priority. Neither an approved new animal drug application (NADA) nor an INAD exemption is required for drugs in this category. Although the FDA is not aware of safety problems associated with the specific uses of these substances, their uses have not been shown to be safe and effective in well-controlled scientific studies. Regulatory action is unlikely if an appropriate grade is used, good management practices are followed, and local environmental requirements are met. Refer to Table 2 for a listing of these compounds. It is unlikely that there will be many additions to this category.

4. Extra-label use of an approved new animal drug. Under the provisions of the Animal Medicinal Drug Use Clarification Act (AMDUCA), licensed veterinarians, within a valid veterinarian-client-patient relationship, can prescribe FDA approved new animal drugs or new human drugs for an extra-label use. Extra-label use of a drug refers to the actual or intended use of an approved new animal drug in a manner not described on the label. This includes, but is not limited to, species, frequency, route of administration, dose, withdrawal time, and conditions of use. This extra-label use is limited to treat modalities when the health of an animal is threatened or when suffering or death of the animal may result if not treated. Extra-label use of FDA-approved new animal drugs or new human drugs that are not prescribed by a licensed veterinarian is a violation of law.

TABLE 2.   Unapproved new animal drugs of low regulatory priority for FDA.

Common name	Permitted use
Acetic acid	Used as a dip at a concentration of 1,000–2,000 mg/L for 1–10 min as a parasiticide for fish.
Calcium chloride	Used to increase water calcium concentration to ensure proper egg hardening. Dosages used would be those necessary to raise calcium concentration to 10–20 mg/L calcium carbonate. Also used to increase water hardness up to 150 mg/L to aid in maintenance of osmotic balance in fish by preventing electrolyte loss.
Calcium oxide	Used as an external protozoacide for fingerling to adult fish at a concentration of 2,000 mg/L for 5 s.
Carbon dioxide gas	Used for anesthetic purposes in cold, cool, and warmwater fish.
Fuller's earth	Used to reduce the adhesiveness of fish eggs in order to improve hatchability.
Garlic (whole)	Used for control of helminth and sea lice infestations in marine salmonids at all life stages.
Hydrogen peroxide	Used at 250–500 mg/L to control fungi on all species and at all life stages of fish, including eggs.
Ice	Used to reduce metabolic rate of fish during transport.
Magnesium sulfate (Epsom salts)	Used to treat external monogenetic trematode infestations and external crustacean infestations in fish at all life stages. Used in freshwater species. Fish are immersed in a solution of 30,000 mg/L magnesium sulfate and 7,000 mg/L sodium chloride for 5–10 min.
Onion (whole)	Used to treat external crustacean parasites and to deter sea lice from infesting external surface of fish at all life stages
Papain	Used as a 0.2% solution in removing the gelatinous matrix of fish egg masses in order to improve hatchability and decrease the incidence of disease.
Potassium chloride	Used as an aid in osmoregulation to relieve stress and prevent shock. Dosages used would be those necessary to increase chloride ion concentration to 10–2,000 mg/L.
Povidone iodine compounds	Used as a fish egg disinfectant at rates of 50 mg/L for 30 min during water hardening and 100 mg/L solution for 10 min after water hardening.
Sodium bicarbonate (baking soda)	Used at 142–642 mg/L for 5 minutes as a means of introducing carbon dioxide into the water to anesthetize fish.
Sodium chloride (salt)	Used as a 0.5–1% solution for an indefinite period as an osmoregulatory aid for the relief of stress and prevention of shock. Used as a 3% solution for 10–30 min as a parasiticide.
Sodium sulfite	Used as a 15% solution for 5–8 min to treat eggs in order to improve hatchability.
Thiamine hydrochloride	Used to prevent or treat thiamine deficiency in salmonids. Eggs are immersed in an aqueous solution of up to 100 ppm for up to 4 h during water hardening. Sac fry are immersed in an aqueous solution of up to 1,000 ppm for up to 1 h.
Urea and tannic acid	Used to denature the adhesive component of fish eggs at concentrations of 15 g urea and 20 g NaCl/5 L of water for

Table 2.   (Continued.)

	approximately 6 min, followed by a separate solution of 0.75 g tannic acid/5 L water for an additional 6 min. These amounts will treat approximately 400,000 eggs.

Note. FDA is unlikely to object to the use of these low regulatory priority substances if the following conditions are met:

1. The drugs are used for the prescribed indications, including species and life stage where specified.
2. The drugs are used at the prescribed dosages.
3. The drugs are used according to good management practices.
4. The product is of an appropriate grade for use in food animals.
5. An adverse effect on the environment is unlikely.

FDA's enforcement position on the use of these substances should be considered neither an approval nor an affirmation of their safety and effectiveness. Based on information available in the future, FDA may take a different position on their use. Classification of substances as new animal drugs of low regulatory priority does not exempt facilities from complying with other federal, state, and local environmental requirements. For example, facilities using these substances would still be required to comply with National Pollutant Discharge Elimination System requirements.

Further Restrictions on Use

New animal drugs that are added to aquaculture feed are subject to FDA regulation and must be specifically approved for use in aquaculture feed. Approved new animal drugs may be mixed in feed only for uses and at levels that are specified in FDA medicated-feed regulations. It is unlawful to add drugs to feed, even under extra-label use provisions, unless the drugs are approved for feed use. The FDA requires that certain mills producing medicated feeds be licensed by the FDA. There are more details on these regulations on the CVM Web site.

Many of the chemicals used in aquaculture are applied directly to water where the FDA or EPA restrictions are determined by the intended use of the product. Although some products may be beneficial when applied to aquaculture systems at low concentrations, they may also act as irritants or even become toxic at higher concentrations. The improper use or application of water treatments can cause severe stress, which can lead to an animal disease outbreak or even death. Some compounds can accumulate in the animal and may cause illegal chemical residues in tissues intended for human consumption. Illegal residues can also result from the improper use of products to control weeds or unwanted fish or to alter water quality. To prevent possible fish losses and illegal chemical residues from excessive treatment levels, always read and strictly follow product label directions.

Product withdrawal times must be observed to ensure that prod-

ucts reaching consumers are safe and wholesome. All federally approved products list any specific required withdrawal times to ensure that products used in aquaculture do not exceed legal tolerance levels in animal tissues. Withdrawal information is found on the product label, package insert, or feed tag. An exception to withdrawal requirements is made for products used in an extra-label manner. Extra-label use may require the same or a different withdrawal time from that listed on the label, depending on the species, treatment, and other conditions. Withdrawal times for the extra-label use of an approved product are not listed on the label and must be determined by the prescribing licensed veterinarian.

Quality Assurance and Record Keeping

Record keeping is a critical element of quality assurance programs. Record keeping may also be required of producers by processors purchasing fish from them to comply with hazard analysis critical control point or HACCP requirements. A good record keeping system helps producers keep track of specific treatments and their results with identifiable, known populations or stocks of aquatic animals, as well as the specific water and land areas involved. Good records provide a basis for sound, cost-effective management decisions. The treatment status of animals, ponds, and other areas is known at all times. Records are needed to determine dosage rates and certify withdrawal times. Processors may require records to demonstrate that all drugs and chemicals have been used properly. Records provide valuable evidence and protection in liability cases. Accurate record keeping also is required for any producer using an INAD exemption in clinical field trials. Quality assurance programs have a high level of participation among many of the segments of the aquaculture industry.

Using Drugs and Chemicals

Product Label

Always read and understand the product label before using any compound. Label directions and information are important for two reasons. First, they describe the conditions of use under which the product can be expected to be effective and safe. Second, labels for approved products describe uses allowed by law. The product label and package inserts provided with regulated products present information on proper storage, mixing, dosage, and administration; date of expiration; diluting or reconstituting the product; safe disposal of the unused product

and product containers; and withdrawal times. Pesticide labels list precautionary statements on environmental, physical, and chemical hazards. Prescribed aquatic-use information is usually not found on the product labels of those substances determined by the FDA to be unapproved new animal drugs of low regulatory priority (LRP) because these substances typically are not marketed specifically for aquaculture use.

Economic Considerations

Drugs, pesticides, and vaccines are used to control or prevent specific diseases, water quality conditions, and pest (e.g., weed) or stress problems. These treatments should be used only when needed. Each treatment has an economic value in terms of treatment cost and expected economic benefit. The proper use of regulated products, some of which are quite costly, can be important in preventing significant economic losses. Such losses are more likely to occur if the actual problem is incorrectly diagnosed, if precautions for treatment are ignored, or if treatments are improperly applied. The use of best management practices in aquatic animal husbandry and water quality maintenance can reduce the use of regulated products. Using regulated products at less than the concentrations or dosages specified on the label can cause the treatment to be ineffective or only partially effective. Of particular concern is the emergence of antibiotic resistant strains of bacteria. Using these compounds at concentrations or in dosages greater than those specified on the label (overdosing or over treating) wastes the product and can cause unwanted side effects, including stress and toxicity problems in aquatic stocks and nontarget organisms, as well as environmental damage. Persons applying regulated products should recognize their legal responsibility for any harm to nontarget aquatic and nonaquatic species and for off-site damage.

Storage and Handling

Always follow label directions for storing, handling, mixing, diluting, reconstituting, and disposing of regulated products and their containers. This preserves the activity and quality of the product and helps prevent misuse, damaging effects on plants and animals, human injury, and environmental contamination. Disinfectants, pesticides, and most drugs should be stored in a locked cabinet in a dry, well-ventilated utility area away from children, animals, food, feed, and living areas. Some drugs and veterinary biologics require refrigerated storage; other products require storage in a freezer or at room temperature. All disinfectants, pesti-

cides, drugs, and veterinary biologics should be stored away from bright light, because light can cause inactivation or deterioration of the product.

Regulated products should be stored in their original containers with the original label attached. Dampness in storage areas can cause paper packages to deteriorate, metal containers to rust, and metal and glass containers to lose their labels. Disinfectants, pesticides, and drugs should not be stored where flooding is possible or in sites where they might spill or leak into the environment. High-temperatures can cause excessive pressure in and bursting of sealed containers. Exposure to high temperatures can also result in product deterioration, shortened shelf life, premature inactivity, and inactivation.

Proper mixing, diluting, and reconstituting are essential for the effectiveness of products requiring such steps, as well as for reasons of safety. Improper dilution may cause the concentration or dosage administered to be too great or too small. Incomplete mixing can cause variations in the concentration or dosage applied or administered, with uneven effects ranging from ineffectiveness to overdose and toxicity. Some veterinary biologics are supplied with diluents that should be used for reconstitution and the proper concentration of materials.

Safety Considerations

The use of any pesticide (and some other regulated products) requires adequate protection from exposure. Users should always read the product label for information on recommended personal protective equipment. Common-sense precautions should be followed, such as wearing gloves, long-sleeved shirts, long pants, socks, shoes or boots, a hat, and goggles, protective glasses, or a face shield. Powders may be harmful or toxic if they are inhaled as dusts; fumes and evaporating ingredients may also be harmful or toxic. Some pesticides may require use of a respirator. Persons mixing or applying pesticide or working in an area where pesticides are being applied or have recently been applied should shower and wash their clothes after actual or possible exposure.

Users should not mix different regulated products unless this is specifically recommended on the label. The combining of products can have many—mostly undesirable—effects. One or both products can be inactivated, and chemical reactions can produce harmful gases or other reaction products and by-products, some of them toxic. Following appropriate precautions can prevent accidental poisoning from pesticide contact with bare skin or from the inhalation of fumes or dust. The pesticide label provides important product-specific information on mixing, diluting, storage, and disposal, as well as on first aid in the event of accidental poisoning. Material Safety Data Sheets, provided by product

manufacturers, are a source of additional information on safety precautions. It is important that unused portions of a regulated product and empty containers be disposed of properly. The best approach is to purchase only the amount of material that will be used within a reasonable time period and to use all of the product for its intended purpose. Empty containers must be disposed of, however, and often a quantity of the product is left over. Product labels provide instructions for safe disposal; these instructions should be followed. Improper disposal can result in toxicity, environmental contamination, and liability problems.

Applicators and persons near treatment areas can be affected by various regulated products through contact, exposure to evaporated material in the air, or exposure to dusts or aerosols. Treated waters or airborne drift can carry restricted products to distant locations, where the products may affect nontarget organisms and sites. Accidental self-injection of some veterinary biologics and injectable drugs can cause local tissue reactions, allergic reactions, or infections. Use of common sense and strict compliance with product label directions can minimize undesirable effects in humans, nontarget plants and animals, and the environment. Seek professional advice when in doubt.

Disease Treatments

In general, it is important to diagnose fish disease problems as early as possible and to begin treatment as soon as practical. If routine disease problems can be recognized early by the hatchery manager, treatment can begin sooner than if a diagnosis is required from a pathology laboratory. Broad-spectrum treatments based on a poor diagnosis are ill-advised, but timely treatments based on keen observation and awareness of signs can mean the difference between losing just a few fish or losing many thousands.

There are two classes of treatments for fish disease—control and therapeutic. Control treatments are measures designed to prevent an outbreak from occurring or increasing in magnitude. Such treatments are used primarily against external parasites, bacteria or fungi. Therapeutic treatments are begun only after clinical signs appear in a significant number of animals. When frequent control treatments are needed for external parasites or bacterial gill disease, it may be an indication of poor hatchery management. The general principle should be to manage first and treat as a last resort. Fish disease treatments are a serious undertaking, and caution should be taken to avoid disastrous results. All drugs and chemicals used to control infectious organisms can be toxic to fish if concentrations are too high. All treatment calculations should be double-checked before being implemented.

Two important principles should be kept in mind regarding the treatment of fish diseases. Firstly, treatment with various medications and chemotherapeutic agents is for the purpose of tipping the balance in favor of the host, not for killing 100% of the disease organisms present. Medications may kill or retard the growth of the pathogen, but in the end, it is the fish's own immune mechanisms that must overcome the disease if the treatment is to be successful. Secondly, there is increasing concern regarding the use of drugs and chemicals in aquaculture. These concerns center around possible environmental harm and the development of antibiotic resistance in aquatic microorganisms that might be transferred to human pathogens. To help allay these concerns, the use of drugs and chemicals should be reduced as much as possible and antibiotic treatments rotated among various compounds and continued for the full treatment period to reduce the development of drug-resistant bacteria.

Treatment Methods

Before making a decision to treat a group of fish, the following questions should be asked:

1. Does the loss rate, severity, or nature of the disease warrant treatment?
2. Is the disease treatable, and what is the prognosis for successful treatment?
3. Is it feasible to treat the fish where they are, considering the cost, handling, prognosis?
4. Is it worthwhile to treat the fish or will the cost of treatment exceed their value?
5. Are the fish in good enough condition to withstand the treatment?
6. Will the treated fish be released or moved soon and is adequate withdrawal or recovery time available?

Before any treatment is started, four factors must be considered. The culturist must know and understand (1) the water supply, (2) the fish, (3) the chemical, and (4) the disease. Failure to take all these factors into consideration can result in a complete kill of all of the treated fish or a failure to control the disease with a resultant loss of many fish and wasted money.

1. *Water supply.* The volume of water in the rearing unit to be treated and the flow rate to the unit must be calculated accurately before any treatment is applied. An overestimation of the water volume or flow rate means too much drug or chemical will be used, which may

kill all the fish. An underestimation of the volume or flow means not enough of the drug or chemical will be used, thus, the disease-causing organism may not be controlled. Often overlooked is the fact that water quality factors, such as total hardness, pH, and temperature, will increase the activity of some chemicals and decrease that of others. In ponds, the amount and type of aquatic vegetation or organic matter also must be taken into consideration before any chemical is applied.

2. *Fish.* Fish of different species and ages may react very differently to the same drug or chemical. Certain species are much more sensitive to a particular chemical than others. The age of fish may also affect how they react to a specific treatment. Regardless of whether or not a particular chemical or drug has been used to treat fish at the hatchery in the past, it is always a good idea to test it first on a small number of fish before an entire pond or holding unit is treated. This can be done in tanks or in small containers such as large plastic wastebaskets.

3. *Chemical.* The toxicity of the chemical should be known for the species to be treated. The effect of water chemistry on the toxicity of the chemical also should be known. Some chemicals break down rapidly in the presence of sunlight and high temperatures and, thus, are less likely to be effective during summer months than during the cooler months of the year. Mixing chemicals may enhance or intensify the toxicity of one of them. Also, certain chemicals are toxic to plants and can cause oxygen depletion if used in ponds at the wrong time.

4. *Disease.* Although the correct diagnosis of a disease may be self-evident, confirmatory testing or a second opinion from a fish health professional is important. Most chemicals used to treat fish diseases are expensive and, generally, are effective only against certain groups of organisms. Use of the wrong chemical or drug usually means that several days to a week may pass before one realizes the treatment was not effective. During this time, large numbers of fish may be lost unnecessarily. In addition, bacterial pathogens may be resistant to one or more antibiotics and this should be determined before treatment is begun.

When it is apparent that a treatment is necessary, the following checklists may be useful:

Pretreatment checklist

1. Accurately determine the water volume, flow rate, and temperature.
2. Accurately determine the number and total weight of fish in the rear-

ing unit.

3. Confirm the identity, purity, age, and stock strength of the drug or chemical to be applied.
4. Double-check concentration figures to be delivered. Beware of co-fusion from mixing metric and English units.
5. Have aeration devices ready for use if needed.
6. Make sure of the route by which chemical solutions are discharged from the holding unit.
7. Test treatment on a pilot group of fish.

Treatment checklist

1. Dilute the chemical with rearing water to the correct concentration before applying it.
2. Ensure the chemical is well-mixed in the units or ponds.
3. Observe fish closely and frequently during treatment for signs of distress.
4. Monitor temperature and dissolved oxygen levels in the rearing unit during treatment (aeration may be required).
5. Discontinue treatment and resume water flow immediately if fish become distressed.

Posttreatment checklist

1. Observe fish frequently for at least 24 h following treatment.
2. Do not stress treated fish for at least 48 h.
3. Recheck fish to determine success of treatment.

Various methods of treatment and drug application have been used in the control of fish diseases. There is no one specific method that is better than others; rather, the method of treatment should be based on the specific situation encountered. Here, experience is exceptionally valuable. A fish health professional or other knowledgeable source should be consulted if one is unfamiliar with the disease or treatment proposed.

Dip Treatments

During the dip treatments, small numbers of fish are placed in a net and dipped in a strong solution of chemical for a short time, usually 15–45 s, depending on the type of chemical, its concentration, and the species of fish being treated. Metal containers should not be used to hold the treatment solution because some chemicals can react with the metal and form toxic compounds, particularly if the water is acidic. This method of treatment is dangerous because the difference between an effective dose and

a killing dose often is very small. Other disadvantages to this method include its high labor costs and stress on the fish due to handling. However, if done properly, it is very effective for treating relatively small numbers of valuable fish.

Flush Treatments

Flush treatments are simple and consist of adding a solution of the treatment chemical at the upper end of a holding unit and allowing it to flush through. It has been used at trout and salmon hatcheries, but is applicable only with raceways, tanks, troughs, or incubators for which an adequate flow of water is available, so that the chemical is completely flushed through the unit or system within a predetermined time. Highly toxic chemicals should be avoided because there is no way to assure a uniform concentration within the unit being treated.

Prolonged Bath

For prolonged-bath treatments, the inflowing water is cut off and the correct amount of chemical is added directly to the unit being treated. After a specified time, the chemical is flushed out quickly with fresh water. This treatment can be used in any unit that has an adequate supply of fresh water that can flush out the treatment within 5–10 min. Several precautions must be observed with this method to prevent serious losses: (1) Because the water flow is turned off, the oxygen concentration of the water may be reduced to the point that the fish are stressed and losses occur to anoxia. The more fish per unit volume of water, the more likely this is to occur. Aerators of some type must be available or installed in the unit being treated to ensure an adequate oxygen supply during the treatment period; (2) In areas with warm weather or intense sunlight, the temperature of the rearing unit may rise significantly putting the fish at risk; (3) Regardless of the treatment time that is recommended, the fish always should be observed throughout the treatment and, at the first sign of distress, fresh water must be added quickly; (4) The chemical must be uniformly distributed throughout the unit to prevent the occurrence of "hot spots" of the chemical. Fish being treated may be killed or severely injured by overdoses if they swim through hot spots. Conversely, fish that avoid these hot spots may not be exposed to a concentration high enough to be effective. The method used for distributing the chemical throughout the unit will depend on the kind of chemical being used, type and size of unit being treated, and equipment and labor available.

Indefinite Bath

Indefinite bath treatments usually are used to treat ponds or other large volumes having low (or no) flow rates. A low concentration of a chemical is applied and left to dissipate naturally. This generally is one of the safest methods of treatment. One major drawback, however, is that large quantities of chemicals may be required that can be expensive to the point of being prohibitive. Another drawback relates to the possible adverse effects on the pond environment. Some treatment chemicals are algicidal or herbicidal and may kill enough plants to ultimately cause an oxygen deficit. Other chemicals, such as formalin, may reduce dissolved oxygen levels as they degrade.

As in prolonged-bath treatments, it is important that the chemical be evenly distributed throughout the culture unit to prevent the occurrence of hot spots. For dry chemicals that dissolve rapidly in water, such as copper sulfate or potassium permanganate, burlap or any coarse-weave bags can be used. The required amount of chemical is put into a bag and towed behind a boat so that the chemical dissolves in the wake of the boat. Liquids and wettable powders can be applied evenly with hand or power sprayers or can be siphoned over the edge of a boat into the prop wash. As with the prolonged-bath method, there is no one correct way to apply a chemical evenly to the unit of water to be treated. Rather, the application will depend on the kind of chemical being used, the equipment available, and the type of unit to be treated. Again, experience is the best guide.

Constant-Flow Treatments

Constant-flow treatments are useful in raceways, tanks, or troughs in situations where it is impractical or impossible to shut off the inflowing water long enough to use prolonged baths. The volume of water flowing into the unit must be determined accurately, and a stock solution of the chemical metered into the inflowing water to obtain the desired concentration. Before the metering device or constant flow siphon that delivers the chemical is started, enough chemical should have been added to the water in the device to give the desired concentration. Upon completion of the desired treatment period, the inflow of chemical is stopped and the unit is flushed by allowing the water flow to continue. The method by which the chemical is metered into the inflowing water will depend on the equipment available and the type of unit to be treated. Although the constant-flow method is very efficient, it can be expensive if large volumes of water must be treated.

Feeding

Treatment of certain diseases, such as systemic bacterial infections and certain internal parasite infestations, requires that the drug be introduced into the fish's body. This usually is accomplished by incorporating the medication in the feed. Treatment by feeding is usually based on body weight; standard treatments are typically given in grams or micrograms of active drug per kilogram of fish per day. Medicated food should be purchased commercially from an approved source. Beginning treatment early is important because once an outbreak is underway, many of the fish may be clinically ill and cease feeding, making it impossible to stop the losses in a timely manner. Once feeding of medicated food is begun, it should be continued for the prescribed treatment period.

Injection

Large and valuable fish, particularly small numbers of them, sometimes can be treated best with injections of medication into the body cavity (intraperitoneal) or into the muscle tissue (intramuscular). Most drugs work rapidly when injected intraperitoneally. For both types of injections, but particularly intraperitoneal ones, caution must be exercised to ensure that internal organs are not damaged. The most convenient location for intraperitoneal injections is the base of one of the pelvic fins. The pelvic fin is partially lifted, and the needle placed at the fin base and inserted until its tip penetrates the body wall. The needle and syringe should be held on a line parallel to the long axis of the body and at about a 45 degree angle to avoid internal organs. One can tell when the body wall has been penetrated by the sudden decrease of pressure against the needle. As soon as the tip of the needle is in the body cavity, the required amount of medication should be injected rapidly and the needle withdrawn. For intramuscular injections, the best location usually is the area immediately ahead of the dorsal fin. The syringe and needle should be held on a line parallel with the long axis of the body and at about a 45 degree angle. The needle is inserted to a depth of about 0.5–1.0 cm and the medication is injected directly into the muscle of the back. The injection must be done slowly, otherwise back pressure will force the medication out of the muscle through the channel created by the needle. For adult salmon broodstock, some drugs (e.g., erythromycin) are injected directly into the dorsal sinus.

Immunization of Fish

Prevention of disease through vaccination is an important adjunct to the maintenance of fish health. In addition to the commercial availability of several new vaccines, it has become increasingly clear that reliance on the use of antimicrobial drugs and chemicals in fish culture will have to be reduced. Firstly, the list of antibacterial drugs and chemicals that can legally be used is extremely small (especially in the United States and Canada) and the effectiveness of the few available antibacterial drugs is being diminished because of the development of antibiotic resistance among bacterial fish pathogens. Secondly, the prospects for enlarging the list are limited due to the extensive testing needed to meet stringent licensing requirements and concerns that antibiotic resistance might be transmissible to microorganisms of public health importance. Finally, none of the viral and many of the bacterial, fungal, or protozoan infections in fish can be successfully treated using the antibiotics or chemicals currently available or anticipated in the near future.

Faced with the foregoing problems, fish culturists should consider other measures that might help to ensure the health of the aquatic animals under their care. One obvious approach is immunization. Advantages of immunization are several. Firstly, immunization does not generate antibiotic resistant microorganisms; secondly, it can be applied to control viral, as well as bacterial diseases; thirdly, fish may be vaccinated economically and conveniently while still very small; and fourthly, protection conferred by vaccination is more durable than that resulting from chemotherapy and persists for a considerable period. Finally, with killed vaccines, at least, the requirements for licensing the vaccines may be less stringent than those required for the registration of antimicrobial drugs.

Initially, the biggest factor working against the widespread use of fish vaccination was the lack of a safe, economical, and convenient technique for vaccinating large numbers of fish. Recent advances have remedied this obstacle and mass-delivery methods are now routinely used. In addition, improvements in adjuvant formulations and the development of multivalent vaccines have led to the widespread use of vaccines for prevention of several of the most important bacterial diseases of fish. Recent advances in the molecular biology of fish pathogens and the development of novel approaches in vaccinology have opened the way for a new generation of fish vaccines that promise further improvements in the ability to protect populations of cultured fish against significant diseases.

Killed Vaccines

The ideal fish vaccine is global in scope (i.e., composed of antigens common to all strains) and capable of stimulating specific, long-lasting, humoral and cellular immune responses that result in solid protection. Traditional fish vaccines have used killed preparations of bacteria, viruses, fungi, or parasites delivered by waterborne exposure or by injection with adjuvants and today, this strategy forms the basis for most of the fish vaccines that have become commercially successful. A new generation of killed vaccines is expected as better delivery systems and new adjuvants become available.

A significant advantage of killed vaccines is their high degree of safety and ease of development. This has also made them relatively easy to license. In fact, the main requirements for autogenous vaccines (killed cultures of a pathogen isolated from animals at an individual farm and restricted to use on that farm) is that they be produced in a licensed facility and used under the supervision of a veterinarian. The disadvantages of killed vaccines are that they are sometimes only effective when delivered by injection in the presence of an adjuvant and that protection is often less solid or of shorter duration than that resulting from natural infections. In addition, killed viral vaccines that must be produced in cell culture are probably not cost-effective for small fish.

Attenuated Vaccines

Attenuated vaccines for fish have been developed by traditional methods of serial passage in culture, by using naturally occurring mutants and cross-reacting strains or by mutagenesis of wild-type organisms. While effective in laboratory trials, acceptance has been slow due to concerns about the release of live organisms into the environment and the perceived potential of the vaccine strain to revert to virulence, to replicate in the fish in unwanted ways, or to infect nontarget species. This means that the testing required for attenuated vaccines can be extensive, making development costs relatively high. In addition, without a genetic marker to distinguish the vaccine strain from natural isolates, diagnostic examinations may be compromised and the vaccine will be difficult to protect commercially. More recently, molecular approaches have been used to circumvent some of these concerns. Following initial development, attenuated vaccines for fish can be produced inexpensively and delivered efficiently by waterborne exposure. Because the attenuated strain replicates in the fish, it will stimulate both humoral and cellular immunity that is often superior to that provided by killed vaccines.

The Next Generation of Fish Vaccines

In the last decade, the techniques of molecular biology have been used to develop a novel set of vaccines for fish. While several of these new approaches have been effective in stimulating specific immunity against challenge in the laboratory, only a few have been tested in field trials, and more work is needed to develop better delivery systems or to overcome potential regulatory concerns. For fish, the most promising of these new approaches include subunit vaccines, recombinant vectors, and genetic immunization.

Subunit vaccines consist of only a portion of a pathogen that will stimulate protective immunity. This is typically a protein that bears a protective antigen or the immunogenic region of such a protein. While subunit vaccines can be created by purification of the native antigen directly from cultures of the pathogen, development of a subunit vaccine by recombinant DNA technology usually involves inserting all or part of the gene coding for the appropriate antigen into a bacterium, yeast, or virus that can produce large amounts of the protein in vitro. Because no infectious agent is present, subunit vaccines are regarded as having a high level of safety, and the cost of producing the vaccine can be quite low after initial research and development costs. Although relatively easy to produce and license, the subunit vaccines that have been tested in fish have generally been less effective than desired. Subunit vaccines can also be made by chemical synthesis of peptides that mimic the antigenic epitope. However, like other subunit vaccines, they are more effective when delivered by injection with an adjuvant and may require coupling to a carrier molecule. Another drawback is that creation of peptide vaccines requires a detailed knowledge of the epitope structure of the protective antigens of the pathogen.

Recombinant vectors make use of DNA technology to insert copies of the genes from protective antigens into a virus or bacterium that will be able to infect the host and replicate without causing disease. While replicating, high levels of the recombinant antigen are produced by the vector that will stimulate the host immune system. One concern is that the method may be somewhat difficult to license owing to the use of a replicating recombinant organism. However, the strategy promises to be highly effective, and several human and animal vaccines are being developed with this approach. A significant advantage of recombinant vectors is that they readily lend themselves to the creation of multivalent vaccines that could stimulate protection against several pathogens simultaneously. Also, because of the replication of the vector, other aspects of host immunity may be co-stimulated.

Genetic immunization by injection of plasmid DNA coding for pro-

tective antigens represents an exciting new area of vaccine development. Demonstrated in laboratory trials to be effective for viral, bacterial, and parasitic diseases of higher animals, the major advantage of this approach is that cells transfected with the plasmid produce the antigen in its authentic form. To the immune system of the animal, these cells appear to be infected and a full immune response is produced resulting in strong, long-lasting protection. Currently, these preparations have to be delivered by injection or by "gene gun" making them either more labor intensive or somewhat less efficient than desired; better delivery methods are needed if this technology is to become widely used in fish. Finally, because this technology is new, the potential for licensing these vaccines is difficult to evaluate; however, most concerns are expected to ease as DNA vaccines are developed for human and animal diseases.

While substantial progress has been made in the molecular biology of fish pathogens and in the construction of novel vaccines that are effective in the laboratory, much remains to be done. The high cost of development and licensing of these vaccines is a major problem, especially if the market for the vaccine is limited to a small geographic area or to a minor species in aquaculture. Other significant problems that need to be overcome are the lack of optimal adjuvants and mass delivery systems for killed and subunit vaccines and an incomplete understanding of critical elements of the fish immune system.

Although it is difficult to predict the future of fish vaccination, recent experience suggests that there will be a trend toward the use of attenuated vaccines, recombinant vectors, and genetic immunization. The genetic engineering of stable attenuations with essentially no likelihood of reversion, no antibiotic resistance genes, and simple genetic markers will provide attenuated vaccines with significantly improved safety and acceptance. The low cost and high efficiency of such attenuated vaccines make them attractive candidates for commercial development. Genetic immunization, likewise, appears highly promising, especially for complex, conformation-dependent epitopes that serve as single protective immunogens; however, improved delivery systems will be required before this method can gain widespread use. Work will also continue on the development of recombinant vectors that can provide mass immunization. Finally, there will be a trend toward use of multivalent vaccines in an effort to lower costs.

Vaccination Methods

Vaccines may be delivered to fish by intraperitoneal or intramuscular injection using a needle and syringe, by oral delivery where the vaccine

is incorporated into the feed, or by direct immersion of fish into the vaccination solution. These methods work well for various commercially available preparations, but further improvements are needed. Not all vaccines are equally efficacious when delivered by these methods and where many small fish must be vaccinated economics dictate that a low-cost method (oral or immersion) must be used. Recent advances in vaccine delivery promise to improve the efficacy of the methods available and to provide additional methods in the future.

Mass inoculation by intraperitoneal or intramuscular injection works well with fish that are 5–10 g or larger, and teams of operators are able to vaccinate several thousand fish per hour. The cost of this delivery method is reasonable for large or highly valuable animals, but the number of fish that can be treated is limited by the manpower available and by technical limitations such as the area available for setting up the anesthetic baths and inoculating tables. In some areas of the world, however, vaccination by injection is practiced on a large commercial scale with substantial efficacy.

Oral immunization has been shown to be effective in the laboratory, but is not yet used commercially. The method is low cost and will be the preferred method for the future. One difficulty has been that the level and duration of protection offered by oral immunization has typically been less than that offered by injection or immersion methods. Microencapsulation of the vaccine to avoid inactivation by the low pH in the stomach and other methods to improve uptake of the antigens in the lower intestine offer new approaches that will improve efficacy of preparations delivered by this method.

The immersion method allows vaccination of several thousand fish quickly and safely in a short period. Weighed volumes of fish are lightly anesthetized and placed briefly into a solution of commercially prepared vaccine diluted in the rearing water. The vaccine is taken up by exposed tissues in the skin, gill, mouth, or anus and transported to cells that process the antigen and stimulate the immune response. Most of the commercially successful vaccines are killed bacterial preparations (bacterins) that can be effectively delivered in this manner.

Today, monovalent and multivalent bacterins against several fish pathogens are licensed for commercial use in the United States (Table 3). While these are highly effective, additional vaccines are needed to prevent other important diseases in aquaculture. Following the relatively rapid development and successful application of the bacterins listed, creation of additional vaccines has proven more difficult than hoped. This has probably been due to the failure of these newer preparations to stimulate the appropriate protective responses in the fish and the need to vaccinate large numbers of small animals economically. Recent re-

TABLE 3. Bacterins and vaccines.

Trade name	Product name	USDA product code	Licensee	Species	Disease
Alphadip 1100, Alphaject 1100	Yersinia Ruckeri Bacterin	2638.01	Alpharma, Inc.	Salmonids	Yersiniosis (enteric redmouth disease)
Alphadip 1100C	Yersinia Ruckeri Bacterin	2638.00	Alpharma, Inc.	Salmonids	Yersiniosis (enteric redmouth disease)
Alphadip 2100	Vibrio Anguillarum-Ordalii Bacterin	2858.02	Alpharma, Inc.	Salmonids	Vibriosis
Aquavac-ESC	Edwardsiella Ictaluri vaccine	1531.00	Intervet, Inc.	Catfish	Enteric septicemia
Autogenous bacterin	Autogenous Bacterin	2051.00	Alpharma, Inc.	Fish	Bacterial diseases
Autogenous bacterin	Autogenous Bacterin	2051.00	Jerry Zinn, Aqua Health, Ltd. (Canada)	Fish	Bacterial diseases
Autogenous vaccine	Autogenous vaccine	1015.00	Alpharma, Inc.	Fish	Viral diseases
Autogenous vaccine	Autogenous vaccine	1015.00	MicroTechnologies, Inc.	Fish	Viral diseases
Biojec 1500	Aeromonas Salmonicida Bacterin	2035.00	Alpharma, Inc.	Salmonids	Furunculosis
Biojec 1800	Aeromonas Salmonicida-Vibrio Anguillarum Bacterin	2037.00	Alpharma, Inc.	Salmonids	Furunculosis, vibriosis
Biojec 1900	Aeromonas Salmonicida-Vibrio Anguillarum-Salmonicida Bacterin	2137.00	Alpharma, Inc.	Salmonids	Furunculosis, vibriosis
Biovax 1200	Vibrio Salmonicida Bacterin	2870.00	Alpharma, Inc.	Salmonids	Vibriosis
Biovax 1600	Vibrio Anguillarum-Salmonicida	2868.00	Alpharma, Inc.	Salmonids	Vibriosis
Ermogen	Yersinia Ruckeri Bacterin	2638.00	Jerry Zinn, Aqua Health, Ltd. (Canada)	Salmonids	Yersiniosis (enteric redmouth disease)
Escogen	Edwardsiella Ictaluri Bacterin	2637.00	Jerry Zinn, Aqua Health, Ltd. (Canada)	Catfish	Enteric septicemia
Fryvacc	Flavobacterium Columnare Bacterin	2974.00	Jerry Zinn, Aqua Health, Ltd. (Canada)	Salmonids	Columnaris disease

Table 3.   (Continued.)

Furogen	Aeromonas Salmo-nicida Bacterin	2035.01	Jerry Zinn, Aqua Health, Ltd. (Canada)	Salmonids	Furunculosis
Furogen 2	Aeromonas Salmo-nicida Bacterin	2035.00	Jerry Zinn, Aqua Health, Ltd. (Canada)	Salmonids	Furunculosis
Furogen B	Aeromonas Salmo-nicida Bacterin	2035.02	Jerry Zinn, Aqua Health, Ltd. (Canada)	Salmonids, Koi carp	Furunculosis, ulcer disease
Lipogen forte	Aeromonas Salmo-nicida-Vibrio An-guilarum-Ordalii-Salmonicida Bacterin	2138.01	Jerry Zinn, Aqua Health, Ltd. (Canada)	Salmonids	Furunculosis, vibriois
Lipogen triple	Aeromonas Salmo-nicida-Vibrio An-guilarum-Ordalii-Salmonicida Bacterin	2138.00	Jerry Zinn, Aqua Health, Ltd. (Canada)	Salmonids	Furunculosis, vibriosis
Multivacc 4	Aeromonas Salmo-nicida-Vibrio An-guilarum-Salmo-nicida Bacterin	2137.00	MicroTechno-logies, Inc.	Salmonids	Furunculosis, vibriosis
Vibrogen	Vibrio Anguillarum-Ordalii Bacterin	2858.02	Jerry Zinn, Aqua Health, Ltd. (Canada)	Salmonids	Vibriosis
Vibrogen-2	Vibrio Anguillarum-Ordalii Bacterin	2858.03	Jerry Zinn, Aqua Health, Ltd. (Canada)	Salmonids	Vibriosis

search on the fish immune system and the powerful methods of molecular biology to produce recombinant antigens and DNA vaccines offer hope that a new generation of vaccines can be created to prevent the most common diseases of fish.

Fish Disease Policies and Regulations

The last 25 years have seen significant changes in disease control with the emphasis shifting from treatment to prevention. International, national, and regional regulations have been passed to minimize the spread of certain contagious diseases of fish, and both agricultural authorities and fisheries agencies have increased their emphasis on protecting the health of aquatic animals raised by both the public and private sectors.

The Office International des Epizooties (OIE) in Paris, France, is the

world animal health organization charged with developing guidelines and procedures for reducing the spread of infectious animal diseases on behalf of more than 150 member countries. In 1960, the OIE created the Fish Diseases Commission in recognition of the increasing importance of aquaculture and the potential for the international spread of important diseases of aquatic animals. The OIE publishes an international aquatic animal code and diagnostic manual for listed diseases of aquatic animals. Member countries are encouraged to use these guidelines to establish disease-free zones and farms in order to reduce the risk of spreading diseases associated with international shipment of aquatic animals. Many of the OIE materials are available via the Internet (see electronic resources at the end of this chapter).

Following creation of the European Community (EC), a series of directives was issued, beginning in 1991, to prevent the introduction and spread of aquatic animal diseases among member states. These directives (91/67, 91/492, 91/493, 92/53, 93/54) specify testing methods and control measures to be applied to fish and shellfish products to be imported into the EC. It is expected that these directives will be updated regularly to ensure that they are consistent with the guidelines of the OIE.

In the United States, the Code of Federal Regulations, Title 50, Chapter 1, Part 16, Injurious Wildlife, was amended in 1993. The revised text states that salmonids and their eggs can only be imported into the United States if accompanied by a certificate stating the shipment is free of the viral pathogens OMV, VHSV, IHNV, and IPNV. Salmon that have been eviscerated, processed by certain methods, or captured commercially in the open sea are exempt. The director of the U.S. Fish and Wildlife Service maintains a list of persons who are authorized to conduct the specified inspections and issue the required certificates. In addition, all live salmonid eggs imported into the United States must be disinfected within 24 h before shipment by immersion for 15 min in an iodophor solution containing at least 75 ppm active iodine. These regulations will also require regular updating.

In 1997, Canada amended the federal Fish Health Protection Regulations that address international and interprovincial movement of salmonids and their eggs. The Canadian regulations deal with all species and hybrids of fish in the family Salmonidae. Live fish to be imported into Canada or shipped between provinces must be tested and found free of any filterable replicating agent able to cause cytopathic effects in specified cell cultures including, but not limited to, VHSV, IHNV, and IPNV. Also listed are the bacterial pathogens *Aeromonas salmonicida* and *Yersinia ruckeri* and the protozoan pathogens, *Myxobolus cerebralis* and *Ceratomyxa shasta*. Live eggs must be tested for viral agents and surface disinfected

before shipment. Dead, uneviscerated fish must be tested and found free of VHSV and *M. cerebralis*. More recently, several other countries have begun to draft regulations affecting the importation or internal movement of aquatic animals. An important goal for the future is to ensure these regulations are harmonized.

Within the United States, disease-control programs are also administered by the Colorado River Wildlife Council, the Great Lakes Fishery Commission, the Pacific Northwest Fish Health Protection Commission, the U.S. Fish and Wildlife Service, several tribes, and numerous states. Most of the state, regional, and national programs include regulations to restrict the movement of fish unless inspected and found free of certain pathogens. While these programs represent important cooperative efforts among the various management agencies, there is a lack of agreement among the various entities as to the pathogens and host species for which inspections must be conducted. It will be important to make the list of pathogens, host species, and inspection methods consistent among the various states and regions of the United States in order to develop a more uniform national program in fish health.

Fish Disease Control Programs

A fish disease control program should emphasize all aspects of good health, including infectious diseases, nutrition, physiology, and environment. The first step of any program must be the establishment of long-range goals. These goals may be broad in concept or may dictate eradication of specific pathogens. The latter is more difficult to achieve and it is possible to have disease control without pathogen eradication.

After the goals of disease control have been established, it is necessary to design a policy that is compatible with other fishery resource priorities. The backbone of the policy should be a monitoring program that will determine the range of serious fish pathogens and detect new outbreaks of disease. Control and containment of fish diseases require the periodic examination of hatchery populations, as well as fish that are free-ranging in natural waters. The health of hatchery fish extends beyond their cultural confinement to natural populations, which they affect either through direct contact or via the effluent from hatchery operations. A monitoring program should include

1. Fish health laboratories equipped to test for disease agents, nutritional deficiencies, and tissue residues;
2. A corps of competent, qualified individuals trained in inspection and laboratory procedures;
3. A training program in fish health for all persons involved in fish

husbandry;

4. Agreements between various government agencies and private groups to establish lines of communication, as well as the storage and cataloging of data derived from the monitoring program;

5. Specific guidelines for laboratory procedures to be followed and for qualifications of persons and laboratories doing the inspections and testing;

6. The development of specific steps for disease reporting and a list of reportable pathogens; and

7. Preplanned courses of action that will be used to control or eradicate a reportable disease when it occurs.

The U.S. Fish and Wildlife Service has established a national program for fish disease control administered through a series of regional fish health centers. The plan provides for determining the distribution of specific pathogens within the National Hatchery System, restricting dissemination of fish pathogens, and eradicating certain disease agents from federal fish hatcheries. The policy also provides for research and training in epidemiology, prevention, control, and diagnosis of various fish diseases. Recently, the agency has initiated a comprehensive national wild fish health survey. Many states, tribes, and regions have developed similar disease control programs for hatchery facilities under their authority.

The Fish Health Professional

Initially, those knowledgeable about matters of fish health were typically hatchery managers or workers who developed an interest in disease diagnosis and treatment in order to be more successful at rearing aquatic species in captivity. In addition, a small cadre of academics was interested in fish parasites and pathogens as objects of study. Eventually, through personal contact and training, usually provided by academic institutions or federal laboratories (e.g., the "short" and "long" courses in fish health taught at the Leetown laboratory), a corps of fish health experts developed who could assist hatchery managers in diagnosis of infectious disease and recommend appropriate treatment.

Today, the fish health professional may be a veterinarian with specialized training in aquatic animal medicine; an academic with research expertise in infectious diseases of fish; a state, federal, or tribal fish biologist with specific training in fish health from various sources; and others who have an interest in this expanding field. The Fish Health Section (FHS) of the American Fisheries Society maintains a certification program for fish pathologists and fish health inspectors who have the required training and experience to provide expert advice and to con-

duct fish health inspections. These professionals often work for federal, tribal, or state agencies in facilities that are specialized for work with fish diseases. Several universities and private individuals have also established laboratories that can help in disease diagnosis and provide inspection services to the private aquaculture industry.

Fish Health Inspections

During an on-site fish health inspection at a hatchery, the fish health professional will collect random samples of fish tissues for analysis. The tests to be conducted will vary according to the type of testing requested and should follow the standardized procedures of the most recent editions of the Fish Health Section of the American Fisheries Society "Bluebook" officially titled *Procedures for the Detection and Identification of Certain Fish and Shellfish Pathogens*, the *Fish Health Protection Regulations, Manual of Compliance* published by the Department of Fisheries and Oceans, Canada, or the *Diagnostic Manual for Aquatic Animal Diseases* published by the Office International des Epizooties in Paris, France.

The inspector typically takes tissues from a specified number of fish from each population at the hatchery. In most cases, each fish sampled must be killed. The minimum sample size from each population will follow a statistical plan that provides a 95% confidence for including an infected fish in the sample where the incidence of infection is equal to, or greater than, a specified level, usually 2 or 5%. The inspector will appreciate some advance preparation on the part of the hatchery manager and assistance in providing equipment needed to sample the rearing units and a place to work.

The sample population is determined on the basis of hatchery variables such as species, age, and water source. Generally, fish are sampled by "lot." A lot being defined as fish of the same species, originating from the same spawning population that have always shared the same water supply. Thus, when fish are held in different water supplies, each group has to be sampled as a separate population. All broodstock of the same species held in a single water supply can be considered one population. Broodstock are usually inspected during the period when eggs are being obtained.

All fish on hand at the time of inspection constitute the population and are sampled accordingly. Samples are collected from each tank or rearing unit. Suspect fish (moribund specimens) are collected along with healthy individuals. Fish should be alive when collected. Necropsy procedures assume that the same fish may provide tissues for the various laboratory tests (bacterial, viral, and parasitic). A modified procedure

may be required for very small fish. Material to be examined for external parasites must be taken before any antiseptic or disinfectant procedures are applied. After the body has been opened aseptically, tissues for bacterial cultures and virus tests are collected. These tissues should be refrigerated or stored on ice and must not be frozen. Finally, any histological samples are taken and placed in fixative.

At least 2 weeks are required for the laboratory analyses to be completed. Upon completion of the tests, a certifying official will issue a report specifying the samples taken, the laboratory tests conducted, and the findings. The exact type of report can vary according to the governmental agency involved and the circumstances of the inspection.

Diagnostic Examinations

Correct diagnosis depends upon accurate and detailed information regarding the fish, the signs observed, the history of the facility, and the conditions under which the fish were raised. The more information that is available, the more likely that the diagnosis will be correct. If space permits, a small area of the hatchery should be devoted to fish health examinations. This space can be used by hatchery personnel to monitor levels of external bacteria or parasites and to perform standard microbiological procedures. The space will also be needed by a fish health professional conducting inspections or diagnostic examinations. In addition to being dry and well-lit, the space should have a bench or table for preparation of specimens, a compound light microscope and various slides, stains, incubator, culture media, and small equipment that may be needed to perform on-site diagnostic tests.

Even after a preliminary diagnosis by the hatchery staff, a fish health professional should be contacted who may visit the hatchery or request that specimens be sent to the laboratory for verification. Although the symptoms may seem typical, another disease may be present and while treatments may be effective for one condition, the other disease may still be uncontrolled. Hatchery personnel should furnish the fish health professional with correctly collected and handled material, including all available information, at the earliest possible date. To assist in obtaining a correct diagnosis, specimens should be collected before any treatment is given or started. Only a few fish need be collected for on-site examination or sent for examination, but these should be collected with the utmost care. Dead fish or fish that appear to be normal are nearly worthless. The most desirable fish are those that show most typically the signs of the disease in question. Moribund, but still living, fish are the best for diagnostic purposes.

Shipping Live Specimens

When it is necessary to ship live specimens for diagnostic purposes, (1) assure that everything possible is done to ensure that the specimens will be received alive, and (2) take extra precautions to ensure that other parcels will not be damaged by water leakage. Shipments should bear the notation "Live Fish—This Side Up" and should be sent by the most rapid means possible.

If air express shipments are needed, packing should allow for gas expansion that occurs at altitude. Plastic bags containing about one-fourth water and half or less air or oxygen usually provide room for expansion. A general precaution is to use a double bag system, one bag filled and sealed within another. It is best to ship a minimum number of specimens. Sick fish and coldwater species, such as trout, require greater volumes of water than healthy or warmwater fish. Twenty volumes of water for each volume of fish usually will be adequate for healthy fish, but greater volumes should be provided for sick fish. During extreme hot or cold weather, insulated containers may be required. Coldwater species usually ship better if ice is provided. The ice should be packed in double plastic bags so that it will not leak when it melts, especially if the ice is made with chlorinated city water and placed in the water containing the fish.

Shipping Preserved Specimens

Preservatives typically are corrosive and odorous. Containers should be unbreakable and absorbent material should be provided in the event leakage occurs. A good procedure is to fix the fish in a proper fixative for a day or two, then place the preserved fish, with a very small volume of fixative, in a plastic bag. The sealed bag should be placed within a second plastic bag, which also should be sealed. This durable package has minimal weight. Select representative specimens. Examine them carefully and record relevant data. Buffered formalin or Davidson's fixatives are preferred to Bouin's solution that contains picric acid that may become explosive when improperly stored.

The volume of the fixative should be at least five to ten times that of the fish or tissue. Fish and tissues should be left in the fixative for at least 24 h and then the fixing solution replaced with 70% ethyl alcohol. However, if alcohol is not available, retain the specimens in fixative. To facilitate fixation, fish should be slit down the abdomen from the anus to the gills. The air bladder should be broken to permit fixation of the kidney. The kidney of fish 15 cm or larger should be split along its entire length. The intestines and other organs should be slit if the fish are larger than

fingerlings. It also is desirable to cut the skin along the back of the fish. If the fish are larger than 15 cm, the cranial cap should be opened to facilitate fixation of the brain. The importance of these incisions cannot be overemphasized. If the fish are too large to ship whole, cut pieces from individual tissues (gill, heart, liver, etc.), and especially any lesions observed. These pieces should not be larger than 1 cm square and 0.5 cm thick.

Commercial formalin also can be used for preserving specimens and should be mixed with nine parts of water to make a 10% formalin solution. Unless the lesions are very clear and obvious, always preserve several healthy specimens of the same size and age as the sick fish and send them at the same time in a separate container.

Decontamination of Equipment and Facilities

Equipment Decontamination

Equipment sometimes must be decontaminated. One of the best and cheapest disinfectants is chlorine. A solution of 200 ppm will be effective against most pathogens in less than a few minutes while one of 10 ppm may require up to several hours for sterilization. Chlorine levels are greatly reduced by organic material such as mud, slime, and plant material; therefore, it is important to thoroughly clean equipment before it is exposed to the solution. A chlorine solution also loses strength when exposed to the air or sunlight, so it may be necessary to add more chlorine or make up fresh solutions during disinfection.

Chlorine may be obtained as sodium hypochlorite in either liquid or powdered form. The latter is the more stable of the two, but it is more expensive. The amount of chlorine added to water depends on the percentage of available chlorine in the product used. As an example, powdered chlorine may contain either 15, 50, or 65% available chlorine, while liquid solutions typically contain from 5.6% (commercial laundry bleach) to 10% available chlorine. The following amounts would be needed to make a 200 ppm solution:

- 100 g (3.53 oz) of 15% available chlorine powder to 75 L (19.8 gal) of water;
- 100 g (3.53 oz) of 50% available chlorine powder to 250 L (66.04 gal) of water; and
- 100 ml (3.4 fl. oz) of 10% available chlorine solution to 50 L (13.21 gal) of water.

Chlorine is acutely toxic to all life. In addition to insuring protection

to workers applying the material, residual chlorine must be completely neutralized before it is allowed to drain or to enter waters containing fish. One hundred liters (26.4 gal) of 200 ppm chlorine solution can be neutralized by 150 g (5.3 oz) of sodium thiosulfate. Neutralization can be easily determined with chlorine test paper or with a standard swimming pool test kit.

Facility Decontamination

With the increase in global trade, the risk of importation of exotic diseases has become an increasingly important threat. Also, the expansion of aquaculture has led to the emergence of new diseases for which control methods are not established. For those diseases that presently cannot be treated (e.g., most viral diseases), the only successful control is complete elimination of all infected fish from a hatchery, thorough decontamination of the facility, development of a new stock of disease-free fish, and maintenance of disease-free conditions throughout all future operations. Hatchery decontamination has been successful in removing pathogens; however, this method is practical only at those hatcheries having a controlled water supply originating in wells or springs that can be kept free of fish or a water supply treated with ozone or ultraviolet light to render it pathogen-free.

Preliminary Operations

Before chemical decontamination of the hatchery is started, a quarantine zone should be established with strict entry and exit controls. This may involve decontamination of all vehicles leaving the facility. The volumes of all incubators, troughs, raceways, or ponds must be known or measured accurately. The areas of all contaminated surfaces in the buildings are calculated, and allowance is made for disinfectant solution to be applied on walls and floors. Then, the quantity of sodium hypochlorite needed for a 200 ppm solution is computed. If the chlorine solution will enter fish-bearing waters after leaving the hatchery, it will have to be neutralized. Commercial sodium thiosulfate, used at the rate of 1.5 g for each liter of 200 ppm chlorine solution, will suffice.

All loose equipment should be brought from storage rooms, scrubbed thoroughly with soap and warm water, and left near a raceway for later decontamination. Such equipment includes nets, buckets, pans, small troughs, tubs, screens, seines, boots, and rain gear. During this operation, any worn-out, unnecessary, or difficult to disinfect equipment (e.g., wooden dam boards) should be burned or otherwise destroyed. The walls

of all raceways and rearing units should be scrubbed and the bottom raked. Particular attention should be given to removing any remaining fish food, pond scum, or other organic substances having a high chlorine demand.

Elimination of Fish

Prior to chemical decontamination, all fish should be destroyed by deep burial and covered with lime. The burial grounds should be so located that leaching cannot recontaminate the hatchery water supply. All stray fish left in pipelines will be killed by chlorine, but it is important that their carcasses be retrieved and destroyed.

Decontamination

Decontamination methods should assure that the full strength (200 ppm) of the chlorine is maintained for at least 1 h and that a concentration of not less than 100 ppm is maintained for several hours. Many hatcheries are so large that total decontamination cannot be completed in one day. Treatment then must be carried out by areas or blocks and started at the upper end of the hatchery.

Before chlorine is added, all ponds, raceways, tanks, and troughs are drained. Additional dam boards, plugs, or caps are installed to hold the water to the very top of each unit. The required quantity of chlorine then is added gradually to the incoming water. The solution flows to the various rearing units, which are allowed to fill and overflow until there is about 5 cm of the chlorine solution on the floor. The incoming water then is turned off or bypassed. The chlorine solution is pumped from the floor and sprayed on the sides and bottoms of all tanks and racks, the walls and ceiling, head trough, and any other dry equipment for 1 h. The same procedure must be used in all rooms of every building. Underground pipelines must be filled and flushed several times. If the hatchery must be decontaminated in sections, the work should be planned and timed so that all buildings, springs, supply lines, and rearing units contain the maximum level of chlorine at the same time, so that no contaminated water can enter parts of the system already treated. While a maximum concentration of chlorine is being maintained, all loose equipment such as pails, tubs, trays, splashboards, and other material may be immersed in the raceways.

Throughout the course of the project, checks should be made on the approximate chlorine strength with a chlorine test kit or chlorine test

paper. If any section holds a concentration below 100 ppm chlorine after 1 h, the solution should be fortified with additional chlorine.

Hatchery Maintenance

After a hatchery has been decontaminated and is pathogen free, recontamination must be prevented. The movement of live fish into the hatchery should only originate from known disease-free stocks at a facility with a strict disease inspection program. Ideally, production should be restarted with disinfected eggs from a certified pathogen-free source. The spread of disease can be prevented only by rigid cleanliness. All hatchery equipment should be double-checked to ensure it has been decontaminated thoroughly before it is used and strict internal quarantine procedures must be followed. At some facilities, this may include the decontamination of vehicles and equipment before they are allowed to enter the hatchery. Finally, the hatchery staff should be impressed with the idea that one mistake may nullify all previous efforts.

Acknowledgments

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Electronic Resources

American Fisheries Society	http://www.fisheries.org
American Veterinary Medical Association	http://www.avma.org
Aquaculture Health Page	http://www.geocities.com/ CapeCanaveral/Lab/7490/ index.html
Aquaculture Network Information Center	http://aquanic.org
Aquarium Aqualink	http://aqualink.com
Center for Veterinary Medicine	http://www.fda.gov/cvm
Fishlink	http://fishlink.com