GREAT LAKES FISHERY COMMISSION

2002 Project Completion Report[1]

A Guide to the Integrated Management of Warm-water and Cool-water Fish Diseases in the Great Lakes Basin

by:

J.A. Plumb[2]

[2]Auburn University
Department of Fisheries
and Allied Aquacultures
Auburn, Alabama
36849

February 2002

[1]Project completion reports of Commission-sponsored research are made available to the Commission's Cooperators in the interest of rapid dissemination of information that may be useful in Great Lakes fishery management, research, or administration. The reader should be aware that project completion reports have not been through a peer review process and that sponsorship of the project by the Commission does not necessarily imply that the findings or conclusions are endorsed by the Commission.

A Guide to the Integrated Management of Warm-water and Cool-water Fish Diseases in the Great Lakes Basin

Prepared by:
John A. Plumb[1], Department of Fisheries and Allied Aquacultures
Auburn University, Auburn, Alabama 36849

Presented to:
Great Lakes Fishery Commission Fish Health Committee at its Annual Meeting, February 25, 2002, Minneapolis, Minnesota

1. Current address: 1203 Nixon Ave., Auburn, Alabama 36830
(334 821-1881; jplumb@acesag.auburn.edu)

Foreword

Fish diseases have been of concern to aquaculture and natural resource agencies in the United States and Canadian provinces contiguous to the Great Lakes for many years. The Great Lakes Fishery Commission has been mandated the responsibility to care for and protect the fishery resources in the Great Lakes basin. To aid in carrying out this mandate *A Guide to Integrated Fish Health Management in the Great Lakes Basin* edited by F. P. Meyer, J. W. Warren, and T. G. Carey was published in 1983 and emphasized diseases of salmonids. *A Guide to Integrated Management of Warmwater and Cool-water Fish Diseases in the Great Lakes Basin* is offered as a companion to the 1983 publication and provides a holistic management approach to warmwater and cool-water diseases.

Acknowledgements

I wish to thank John Hnath, Paul Bowser, John Schachte, Brian Souter, and Bill Rogers for reviewing all or parts of the manuscript and providing valuable suggestions for its improvement. Thanks are also expressed to Iowa State University Press for allowing extraction of material from "*Health Maintenance and Principal Microbial Diseases of Cultured Fishes*" by J. A. Plumb and published in 1999.

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Introduction

The aquaculture industry in North America and throughout the world has increased significantly during the last three decades and no segment has grown more dramatically than the culture of warmwater fishes. Channel catfish culture has been responsible for much of the expansion in North America, but the culture of tilapia, striped bass, largemouth bass, walleye, sturgeon, and other species has become increasingly popular. In the past, most warmwater aquaculture occurred in ponds, however, due to a greater demand for fish and the limited availability of land and water, use of intensive cage, raceway, and recirculating culture systems are on the rise. While channel catfish do not readily adapt to raceway and recirculating systems, tilapia and striped bass (and striped bass X white bass hybrids) do adapt well. Mechanically heated water now allows intensive culture of species in climates previously not suited to conventional production. As a result of more intensive aquaculture an increase in acute fish health problems has been noted and previously unknown pathogens are emerging (Plumb 1999).

Growth and expansion of aquaculture involving the movement of fish within North America and internationally may result in an inadvertent transfer of disease agents from one local to another (Moffitt et al. 1998). Newly introduced diseases may adversely affect indigenous fishery resources; whereas, enzootic disease agents may also affect newly introduced fish. Only recently has transferring warmwater and cool-water fish diseases through interstate, interregional or international trade become an issue. This can be attributed to a lack of concern for infectious diseases on the part of many warmwater aquaculturists; a belief that diseases are inevitable and nothing can be done to prevent them; many pathogens that infect warmwater fish are ubiquitous; non-obligate pathogens occur in all surface waters; and most warmwater and cool-water aquaculture facilities have open water supplies that are not conducive to disease eradication.

Available drugs to treat fish diseases are limited and the outlook for future drug development is not promising because of public health constraints and the fact that some pathogens become antibiotic resistant. Therefore, most infectious fish disease control must be approached through best management practices which involves the use of improved feeds, maintaining a high quality aquatic environment, moderated stocking densities, improved methods of fish handling and transport, vaccination, and judicious use of chemotherapy as prophylaxis and curative treatment. Also, selective breeding and genetics, developing specific disease free stocks,

and controlling the spread of selected diseases through stringent regulations concerning the importation of exotic disease agents into a region, and controlling the spread of certain existing diseases within a specific region must also be taken into consideration.

The objective of this guide is to present integrated management practices for warmwater and cool-water fish diseases, address current diseases of these fishes in the Great Lakes basin, discuss which pathogens may be introduced through importation and how they can affect fishery resources of the region, and to propose amendments to the current Great Lakes Fish Disease Control Policy and Model Program to include certain disease agents of warmwater and cool-water fishes. Part I addresses principles of disease management, general epizootiology, chemotherapy, vaccination, and recognition of diseases. Part II discusses individual disease distribution, disease signs, pathogen characteristics, methods of detection, species susceptibility, epizootiology, management, and the significance of each disease to the Great Lakes basin. Part III, the Appendix, includes suggestions on which diseases are of greatest concern and should be melded into the current Great Lakes Fish Disease Control Program.

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Part I

Management

1. Principles of Fish Health Management

2. Epizootiology

3. Chemotherapy

4. Vaccination

5. Disease Recognition and Diagnosis

Chapter 1. Principles of Fish Health Management

Fish health management is an “applied art” or “science”, depending on one’s perspective, or a melding of the two. Fish health management, or best management practices, through environmental manipulation may not totally eliminate infectious disease in cultured fish, but when disease does occur its impact will be minimized. The premise of health management is a holistic approach which includes manipulation of the aquatic environment to optimize growth, feed conversion ratio efficiency, reproduction, and survival while minimizing problems related to infectious, nutritional, and environmental diseases; all within an economical context. Health management in aquaculture involves daily intervention in the growth process of fish and other organisms in an aquatic environment. As culture systems become more intensive, the need for intervention increases accordingly (Plumb 1999).

Maintaining Health

The goal of health management is to improve the health and well-being of animals that generally appear to be healthy. If sound health maintenance principles are applied on a regular basis the result will be fewer environmental and disease problems and optimum production of a healthier and more desirable product. Obviously all activities, policies, and changes must be based on sound economic and/or overriding epizootiological factors.

A profound relationship exists between environmental quality and the disease status of fish. The aquatic environment is in a continuous state of flux and as environmental conditions deteriorate, incidence and severity of infectious disease increases. Because fish are poikilothermous and body functions are dependent on temperature, they must constantly adapt physiologically to a multitude of environmental or physical changes ("stressors") or they will become "stressed". “Stress” is difficult to define because it is used in conjunction with many adverse situations which affect the well being of individuals, but generally it is the reaction of an animal to a physical, physiological, or chemical insult (Barton 1997). Stress manifests itself in reduced weight gain, higher feed conversion ratios, reduced immunity and natural disease resistance, higher disease incidence, increased mortalities, lower hardiness, and reduced productivity in general.

Fish health management is a proactive concept which considers interactions of the host with husbandry practices, environmental quality, nutritional factors, physiological status of the fish, and the pathogen. These factors interact individually and collectively like a "spider web" affecting the health status of the entire fish population (Figure 1.1). The various segments of the web are not autonimous and they interact and affect one another, therefore, if one remote area of the web is disturbed other areas sense the tremor and the health of the fish is affected.

Some commonly known stressors are: organic pollutants which can lead to unhealthy levels of un-ionized ammonia, nitrites, insufficient oxygen, and high concentrations of carbon dioxide; rapidly changing or extremes in water temperature; external salinities, improper nutrition, and high fish density; and chronic
low concentrations of pesticides or heavy metals (Barton 1997; Austin 1999). Also, extremes in pH or low water alkalinity and hardness are not conducive to good fish health. Many of these environmental factors are influenced by quality of pond soils, water supply, quality and quantity of feed, and organic waste accumulation. Health maintenance programs should reduce and modify stressful conditions.

Best management practices that help reduce stress as it relates to increased disease susceptibility are divided into fish handling and stocking, feed and feeding, water flow and temperature, supplemental aeration, other environmental problems, and waste management. However, these problems do not occur independently, therefore, all must be taken into consideration when discussing disease susceptibility (Figure 1.1).

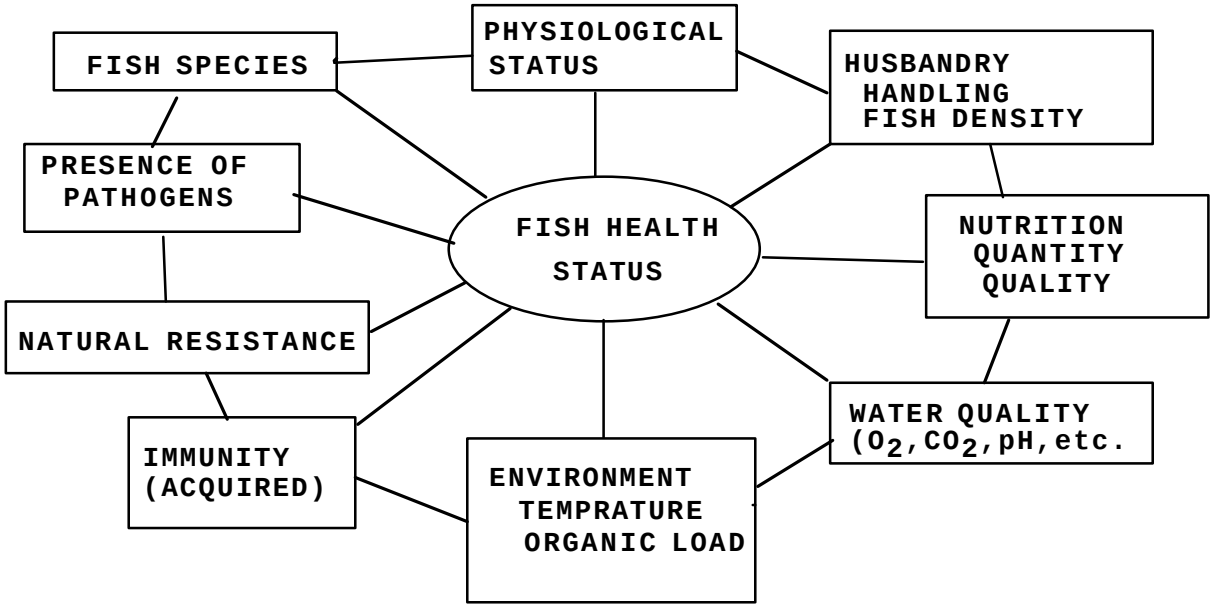

Figure 1.1. Schematic of environmental conditons, biological factors and management practices and their relationship to health status of fishes.

Fish Handling and Stocking

Proper handling during transport, sorting, spawning, and stocking is critical to disease resistance and/or susceptibility. Skin mucus helps prevent facultative pathogens from colonizing on the skin, thus injury to this protective material provides an opportunity for pathogens to become established. When fish are transported or held in tanks, a prophylactic treatment to help prevent secondary infections is advised.

In most cases, when moving fish from one environment to another, tempering allows the fish's physiology to adapt to its new environment. Required tempering time varies depending upon fish species and temperature differential. The greater the temperature differential the longer the required adjustment time but fish generally adapt to lower temperatures more quickly than to higher ones.

Crowding adversely affects the general health of fish populations and must be a consideration when stocking (Wedemeyer 1996). The carrying capacity of a raceway can be elevated by increasing water flow rates, but in ponds with little or no capacity for flushing, fish density is determined by quality and quantity of feed required. Depending upon fertility, the carrying capacity of most natural bodies of standing surface water is about 34 to 196 kg/ha (60 to 175 lb/acre) but aquaculture units may have a standing crop 100 times greater than normal. If nutrients are added to support higher fish densities accumulated uneaten feed and metabolic waste may cause water quality deterioration and increase potential for disease.

Increasing fish density does not always result in improved productivity or profits (Table 1.1). For example channel catfish ponds stocked at 11,120 fish/ha (4,500 fish/acre) produced 5,200 kg/ha (4,639 lb/acre) per year and ponds stocked at 19,770 fish/ha (8,000 fish/acre) produced 6,670 kg/ha (5,880 lb/acre) per year (Tucker et al. 1992). Although the higher stocking density resulted in greater weight gain, there was a deterioration of water quality, the fish did not grow as well, had greater size variation, poorer percentage of survival, and higher feed conversion ratios. The increased potential for low dissolved oxygen in more heavily stocked ponds also increased the need of aeration. Actual net profit in each system was about the same, however, management problems were significantly greater in the more densely stocked ponds.

Table 1.1. Projected production in a 116 hectare catfish farm stocked at two fish densities and harvested annually for 3 years (4 ponds/treatment) based on experimental data from studies on a smaller scale (Tucker et al. 1992).

Criteria	Treatment A[a]	Treatment B[a]
Stocking density/hectare	11,120	19,770
Average No. harvested/year	8,800	13,500
Mean survival	79%	68%
Net Harvest kg/year	5,200	6,670
Average weight (kg)	0.68	0.53
Feed Conversion Ratio	1.37	1.56
Aeration hours	1,606	2,120
Net Revenue/hectare	$3,559	$3,549

a. Each pond was harvested annually and number of fish removed were replaced with equal number of seed stock.

Feed Management

Feed quality is a primary consideration in feed management. Feed should always be fresh, of the highest quality, and consistent with nutrient requirements of a particular species, fish size, and culture unit. The amount and frequency of feed applied per unit is important because accumulation of uneaten feed will contribute to water quality problems and create an increased oxygen demand.

Proper nutrition is essential for fish survival, growth, and reproduction (Lovell 1989). Manufactured feeds which contain minimum levels of proteins, fats, carbohydrates, fiber, vitamins, amino acids, and minerals are recommended. A deficiency in any of these components can result in nutritional disorders and possible lowered disease resistance.

Certain nutritional deficiencies can result in specific pathological conditions (Lim and Webster 2001). A lack of sufficient riboflavin can cause eye cataracts in rainbow trout and possibly channel catfish. A niacin deficiency increases sensitivity to ultraviolet irradiation resulting in "sun burn." Nutritional gill disease of trout is associated with pantothenic acid deficiency and can progress into bacterial gill disease if the deficiency is not corrected. Injury to the spinal column of channel catfish (broken-back syndrome) results from a vitamin C deficiency.

Also, severe anemia in channel catfish is linked to a dietary deficiency of folic acid or presence of pteroic acid, a folic acid antagonist (Butterworth et al. 1986). The ratio of vitamin C and folic acid in the diet is also important in disease resistance and possibly affects immune response (Duncan and Lovell 1993). The presence of fungal toxins in feed can cause tumors and other health problems (Manning 2001).

Ironically, starved or lightly fed fish may actually be more resistant to some infectious agents. Anecdotal observations indicate that channel catfish susceptibility to CCV is decreased following starvation for 2 weeks (unpublished). Kim and Lovell (1995) showed that channel catfish which were not fed at all during winter were significantly less susceptible to *E. ictaluri* than fish fed more frequently or to satiation during the winter. Also, withholding feed from channel catfish during enteric septicemia epizootics has become the preferred management practice throughout the catfish industry (Wise and Johnson 1998).

Generally when water quality becomes critical with high organic fertility and low DO, reduced feeding can hasten a reversal of this trend, therefore, flexibility in feeding can be a valuable tool in aquaculture management.

Water Flow and Temperature Management

In some culture systems (i.e. trout raceways) specific water quality problems may be overcome by increasing water flow volume to maintain adequate temperature and/or dissolved oxygen concentrations. However, in some instances involving large earthen warmwater ponds routine continuous fresh water exchange may actually be harmful. Indications are that excessive water flow in ponds removes more nutrients than desirable and continuous water flow does not deter infectious disease (Lorio 1994). However, during periods of poor water quality, particularly oxygen depletion, addition of good quality water can be beneficial and may reduce severe stress and disease susceptibility.

Maintaining water temperature at a proper level for species such as tilapia and catfish is imperative for maximum growth and good health. In regions where water temperatures normally fall below optimal levels for growth and health of a cultured species, water from geothermal aquifers, heated water from power plants, or artificially heated water can be used to raise water temperatures in raceways or recirculating systems. The use of artificially heated water (electrical,

fossil fuel or solar) for the sole purpose of aquaculture is seldom economical, however, heated water as a by product of some other activity may be.

Aeration Management

Mechanical aeration is a major factor in the rapid growth of warmwater aquaculture because it makes it possible to maintain a higher quality environment in spite of increased respiration demands. To determine if mechanical aeration is needed, oxygen levels should be monitored 24 hours a day during warm weather or extended cloudy periods when oxygen demand is greatest and concentrations are generally at their lowest. To estimate what a pond's lowest oxygen concentration will be just before dawn, measure the dissolved oxygen at dark and 3 hours later, and extrapolate a straight line to determine the predicted oxygen concentration at dawn (Boyd et al. 1978). If the pre-dawn oxygen concentration is projected to be below 3 mg/L, aeration is required during the night to avoid oxygen depletion and possible fish loss. Routine nightly aeration during warm weather prevents oxygen depletion that can lead to stress and increased disease incidence. A study by Lai-Fa and Boyd (1988) showed that during the growing season, 6 hours of nightly aeration almost doubled channel catfish production and lowered feed conversion ratios from 1.75 to 1.32.

A variety of aerators (paddle wheels, agitators, or sprayers) are available with each having unique advantages. Aeration in closed or recirculating systems can be achieved with compressed air, liquid oxygen, pure oxygen, or by mechanical agitators, but bubbling air or oxygen into the culture system or pipeline manifold is probably not as efficient as injecting oxygen or air into a sealed column of water.

Other Water Quality Parameters

Other water quality parameters important to the health of cultured fish include maintaining proper pH, alkalinity, and water hardness as well as low levels of carbon dioxide, nitrites, and ammonia (Boyd 1990) (Table 1.2). In healthy ponds, carbon dioxide seldom poses a problem because algae and plants utilize it almost as fast as it is produced. However, high concentrations of CO_2 can be a problem in closed systems.

Water acidity, which is dependent upon its buffering capacity (alkalinity), should be pH 6.5 to 9. For aquaculture purposes, both total alkalinity and total hardness should be above 20 mg/L ($CaCO_3$) with preferred values of over 50 mg/L (Boyd 1990). Low alkalinity and hardness, or a widely fluctuating pH in ponds, can be partially corrected by adding agricultural lime. In recirculating or flow through systems these deficiencies can be corrected by dripping lime into the water or passing water through a bed of oyster shells. Un-ionized ammonia, is seldom a problem in open ponds but will accumulate in closed systems where it can be controlled with biological filters.

Table 1.2 Desirable water quality criteria for health management of warmwater fishes (Boyd 1990).

Element	Concentration (mg/L except pH)
Oxygen	5-saturation
pH	6.5-9
Ammonia (un-ionized)	0-0.02
Calcium	10-160
Carbon dioxide	0-15
Hydrogen sulfide	0-0.002
Iron (total)	0-0.5
Manganese	0-0.01
Nitrate	0-3.0
Phosphorus	0.01-3.0
Zinc	0-0.05
Total hardness ($CaCO_3$)	10-200
Total alkalinity ($CaC0_3$)	10-400
Nitrogen (gas saturation)	<100%
Total solids	50-500

Some water quality problems are the result of high fish densities and can be partially corrected by using manipulative management: reducing the standing crop, limiting quantity of feed, or increasing aeration. Uneaten feed, accumulated fecal waste, and dead plant matter in a culture system elevates organic content. In trout raceways with high water exchange rates, most waste material is flushed out; however, in warmwater culture ponds solid waste accumulates on the bottom where it decomposes. This waste can best be reduced by periodic de-watering and allowing a pond bottom to dry. To enhance respiration in dry ponds, either calcium hydroxide or calcium carbonate can be added to the soil to raise pH to a more desirable 7.5 to 8.0. Tilling and pulverizing the crust of a pond bottom will accelerate this process (Boyd and Pippopinyo 1994). In closed recirculating systems organic material can be removed by mechanical scrubbers and biological filters.

Where vegetation is a problem, routine but judicial application of Environmental Protection Agency (EPA) registered herbicides (i.e. copper sulfate or Aquazine) can be used to control phytoplankton or filamentous algae in ponds. Also, herbivorous fish (grass carp eat vascular plants; silver carp eat phytoplankton) can help control vegetation in environments with high fertility and an over abundance of vegetation. When practical, aquatic vegetation should be controlled with herbivorous fish rather than chemicals as benefits are usually longer lasting, more economical, and more user friendly. However, precautions must be taken to avoid the escape of exotic species into natural waters where they could be ecologically detrimental. Exotic carp are prohibited by law in some states but stocking sterile triploid grass carp may be allowed.

Health and management decisions should not be made independently because a change in one area may adversely affect another. If a fisheries manager, biologist, or diagnostician is to understand infectious fish diseases, he/she must understand how and to what extent the environment affects the host and/or disease agent so that remedial action can be taken to reduce or avoid environmental impacts. It is not enough to simply culture and/or identify a pathogen, but it is equally important to be able to identify environmental stressors which may enhance fish susceptibility to specific pathogens. When stressors are known preventive or corrective measures can often be initiated to help prevent or minimize a reoccurrence of the condition.

Stress on fish increases when environmental conditions approach the host's limit of tolerance. For example, if water temperature is critically high and oxygen concentration is

adequate, fish may adjust and survive, and if oxygen is critically low and water temperature is normal, fish may also adjust. However, when multiple parameters approach stressful levels but are not individually lethal, the problems are synergistic and fish are more likely to become stressed. The relationship between dissolved oxygen (DO) and carbon dioxide (CO_2) in ponds serves as an example. Channel catfish can adapt to an elevated level of CO_2 (20 to 30 mg/L) if the DO concentration is optimal (Boyd 1990). However, if the CO_2 is critically high and DO is low, fish cannot eliminate CO_2 and will become listless (narcotized) and may die. If fish do adapt to these environmental stressors and survive, resistance to disease is often compromised. Snieszko (1973) theorized that a host/pathogen/environment relationship exists with regard to infectious fish diseases. This theory is based on the premise that if a host and pathogen are present an unfavorable environmental condition will often trigger disease.

Water quality deterioration in warmwater ponds can result in bacterial infections in channel catfish (Plumb et al. 1976). A die-off of blue-green algae in a channel catfish pond was followed by reduced dissolved oxygen, low pH, and increased CO_2 and NH_4 that resulted in an "oxygen depletion" fish kill. The phenomenon was described by R. Schmittou (Department of Fisheries and Allied Aquacultures, Auburn University, Alabama, personal communication) as "low dissolved oxygen syndrome" (LODOS). When the DO dropped below 1 mg/L fish began to die. Initially these fish harbored no significant pathogens either in skin/muscle lesions or visceral organs, however, 4 days after the oxygen depletion *Aeromonas hydrophila* was isolated from lesions and visceral organs of surviving fish. The addition of fresh water and remedial aeration arrested mortality and clinical signs of infection. It was theorized, that while the water was in a state of hypoxia, some muscle areas also became hypoxic which led to tissue necrosis, hemorrhaging, and skin depigmentation. With loss of epithelium integrity the omnipresent *Aeromonas hydrophila* invaded muscle beneath injured skin and focal infections were established which progressed into septicemia. It was subsequently shown experimentally that either low oxygen, low pH, high ammonia, or high $C0_2$ alone did not lead to bacterial disease, however, if two or more of these conditions occurred simultaneously, bacterial infection was much more likely to occur.

Avoiding Exposure

Theoretically the ideal approach to preventing infectious fish diseases is to avoid exposure to pathogenic agents whenever possible, thus avoiding most devastating disease problems. However, when dealing with an aquatic environment, it is impossible to prevent fish exposure to all potential pathogens, especially in open waters that typify most warmwater or cool-water systems. Many fish pathogens are enzootic in most waters and are opportunistic, facultative organisms that remain viable under a variety of conditions. Some parasites have complex life cycles that involve fish-eating birds, snails, or copepods. To control infestations by these parasites the life cycle of the non-fish vectors must be broken, a nearly impossible task.

While maintaining specific disease free (SPF) fish populations in salmonid culture is standard procedure (Meyer et al. 1983; Anonymous 1984; Thoesen 1994), it is not practiced in warmwater culture due to the facultative nature of many fish pathogens. Nevertheless, there are several pathogens in North America currently being considered for this approach to warmwater disease control and include largemouth bass virus, *Edwardsiella ictaluri* (enteric septicemia of catfish), and *Bothriocephalus acheilognathi* (bothriocephalosis or Asian tapeworm) and in cool-water fish the white sturgeion viruses. However, the means to accurately identify pathogen carriers has not been perfected.

Quarantine, routine water disinfection, and destruction of populations infected with specific disease organisms are tools that can be used to avoid exposure. Each disease outbreak should be considered individually and rarely will a general policy be applicable. Therefore, "avoiding exposure" decisions must be based on biological facts and common sense, with an eye to the overall good of the fishery resource.

New Arrivals

Newly arrived fish may harbor pathogens either in an active or carrier state. Therefore, it is recommended that fish not be introduced into an existing population until it can be reasonably ascertained that they do not harbor exotic pathogens. To reduce the risk of introducing a pathogen with new arrivals, their health history should be well documented and when possible the use of SPF eggs or seed fish from inspected hatcheries should be used (Anonymous 1984; Thoesen 1994). If fish are not treated for ectoparasites, or eggs not treated for pathogenic

bacteria prior to shipment they should be treated prophylactically with appropriate registered chemicals upon arrival. If possible, new fish should be segregated (quarantined) from the resident population until shown to be specific disease free. Also, extreme care must be practiced to insure that no new pathogens are introduced into a facility when replenishing broodstock from outside sources.

Breeding and Culling

Few studies have dealt directly with the development of disease resistant fish through selective breeding and/or genetic manipulation. Most fish genetic studies have emphasized increased growth rates, fecundity or improvement in feed conversion ratios. However, aquaculturist can gradually improve fish stocks by selecting fish that are less affected by specific diseases. The routine culling of physically inferior or poor spawning individual broodfish or those that repeatedly produce diseased offspring, will also improve performance.

Using young adults for reproduction before they become heavily parasitized can reduce the effects of metazoa parasites. At some hatcheries in Southeastern United States replacing old largemouth bass broodstock with young adults has successfully prevented the build up of bass tapeworms (*Proteocephalus ambloplitis*) which infect ovaries and reduce egg production (W. A. Rogers, Auburn University, Alabama, personal communication).

Eradication, Prevention or Control

Eradication, prevention, and control are defined as follows: *eradication* is complete elimination of a disease causing agent from a facility or specific geographical region. *Prevention* is avoiding introduction of a pathogen into a region or facility and/or stopping a disease process before it can become a problem. *Control* reduces a disease to a level that is economically and biologically manageable, and/or confinement of a problem to a defined area.

Eradication of a fish disease agent from a facility, watershed, or region is desirable but difficult to accomplish. To date, there are no reported examples of a fish disease agent being totally eradicated from a large geographical region (epizootic epitheliatropic disease of lake trout in the Great Lakes basin may be one exception). Practical eradication of some diseases of trout, such as infectious pancreatic necrosis, furunculosis, and bacterial kidney disease from individual

hatcheries and farms in the United States has been accomplished by depopulation and sterilization of facilities with chlorine or formaldehyde gas. These select facilities had closed water supplies with no indigenous fish populations to serve as disease reservoirs. Precautions were also taken to prevent reintroduction of disease agents by using disinfected or SPF eggs to repopulate the facilities and access of fish transport trucks and equipment was restricted at the disinfected culture units. Opportunities to eradicate a disease agent are rare among warmwater aquaculture facilities because generally there is little or no control over an entire water supply.

Disease prevention is primarily a farm management policy and consideration of it should begin with the design and construction of a culture facility. Considerations should include site selection, development of water supply, facility construction, and selection of the source of fish to be stocked. If feasible, initial fish stocks, and any subsequent fish brought onto the facility, should come from SPF populations. Vaccination should be considered as a disease prevention tool when available. It is recommended that each culture unit have separate handling equipment that is held in properly maintained disinfection baths to minimize the potential for spread of disease agent(s) between culture units.

Control of some diseases can be accomplished by effective use of chemotherapy when practical in conjunction with elimination of environmental stressors. Only those drugs registered by the government regulatory agency should be used on food fish.

When dealing with fish diseases, biological and economic constraints usually dictate the appropriate approach. Eradication may be applicable in cases involving obligate pathogens, but only if a facility can be maintained free of the pathogen after sterilization. Eradication verses control is determined on a case by case basis with consideration given to economic and regional management consequences. When considering fish health policies that include destruction of whole populations the statement by S. F. Snieszko must be considered; "A disease management practice should not destroy more than it saves", in other words the cost/benefit ratio must be positive.

<u>A Clean Environment</u>

Maintaining a clean environment often requires greater diligence in warmwater aquaculture than in cold-water raceways. A clean and neat facility still may have disease problems, but they tend to be fewer and less severe when they do occur.

Water is an excellent medium for transmitting disease agents, therefore, sick or dead fish should be removed to reduce pathogen reservoirs. Controlling vegetation at waters edge will not allow sick or dead fish to go unnoticed. Also, the possibility of scavengers carrying infected carcasses to other ponds and spreading disease agents is reduced. Accumulation of feces, uneaten feed, and other organic detritus is a major problem in ponds and recirculating systems and can serve as a nutrient substrate for facultative pathogens. These problems can be reduced, avoided, or corrected by improving feed quality, regular cleaning, periodic draining, drying and removal of accumulated sediments from ponds, and disinfection.

Maintaining a clean aquaculture facility will pay dividends by providing a safer work place, reduced disease incidence and subsequent loses, increased production, and generally healthier fish, all of which translates into more efficient production and higher profit.

Summary

Health management, or best management practices, must be a primary goal of any overall plan as it provides the greatest deterrent to development of infectious fish diseases and environmental problems. Infectious diseases will not be completely eliminated, but if good health management techniques are incorporated and diligently adhered to, disease incidence and severity will be diminished and result in increased production of a healthier product.

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Chapter 2. Epizootiology

Understanding the dynamic aquatic environment and its role in fish health is imperative to management of infectious diseases. In a broad sense, epizootiology, is the study of infectious diseases of animals, which includes spread of pathogens, mode of infection, effect on the population, and other factors that affect the disease.

Disease is a deviation from normal or good health and may result from infectious agents, nutritional deficiencies, toxicants, environmental factors, or may be genetically based. In discussing epizootiology, it is important to differentiate between "infection" and "disease". Infection is the presence of a pathogen in a host that may or may not be diseased and is often a normal state (Coutant 1998). Disease is the condition in which a pathogen is present in sufficient numbers to affect the animal's well being. Fish may be infected without being diseased and can serve as a reservoir for a particular pathogen or pathogens. Many fish disease organisms are enzootic (present all the time) in the aquatic environment co-existing with the host but not causing disease. However, if conditions become unfavorable for the host and if its immune system or natural resistance are compromised by some stressor, disease may result.

In terms of the host/pathogen relationship two basic types of organisms are involved in communicable disease: (1) *obligate* pathogens and (2) *non-obligate* (facultative) pathogens. Obligate pathogens require a host to provide a system for replication or an organic nutrient source for reproduction and survival. Examples of fish obligate pathogens are: all viruses, the bacterial pathogen *Renibacterium salmoninarum*, and the parasitic protozoan *Ichthyophthirius multifiliis*. Facultative (non-obligate) pathogens can live and multiply in a host or live freely deriving nutrients from organic matter in water. *Aeromonas hydrophila* (motile *Aeromonas* septicemia) and *Flavobacterium columnare* (columnaris) are examples of facultative pathogens. The fungus *Saprolegnia* spp. is saprophytic and derives its nutrients from living or dead organic material.

Most infectious agents require specific levels of infective units before adversely affecting a host's health status; therefore, losses are minimized if infectious organisms can be maintained at levels below the disease threshold. Many parasite populations can be reduced with prophylactic chemotherapy and good health re-established by restoration of environmental quality.

<u>Seasonal Trends</u>

Diseases of warmwater fish generally occur seasonally and tend to fluctuate with temperature changes, presence of young susceptible fish, and environmental conditions that affect immunity and natural resistance (Meyer 1970; Warren 1991; Plumb 1999). Disease incidence in cultured and wild fish populations in the southern United States is low from November through February, increases during March through June as waters begin to warm, decreases during the warm summer months, and increases again in autumn (Meyer 1970; Plumb 1976). A similar pattern can be noted as one moves northward; however, incidence of disease occurs later in the spring and earlier in the fall.

Most water quality problems occur during summer and are correlated to high water temperatures, solar radiation, accumulation of organic matter, increased nutrient input, peak primary pond production, and high standing crop (Tucker and van der Pfloeg 1993). Stressful conditions during the summer may actually predispose fish to disease later in the year.

Factors in Disease Development

Factors that affect disease severity are: (a) source of infection, (b) mode of transmission, (c) portal of entry, (d) virulence of the pathogen, and (e) resistance of the host. If one of these factors can be altered in favor of the fish host through management, disease may be eliminated or its impact reduced.

Source of infection: An infectious disease requires a pathogen source that may be dead, moribund, or carrier fish showing no clinical signs of disease; contaminated eggs from infected broodstock; or contaminated water. Transmission of many pathogens can be prevented by disinfection of equipment, filtration of water, adjustment of stocking densities, removal of dead and moribund fish, utilization of seed fish from specific pathogen free (SPF) broodstocks, and use of proper feed.

Mode of transmission: Transmission of fish diseases is closely related to the source of infection. Water provides a nutrient rich environment in which opportunistic fish pathogens can grow and proliferate, thus serving as a pathogen source as well as a primary mode of transmission.

Reproductive products from disease-carrying broodstock are often responsible for vertical transmission of viral and possibly some bacterial agents from one generation to another; however,

this is not well understood in warmwater fish. Also, intermediate hosts such as birds, snails, and crustaceans serve as vectors for some pathogens. Transmission of fish diseases is often aided by man via fish transport equipment, and on seines, nets, boots, and other utensils. Management practices can help disrupt disease transmission by using SPF fish and eggs, prophylactic treatments, control of intermediate hosts, use of water filtration, water treatment with ozone or ultraviolet light, use of a fish-free water source, and disinfection of equipment.

Portal of entry: Each disease organism has an optimal entry point to the host. By identifying these entry points management can help prevent infection. Common disease entry points in fish are the intestines, gills, and skin. Injury or disruption to the mucus covered epithelium can facilitate pathogen entry; therefore, proper handling and prophylactic treatments will often reduce or prevent epithelial infections.

Virulence of the pathogenic organism: Virulence, the measure of an organisms ability to cause disease, may range from low to high. Facultative bacteria can vary greatly in virulence from location to location. Virulence of a pathogen in nature cannot be manipulated, but proper management can help prevent severe disease outbreaks by optimizing environmental conditions for the host. Additionally, virulence of some pathogens can be reduced or eliminated by frequent subcultivation in the laboratory, or by permanently removing their pathogenic capability through biotechnology (Leong and Fryer 1993; Klesius and Shoemaker 1999). These resulting live avirulent organisms can then be used as vaccines.

Resistance of the host: Natural resistance is related to a host's inherent ability to subdue a pathogen to such a degree that clinical disease will not occur (Chevassus and Dorson 1990). Intrinsic factors which influence a fish’s natural resistance to disease are nonspecific phagocytic activity of neutrophils and macrophages, nonspecific serum components (interferon, complement, etc.), and tissue integrity. However, extrinsic influences such as diminished nutritional well-being and poor environmental conditions can adversely affect these traits. Natural resistance is related to species, strain, and age of fish. Adult fish often have reduced disease resistance during spawning season when they may stop feeding and energy is diverted into reproduction, or during times of environmentally induced stress. In temperate zones, both adult and juvenile fish have reduced resistance to disease in spring; adult fish due to over wintering and young fish because they have not yet acquired a natural resistance. Fish health management practices should take

advantage of natural disease resistance by culturing disease resistant strains and managing the environment to reduce stressful conditions.

Host/Pathogen Relationship

The host/pathogen relationship is a fundamental concept in the epizootiology of fish diseases (Hedrick 1998). In warmwater fish, disease organisms tend to cause infections when a host/pathogen imbalance occurs and the aquatic environment deteriorates to such a degree that a fish's natural resistance is compromised and "infection" progresses into "disease." Best management practices will help in maintaining the proper host/pathogen balance thus minimizing clinical disease outbreaks. However, some pathogens can still cause disease without any triggering stressors (i.e. channel catfish virus, *Edwardsiella ictaluri*, *Ichthyophthirius multifiliis*).

Degree of infection

Primary infections, usually caused by obligate pathogens, can produce disease without any extrinsic or intrinsic factors being present. However, any adverse environmental factor can synergize infection to a more serious level (Wedemeyer 1997). Channel catfish virus, *Edwardsiella ictaluri*, and *Renibacterium salmoninarum* are examples of primary pathogens that infect fish.

Secondary pathogens are generally free-living, facultative or saprophytic, opportunistic organisms that infect a host when its defenses have been compromised by other pathogens or stressors. These infections usually follow mechanical or physiological injury, presence of other organisms that render the host weakened, environmental stressors such as low oxygen levels, temperature shock, and excessive or improper handling. *Aeromonas hydrophila*, *Flavobacterium columnare*, and *Saprolegnia* spp. are examples of secondary pathogens that affect warmwater and cool-water fish; however, occasionally these organisms may also be primary pathogens.

Summary

One must understand the epizootiology of fish diseases in order to establish an integrated management plan to control or minimize their impact. Seasonal trends, disease severity,

host/pathogen relationships, and degree of infection are all important in formulating a management plan.

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Chapter 3: Chemotherapy

Global expansion of aquaculture during the past 30 years can in part be attributed to the incorporation of drug and chemical therapy into fish disease management programs. However, chemotherapy should not be relied upon exclusively to solve aquacultural health problems or to drastically increase carrying capacity of culture units.

Advantages of using drugs in treating infectious fish diseases include availability, can be purchased in advance and administered in several ways and under a variety of conditions, and some may be effective on multiple diseases. Conversely, the effectiveness of some drugs is often short term and once the drug is withdrawn disease may reoccur, especially if pathogens are not completely eliminated or disease predisposing factors are not corrected. Also, some bacteria may develop resistance to antimicrobials if used improperly, too frequently, or over an extended period of time (Acar and Röstel 2001). Often by the time chemotherapy is applied, the target pathogen has already taken a toll on the fish population either in mortality, growth reduction, and/or productivity. Chemotherapy presents a variable cost problem because it cannot be predetermined how often its use will be required and some drugs used on fish may be environmentally hazardous or toxic to animals being treated, the individual applying the drug, or the consumer who eats the product.

Treatment Process

Disease treatments and procedures discussed here are designed for cultured fish. Although disease occurs in wild populations, treatment is usually not practical or economical. Since drugs seldom completely eradicate all pathogens, their application is often a matter of "buying time" until the fish can overcome infection via their own defense systems.

Wellborn (1985) proposed a critical question to be considered before treating fish: What is the prognosis if treatment is applied or withheld, and do potential losses justify treatment? If treatment is still indicated after answering this primary question, four additional criteria apply: (1) know the water, (2) know the fish, (3) know the chemical, and (4) know the disease. Ignoring any one of these factors can result in inappropriate, ineffective, or over treatment.

*Know the Water***:** It is important to know the water volume to be treated in order to prevent a lethal or ineffectual chemical application. Oxygen concentration, alkalinity and

hardness, pH, organic load, and water temperature will influence efficacy and toxicity of some drugs.

Know the Fish: Chemical toxicity may vary from species to species, and between different age groups. If the drug of choice has not been previously used in a particular water supply to treat a specific fish species or age class, sensitivity to the drug should be tested by placing a few fish in a small vessel of water to be treated that contains the desired drug concentration and observe the results.

Know the Chemical: A drugs toxicity and percent active ingredient must be known so that the proper amount to be used can be calculated. Some drugs are affected by sunlight, pH, temperature, organic content, and alkalinity and may become toxic to plants or contribute to a chemically generated oxygen depletion.

Know the Disease: It is essential that a disease be accurately diagnosed. Multiple infections involving different pathogen species often occur and require different treatments beginning with the most serious pathogen. An incorrect diagnosis can lead to an ineffective or disastrous treatment.

Therapeutic Applications

The most effective and economical method of drug application is determined on a case by case basis and is dependent upon the disease, drugs prescribed, type of unit to be treated, and age and species of fish. Six basic methods for treating fish diseases are: (1) dip, (2) flush, (3) prolonged bath, (4) indefinite bath, (5) orally in feed, and (6) injection.

Dip: For a dip treatment netted fish are immersed in the drug solution for 15 to 60 seconds. Treatment concentrations are expressed as percentage of material, milligrams per liter (mg/L), or microliters per liter (µL/L) [equivalent to parts per million (ppm)], or a ratio of chemical to volume of water (i.e. 1:5,000). This treatment method requires caution because of high drug concentrations used and potential for toxicity. Time of immersion is critical, therefore, a few fish should be treated to determine their reaction before exposing an entire population. The dip method is usually used to treat small numbers of easily confined fish which are infected with external parasites or bacteria.

Flush: When applying a flush treatment a stock solution of drug is introduced by a constant and continuous flow delivery system assuring a desired concentration during the entire treatment period which can last from a few minutes up to 1 hour. Drug concentrations are expressed as mg/L, µL/L, ppm or as a ratio of drug to volume of water (i.e. 1:5000).

Prolonged Bath: Prolonged bath treatments are usually used to treat fish with ectoparasitic or external bacterial infections while fish are being held in tanks or raceways. Water flow is stopped and the drug is added to the holding unit at the desired concentration (mg/L, µL/L, ppm or as a ratio of drug to volume of water) and mixed to avoid "hot spots". The drug is then left for a predetermined time, usually 1 hour, with aeration. When the treatment is terminated water flow is resumed and the drug quickly flushed out. However, fish should be observed continuously during treatment and if any signs of discomfort, such as gasping, "flashing," or loss of equilibrium are noted, the drug is flushed out immediately regardless of exposure time.

Indefinite: When applying an indefinite treatment a drug is introduced into a pond or static tanks at comparatively low concentrations for an undetermined length of time and allowed to dissipate naturally. Drug concentrations are usually measured in mg/L, µL/L or ppm, and in ponds large quantities of drugs are often required. Dry chemicals, such as potassium permanganate or copper sulfate, are first dissolved in water and then dispensed by hand, a boat bailer, siphon, or sprayer. The drug must be uniformly applied to avoid “hot spots” and supplemental aeration should always be available. Indefinite treatments can be used for ectoparasitic or bacterial infections.

Oral: Treatment of systemic bacterial infections requires incorporation of antimicrobials into feed by licensed feed manufacturers. Heat resistant drugs can be incorporated into floating feeds but non-heat resistant drugs must be incorporated into sinking pellets. Drugs are added to feed at a concentration that delivers the desired dose per unit of fish weight per day at a specific feeding rate for a specified period of time. Standard units of treatment are in grams of active ingredient per 45 kg (100 lb) of fish or in milligrams active ingredient per kilogram (mg/kg), or pound, of body weight per day for a defined number of days. Prophylactic feeding of an antimicrobial for short periods of time or continuously at low dosage rates is not advised because of the potential for developing bacterial drug resistance. A “withdrawal time”, the number of days

required to elapse between the last day a drug is fed and the day of slaughter, is required to insure that no drug residues remain in the animal's flesh. Withdrawal time varies with drug, fish species, and water temperature.

Injection: Broodfish, or small numbers of valuable fish can be treated for certain bacterial infections by injection. Drug dosages to be injected are measured in either international units (IU) or milligrams (mg) of active drug per kilogram (or pound) of fish and are administered intraperitoneally (IP) or intramuscularly (IM). Intraperitoneal injections are administered in the posterior body cavity by inserting a needle through the peritoneal wall at a 45° angle at the base of the pelvic fin, taking care not to puncture internal organs. Intramuscular injections are given slowly in the thick dorsal musculature near the dorsal fin. The need to handle individual fish may increase stress if fish are already in poor health. Caution should be taken by persons administering drugs by injection to eliminate the possibility of injecting oneself.

Drugs for Fish

In the past, drug and chemical use in aquaculture was extensive, however, approved chemotherapeutics available for food fishes today is now quite limited (Alderman and Michel 1992; Stoffregen et al. 1996; Anonymous 2000; Anonymous 2001). Also, when compared to the quantity of drugs used in the poultry and livestock industry, the demand for pharmaceuticals in aquaculture is small.

The United States Food and Drug Administration's Center for Veterinary Medicine (FDA-CVM) is responsible for approving drugs and chemicals used for disease control in all food animals in the United States. The Veterinary Drug Directorate of Health Canada has the same responsibility in Canada. All candidate drugs to be used on fish must go through an Investigational New Animal Drug (INAD) application in the United States and a similar process in Canada. While drug regulations do vary among countries they have become more cosmopolitan because of increased international trade of aquaculture products (Schnick 1992).

A major concern of the health care industry is the potential for bacterial pathogens to develop antibiotic resistance to them. There is always the possibility that antibiotic resistance in pathogens of husbandried animals may be transferred to pathogens that affect humans, thus reducing the effectiveness of some drugs for treating human diseases. Acar and Röstel (2001)

defined the phenomenon of antimicrobial resistance as the ability of bacteria to survive an adequate antibiotic treatment. Already resistance to Terramycin and Romet-30 by *Aeromonas* spp. and *Vibrio* spp. in salmonids and *Edwardsiella* spp., *Aeromonas* spp, and *Flexibacter columnare* in warmwater fishes has been reported.

Pathogen resistance usually occurs as a result of its extended exposure to subtherapeutic drug levels or to its use for longer periods of time than recommended. Resistance may also result from chromosomal or plasmid mutation or resistance carrying genes by the pathogen. Genes carrying drug resistant traits or plasmids of one species or pathogen strain can be transferred to subsequent generations or to other species or strains. Acquired antimicrobial resistance is a natural phenomenon but steps can be taken to reduce its occurrence in aquaculture. First and foremost, antimicrobials to treat fish should be used only when required for a clinical disease and never prophylactically. They should always be applied in accordance with label instructions, at recommended concentrations, proper method of application, for the prescribed period of time, and for only the prescribed target pathogen.

The FDA-CVM classifies drugs as: (1) Approved New Animal Drugs, (2) Unapproved Drugs of Low Regulatory Priority (LRP), (3) U.S. Environmental Protection Agency (EPA)-Registered Pesticides for Aquaculture/Aquatic Sites, (4) Investigational New Animal Drugs (INAD), and (5) Extra-label Use of an Approved New Animal Drug (Anonymous 2001). All other drugs or chemicals are considered illegal for use on fish.

Because some drugs are dangerous, especially in concentrated form, rubber gloves, breathing masks, protective clothing, and safety glasses are essential safety precautions when weighing, measuring, and applying them. Skin should be immediately and thoroughly washed if it comes in contact with any drug.

Approved New Animal Drugs

A small number of drugs are registered in the United States and Canada for use on fish (Table 3.1). These drugs are generally registered for a specified application method for a

specified disease of a particular species or group of fish, and technically their use for other diseases or other non-listed fishes is illegal unless used under an INAD or Extra Label Use.

Table 3.1. New animal drugs approved for food fish by the United States Food and Drug Administration and Vetrinary Drug Directorate of Health Canada.

Drug (Manufacturer)	Indication	Dosage
UNITED STATES and CANADA[a]		
Finquel[b] (Argent Chemical)	Anesthetic	50 - 100 mg/L (21 d)[c]
Formalin[d]		
Formalin F	Ectoparasites,	Raceways, tanks: >10°C 170 µg/L, 1h, <10°C 250
(Natchez Animal Supply)		µg/L, 1 H 1h, <10°C 250 µg/L, 1 h Ponds: 15 to 25 µl indefinitely
Paracide-F (Western Chemical)	Fungiside on eggs	Eggs: 1000 to 2000 uL/L for 15 min
Romet-30[e] US, C (Alpharma)	Furunculosis, Enteric redmouth in salmonids	50 mg/kg fish per day for 5 d (42 d)
	Enteric septicemia of Catfish	50 mg/kg fish per day for 5 d (3 d)
Terramycin[f] US,C (Phibro Animal Health)	Furunculosis, Enteric redmouth in salmonids Motile *Aeromonas* and Enteric septicemia in Catfish	50 - 75 mg/kg fish/day in for 10 d (21 d)
AVAILABLE IN CANADA ONLY		
Drug (Manufacturer)	Indication	Dosage

Aqua Life TMS[b] (Syndel)	Anesthetic	40-50uL/L (5 d)
Aquaflor[g] (Schering-Plough)	Furunculosis in salmonids	10 m/kg for 10 day (12 d)
Parasite-S[d] (Syndel)	Ectoparasites and Fungicide on Eggs	Raceways, tanks: >10°C 170 µg/L, 1 h, <10°C 250 µg/L, 1 h 1000 to 2000 uL/L for 15 min
Perox-Aid[h] (Syndel)	Fungicide on eggs	500 µL/L for 60 min
Tribrissen[i] (Schering-Plough)	Vibriosis in salmonids	7.5g/100kg/day for 7-10 d (80 d)

Source: Schnick et al. (1989), Anonymous (2000), Anonymous (2001), Canadian Product labels

a. All available in US, Available in Canada (C)

b. Tricaine methanesulfonate - "tricaine"

c. ( ) Withdrawl time if applicable

d. 37 - 39% formaldehyde gas

e. Sulfadimethoxine/ormethoprim in 5 to 1 ratio

f. Oxytetracycline hydrochloride

g. Florfenicol

h. Hydrogen peroxide

i. Sulphadiazine/trimethoprim in 5 to 1 ratio

Finquel: Although not a drug for treating diseases, Finquel (tricaine methanesulfonate, MS-222, tricaine) is registered in the United States and Canada (TMS) as a fish anesthetic for catfish, salmonids, esocids, and percids.

Formalin: Three registered formalin products can be used as an ectoparasiticide for fish and as a fungicide on eggs: Formalin F and Paracide-F are registered in the United States; Parasite-S is registered in the United States and Canada. Formalin is a clear liquid containing 37 to 40% formaldehyde gas but in calculating concentrations it is considered 100% active. Formalin is approved for salmon, trout, catfish, bluegill, and largemouth bass. Concentrations of formalin used in tanks, troughs, and raceways are 170 µL/L (1:6000) for 1 hour as a prolonged treatment at temperatures above 10°C and 250 µL/L (1:4000) for 1 hour at temperatures below 10°C. Formalin is used in ponds as an indefinite treatment at 15 to 25 µL/L, but care must be taken during warm weather because an oxygen depletion requiring aeration can occur several days after application. Formalin is applied as a flush treatment for salmonid and esocid eggs at 1,000 to 2,000 µL/L for 15 minutes to control of Saprolegniaceae (Rach et al. 1997).

Romet-30: Romet-30 is a bactericidal potentiated sulfonamide powder which combines sulfadimethoxine and ormetoprim in a ratio of 5:1. It is registered in the United States and Canada for treating furunculosis in salmonids and enteric septicemia of catfish (ESC) in the United States. Being heat resistant, Romet-30 is incorporated into extruded floating pellets and fed at 50 mg/kg of fish per day for 5 days followed by a withdrawal time of 42 days for salmonids and 3 days for catfish. Mortalities are reduced quickly when fish consume feed containing Romet-30, however, a low incidence of drug resistance by *E. ictaluri* has occurred with frequent recrudescence of infection (Plumb et al. 1995).

Tribrissen: Tribrissen, a combination of sulphadiazine and trimethoprim mixed in a 5 to 1 ratio, is incorporated into feed for salmon infected with *V. anguillarum*. It is fed at a rate of 30 mg/kg of fish per day for 7 to 10 days followed by an 80 day withdrawal time. Tribrissen is registered in Canada but not the United States.

Terramycin: Terramycin, or Terramycin Aqua (oxytetracycline) is a bacteriostatic antibiotic which is added to fish feed to control furunculosis, vibriosis, columnaris, cold-water disease and enteric redmouth in salmonids in the United States and Canada; and motile *Aeromonas* septicemia, *Pseudomonas* septicemia, and ESC in the United States. Terramycin is

fed at a rate of 50 to 75 mg/kg or 2.5 to 3.75 g/45 kg (100 lb) of fish per day for 10 consecutive days with a withdrawal time of 21 days. According to Schnick et al. (2001) FDA-CVM has accepted food safety data for juvenile northern pike and walleye with no withdrawal time required.

Terramycin has been used so extensively in aquaculture (some times improperly) that a high percentage of clinical target isolates, especially motile *Aeromonas* spp. and typical *A. salmonicida* (furunculosis) are resistant to it. Another problem, especially when treating channel catfish, is the fact that Terramycin is only available in sinking pellets because of it's heat sensitivity.

Aquaflor: Aquaflor, a florfenicol product, is registered in Canada only as a feed additive to treat *Aeromonas salmonicida*, *Vibrio salmonicida*, *V. anguillarum*, and *Yersinia ruckeri* in trout and salmon. The drug is fed at a rate of 10mg/kg of body weight for 10 consecutive days followed by a 12 day withdrawal.

Perox-Aid: Perox-Aid (hydrogen peroxide) is registered in Canada for treating salmonid eggs for fungus at 500 µL/L for 60 minutes every other day. Although hydrogen peroxide is not yet approved in the United States it is on the low priority list under an INAD as a fungicide for treating fish eggs and an ectoparasiticide for treating fish. The Center for Veterinary Medicine is requiring no tolerance, regulatory methods, or withdrawal times for fish and/or eggs treated with hydrogen peroxide when used at 100 to 500 µL/L (based on 100% activity) to control fungi on all fish species and at all life stages (Rach et al. 1998; Schnick et al. 2001). The optimum dosage to treat fungal infection in catfish eggs is 75µL/L; however, 50 µL/L has been shown to be effective (Howe et al. 1999). Hydrogen peroxide is effective in eliminating *Ambiphrya* and *Gyrodactylus*, but not *Trichodina*, on rainbow trout (Rach et al. 2000). Warmwater and cool-water fish species, especially walleye, are supposed to be more sensitive to hydrogen peroxide than are cold-water species indicating that concentrations should not exceed 150 µL/L for 1 hour (Gaikowski et al. 1999).

Unapproved Drugs of Low Regulatory Priority

Some compounds categorized as drugs of Low Regulatory Priority are used in aquaculture as well as in our everyday lives. These drugs include acetic acid, calcium chloride, calcium oxide, carbon dioxide, Fuller's earth, garlic (whole), ice, magnesium sulfate (epsom salt), onion (whole), papain, potassium chloride, povidone iodine compounds, sodium bicarbonate (baking soda), sodium chloride (salt), sodium sulfite, urea, and tannic acid. However, only a couple of these are used to actually alleviate infectious diseases. The FDA is unlikely to object to their use if the following conditions are met (Anonymous 2001): (1) used for prescribed indications, including species and life stage where specified; (2) used at prescribed dosages; (3) used according to good management practices; (4) the product is of an appropriate grade for use in food animals; and (5) an adverse effect on the environment is unlikely. The position of the FDA on the use of these substances does not constitute approval or endorsement of their safety or efficacy. The following are the most widely used low regulatory priority compounds used for aquatic animal health. (Table 3.2????)

Povidone iodine: Betadine, Wiscodyne, and Novadine-iodine are povidone iodophores used for fish egg disinfection. The iodine compounds are applied at concentrations of 50 mg/L during water hardening for 30 minutes or 100 mg/L after water hardening for 10 minutes.

Sodium Chloride: Sodium chloride (NaCl) is used as a prophylactic ectoparasiticide and to relieve stress in freshwater fish following handling. Depending upon the species, fish may be held for a short period or indefinitely in 0.5 to 1% NaCl for relief of stress and to prevent shock. Fish may also be dipped in 3% NaCl for 30 seconds or until loss of equilibrium.

Environmental Protection Agency Registered Algicides

The EPA has over 100 trade name algicides and herbicides registered for use in the aquatic environment. Only two of these have any significance in controlling disease in aquaculture and another is used to relieve "off flavor" in catfish. As long as these chemicals are used according to EPA approved labels no objections will be made by FDA-CVM if the products have an incidental effect on fish health (Anonymous 2001). (Table 3.3 ???)

Copper Sulfate: Copper sulfate ($CuSO_4$), also known as bluestone, comes in two formulations, “crystal” or “snow”, both of which are 100% active or in liquid form. Copper sulfate is registered by the EPA under a variety of trade names as an herbicide that can be used in water harboring food fish. It is moderately effective for external bacterial and/or ectoprotozoa infections. Water quality determines whether copper sulfate produces an effective drug treatment or whether it is toxic to fish, therefore, it is most often used in low concentrations as an indefinite treatment. Toxicity of copper sulfate varies with its formulation, water pH, total alkalinity (TA) and water hardness. Generally 1.0 mg/L of copper sulfate can be used in water for each 100 mg/L TA. More specifically, when TA is 0 to 49 mg/L, a toxicity test should be run before use; when TA is from 50 to 99 mg/L, use 0.5 to 1 mg/L of $CuSO_4$; 100 to 149 mg/L TA, use 1 to 2 mg/L of $CuSO_4$; and 150 to 300 mg/L TA, use 2 to 3 mg/L of $CuSO_4$. Copper sulfate is ineffective in water with greater than 300 mg/L TA or in salt water. Chelated copper is less toxic than in the sulfate salt form and should not be used unless the aforementioned water chemistry characteristics are known.

Diquat: Diquat has some efficacy against external bacterial infections, especially bacterial gill disease and columnaris. It is applied at 0.25 to 2.5 mg/L indefinitely or 2 to 4 mg/L for 1 hour as a prolonged bath. Diquat is used only in culture systems where it cannot escape and terrestrial animals have no access to the water for 14 days.

Diuron: Diuron (Nautilus Aquatic Herbicide; Drexel Diuron 80) is a herbicide used in catfish ponds to eliminate or reduce bluegreen algae and to prevent or cure "off flavor" in the flesh of marketable channel catfish. It is applied in ponds at a rate of 0.5 oz/acre foot of water every 7 days for a maximum of 9 treatments during the growing season. Annual emergency approval by the EPA is provisional on a state-by-state basis.

Others Drugs

Several new drugs have been investigated for potential use in treatment of fish diseases and are under the Investigational New Animal Drug category by FDA. One of these is Chloramine-T which is an antimicrobial used as a prolonged bath to treat bacterial gill disease in trout. It is applied at 8 to 10 mg/L for 1 hour for 3 consecutive or alternate days. Chloramine-T is now under CVM review pending results of residue studies.

Potassium permanganate is a chemical on which the FDA has deferred regulatory action while data are gathered on its environmental impact and if there is a bioaccumulation of manganese from potassium permanganate exposure in edible tissue; however it is widely used in warmwater aquaculture. Griffin et al. (1999) have reported that bioaccumulation of magnesium in fish flesh does not occur. Potassium permanganate ($KMnO_4$) is a purple crystalline material that is considered 100% active. It is effective for treating some ectoprotozoa and bacterial infections, especially columnaris, and is applied in tanks at 5 to 10 mg/L for 1 hour as a prolonged bath with aeration. Fish should be observed continuously during treatment and at the first hint of discomfort fresh water should be introduced. Pond application rate is 2 to 4 mg/L indefinitely depending on organic content of the water; therefore, for efficacy concentrations must be 2 mg/L over the oxidizing demand of water to be treated (Lau and Plumb 1981). This oxidizing demand, which depends on organic content of the water, can be determined by the concentration of $KMnO_4$ that is necessary to turn water from reddish-purple (active) to brown (inactive) within 15 minutes (Tucker and Boyd 1977); however, the burgundy color should remain for 12 hours after application to be of therapeutic value. The toxicity margin of $KMnO_4$ over the oxidizing demand is narrow; therefore, caution is required at higher application rates. If $KMnO_4$ is used too often on the same fish (minimum of 5 day intervals) severe gill injury will develop. Application of $KMnO_4$ may also cause a reduction of phytoplankton in ponds that results in a temporary lowering of oxygen levels. High cost is also a factor to consider when treating large volumes of water with $KMnO_4$.

Extra-label Use of an Approved New Animal Drug

Extra-label use of a drug is specified by the Administered Drug Under Animal Use Clarification Act (AMDUCA), which allows use of an approved drug in an animal group different from that specified on the label and in a manner that is not in accordance with the approved label directions (Anonymous 2001). These drugs can be prescribed only by a licensed veterinarian who also should be knowledgeable in aquatic animal health.

Summary

While drugs and chemicals are an integral part of aquaculture, success of an enterprize should not be overly dependent upon them. There are fewer available drugs now than in the past and the potential for acquiring new ones is not promising. Drugs that are now available should be used judiciously, discriminately, and according to label instructions and if used properly they can be a benefit to aquaculture in controlling disease and in maintaining of good fish health.

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Chapter 4. Vaccination

Vaccination as a tool for disease prevention is a relatively recent innovation in fish health management and only in the last 10 years has much attention been given to developing immunogens for warmwater fish. Attributes and characteristics of an effective vaccine are (Leong and Fryer 1993):

1. Provide adequate immunoprotection for a specific disease under intensive rearing conditions.

2. Provide protection when the animal is most susceptible to disease.

3. Provide protection of long duration.

4. Protect against all serotypic variants of the disease agent.

5. Easily administered; preferably orally, immersion, or spray and application should minimally disrupt the normal management routine.

6. Safe for the vaccinated animal.

7. Economical to produce and license and cost effective.

Vaccines present distinct advantages over drugs by reducing the impact of disease, decreasing the need for drugs, providing long term protection which often continues until slaughter, and may provide a more fixed-cost disease prevention expenditure. In many instances vaccinated fish have a more favorable food conversion ratio and better growth than unvaccinated fish, and no residue remains in edible flesh unless adjuvants are used.

Vaccines do not always completely eliminate pathogens or prevent target organisms from being present in vaccinated populations; therefore, some vaccinated fish may become carriers and reservoirs for pathogen. Vaccines can be expensive to make and apply but generally a low percentage of improved survival justifies the cost. For example, an improved survival of about 5% in channel catfish vaccinated against *Edwardsiella ictaluri* can economically justify the expense.

Antigens: The U. S. Department of Agriculture lists 15 different vaccines for use in fish (Anonymous 2001). To date most bacterial vaccine research, development, and application have

targeted salmonid pathogens *(Aeromonas salmonicida, Yersinia ruckeri, Vibrio anguillarum*, and *V. salmonicida*). Current vaccine research and development for nonsalmonids target *E. ictaluri*, *Photobacterium damsela* subsp. *piscicida*, *Streptococcus iniae*, *Flavobacterium columnare*, and *Ichthyophthirius multifiliis*. Only AQUAVAC-ESC (Intervet), containing attenuated *E. ictaluri* to prevent enteric septicemia of catfish, is currently available as a vaccine for warmwater fish in North America.

Fish virus vaccine research has lagged behind that for bacterial pathogens, however, some are now being developed with attenuated, chemically inactivated, and subunit (recombinant DNA) viral preparations. These include vaccines for infectious pancreatic necrosis virus, viral hemorrhagic septicemia virus, infectious hematopoietic necrosis virus, infectious salmon anemia virus, channel catfish virus, and spring viremia of carp virus.

Vaccine Preparations: Most bacterial fish vaccines are bacterins to which a substance (usually formalin) has been added to kill the pathogen. Bacterins are relatively easy to make but formalin or other additives can alter the organisms antigenic quality, thus reducing its efficacy. Vaccines with attenuated organisms are potentially more effective than killed bacterins because they stimulate cell mediated and humoral immune response (Shoemaker et al. 1997). In some channel catfish diseases (i.e. *E. ictaluri*), and possibly others, live bacterial cells may be essential to stimulate a cell mediated response and elicit protection. Also, because many pathogens of warmwater fish are facultative, vaccines against them may be of little or no value.

Adjuvants: Immunostimulants (adjuvants) in fish vaccine preparations have received a lot of attention. Anderson (1992) listed 19 different adjuvants, or vaccine carriers, but not all were equally suitable or effective for fish. These substances include glucans and other yeast extracts, extracts of abalone, lipopolysaccharides (LPS), rough mutants, natural or synthetic oil-based materials, and pre-vaccination salt baths. Adjuvants are added to oral, injection, or immersion vaccines to enhance immune response, increase longevity, and/or broaden effects of the vaccine, while decreasing antigenic specificity of immunity.

Vaccine Administration: Fish vaccines are applied by injection, immersion, spraying, or orally in feed. Univalent injectable vaccines provide the highest level of protection, require relatively small amounts of vaccine, and are economical for use with larger and highly valuable fish. Injectable multivalent vaccines become more cost effective when the broad protection they

provide is taken into account (Press and Lillehaug 1995). Although, semiautomated equipment is available, vaccination by injection is labor intensive and requires handling of individual fish.

Immersion vaccination constitutes dipping fish in a solution of diluted vaccine for seconds to minutes while the antigen is absorbed through the skin, across the gill membrane, and/or by ingestion. Immersion vaccination is easily incorporated into the culture routine, can produce an acceptable level of protection, and fish are less stressed than when injected. However, immersion does require handling fish, can be labor intensive, and vaccination of larger fish is not economical.

Spray or shower is a modification of immersion vaccination which can accommodate a higher weight of fish per unit of vaccine volume. Disadvantages include a need to handle fish and it is labor intensive, but specialized semiautomated equipment can expedite the vaccination process.

Oral vaccination is logistically easy and fish do not have to be handled, however, protection varies from poor to moderate. Oral vaccines may be useful as a secondary or booster vaccination (Thune et al. 1997). The efficacy of orally delivered fish vaccines is hindered by the potential for antigenic proteins to be denatured by stomach acidity before they can be absorbed by the intestine and gain access to immunologically competent tissue. This problem can be circumvented by encapsulating the antigen before it is incorporated into feed.

Problems

Factors that influence successful vaccination and protective immunity in fish are: vaccine characteristics, physiological condition and nutritional well being of the fish, and quality of environmental conditions, age and/or fish size, temperature at which they are vaccinated, and level and duration of protection. Most fish have a minimum age or size at which they become immunocompetent. Channel catfish as young as 7 days post hatch are immunocompetent when exposed to a live modified immunogen (Shoemaker et al. 1999).

The ability of the immune system to respond to antigens can be compromised by poor water quality (low oxygen, high ammonia, etc.) and stressful conditions which cause fish to produce corticosteroids that suppress immune response (Pickering and Pottinger 1985). Sublethal concentrations of toxicants, particularly phenols, severely reduce immunity and render aquatic animals more susceptible to infectious disease (Ellis 1988). It has been reported in the

United Kingdom that trout vaccinated against *Y. ruckeri* failed to be protected when environmental conditions were less than favorable or when fish were in poor condition (Rogers 1991). High population density also reduces immune response. Channel catfish vaccinated against *E. ictaluri* and stocked at high density had poorer survival than vaccinated fish stocked at lower densities (Plumb et al. 1993).

Water temperature is the principal immunomodulator in fish (Avtalion 1981). Temperature does not determine if an immune response occurs but regulates the rapidity and degree to which it develops. The optimum temperature for immunization corresponds to the optimum temperature in which the fish normally lives. Generally, salmonids respond better when vaccinated at 15 to 18°C, while warmwater fish respond better when vaccinated at 20 to 30°C. Bly and Clem (1991) demonstrated a temperature dependent immunity and suggested that channel catfish are immunocompromized during winter when low temperatures may actually suppress immune response. However, a secondary response occurs when fish are again exposed to the target antigen at a warmer temperature.

Summary

Vaccination does not as yet play an intricate role in the health maintenance of warmwater and cool-water fishes but may eventually become a more routine and accepted part of warmwater aquaculture. While there have been a few vaccination successes reported in warmwater fish many problems must still be overcome before it becomes standard operating procedure. In many cases for optimum success, suitable antigens, vaccine preparation, delivery methods, optimum fish size, and suitable environmental parameters for vaccination must be determined. Vaccines are another valuable tool to be used in aquaculture but will never replace the need for good health management. Stressful culture conditions have the potential to override any protective benefit that vaccines may provide.

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Chapter 5: Disease Recognition and Diagnosis

Because many diseases of warmwater fish are caused by secondary pathogens or multiple infections and they share similar clinical signs, a complete and accurate diagnosis is essential. Suspected diseased fish should be sent to a state or provincial diagnostic laboratory where the disease occurred or to a regional federal fish health laboratory. The laboratory should be contacted for instructions on the proper method of submitting suspect fish. Live or moribund specimens are preferred but fish placed on ice and delivered within a few hours are usually acceptable for most diagnostic procedures.

Although infectious agents play a part in the overall health status of fish, other factors must also be considered when making a diagnosis (Meyer and Barclay 1990; Noga 1996; Plumb 1999). In conjunction with identification of parasitic, bacterial, or viral agents a history of events preceding disease should be provided (Lasee 1995).

History: The history of a fish population exhibiting morbidity or mortality should include species, origin, size, age, number, previous diseases, type of holding facility, and how long clinical disease signs have been noted; mortality, behavior and feeding patterns; and type of any administered treatments. Desirable environmental and water quality data should include temperature, dissolved oxygen, carbon dioxide, ammonia, and nitrite concentrations; any recent change in water color, clarity, or flow; size of culture unit and stocking density; weather conditions just prior to mortality and agricultural activities in the area.

Mortality Pattern: Mortality pattern may indicate if cause of death is due to a communicable agent, poor water quality, or a toxicant. Infectious diseases seldom result in "overnight" mass mortality, but more often are manifested in a gradual increase in subacute or chronic mortality which results in cumulative losses of 30 to 40% or less over days or weeks. Mortalities due to infectious agents occasionally exceed 80% when highly virulent pathogens infect susceptible fish. When the majority of a fish population dies overnight or in a 24-hour period, oxygen depletion, chemical toxicants, or other environmental causes should be strongly considered. Mortalities due to nutritional deficiencies are usually very protracted.

Clinical signs: “Clinical signs” in warmwater fishes include behavioral, physical, or other external pathological changes which, when combined with gross internal pathology can aid in diagnosing disease. However, in most cases, it is difficult to diagnose a specific disease or determine a specific etiological agent based solely on clinical signs because very few of these signs are disease specific, especially in warmwater fish.

Initial fish reaction to onset of disease is often a change in feeding behavior but stress of any type can cause fish to "go off feed." Diseased fish may swim lethargically into shallow water, gasp at the surface, lie listlessly or float on the surface of the pond or tank bottom, swim erratically, or rub against underwater structures. Some disease agents cause fish to swim in a longitudinal spiral or "tail chase" in a circle. Crowding around a water inlet to take advantage of oxygenated water may indicate diseased gills or low dissolved oxygen concentrations. Sick fish may gather in a tight school, ride high in the water, or disperse evenly throughout the water.

Gills are adversely affected by viruses, bacteria, and parasites. They can become frayed and necrotic (gray or white), pale (anemic), swollen as a result of hyperplasia, or can produce excessive mucus which interferes with oxygen absorption from the water, thus causing affected fish to crowd around water inflow.

Skin lesions of diseased fish are diverse and usually nonspecific. Ectoprotozoa or monogenetic trematodes stimulate excess mucus production that will give fish a grayish appearance. Lesions resulting from bacterial infections may appear as slightly raised or swollen depigmented areas. In advanced stages of infection, or when pathogens severely affect the skin, the epithelium and dermis can be totally missing (necrotized and sloughed into the water) leaving exposed musculature. Necrotic lesions accompanied by areas of deep ulceration can occur in muscle, or on the opercle, head, or mouth. Margins of necrotic, ulcerative lesions are often surrounded by inflammation or hemorrhage in the epithelium.

Infected fins become hyperemic or pale and necrotic. Injury may progress until soft fin tissue is destroyed leaving only hard spines or fin rays. Bacterial and viral infections are often characterized by raised scales, a swollen body due to fluid accumulation in the musculature (edema, hydropsy), and swollen abdomens (fluid in the body cavity - ascites). Growths on the body and fins may appear as cotton-like material (fungus); solid tumors of various size, color and

texture; or as parasites embedded in/or beneath the skin. Embedded helminthic parasites appear as raised yellow, white, or black spots in the skin.

Fish with systemic bacterial infections often have a prolapsed swollen and red anus. Protruding (exophthalmic), hemorrhaged, or opaque eyes may be associated with an accumulation of fluid (edema) or inflammatory exudate behind the eye caused by viral or bacterial infection, helminth parasites, or supersaturation of gas in the water. Opaqueness of the eye may also be due to trematode or nematode infestations, dietary deficiency, or bacterial infection.

Mortalities caused by acute water quality changes or toxicants generally affect most species of fish present over a short period of time. These fish generally show no external lesions or clinical signs other than flared gill covers and gaping mouth at death; but some chemical toxicants will cause hemorrhagic inflammation of the skin and fins. Fish suffering from oxygen depletion will gasp at the surface and will have dark red gills. Fish deformities involving the head and/or spine are common. In warmwater fish these deformities are usually dietary (vitamin C deficiency) or pesticide related, or the result of trauma.

Gross Internal Lesions: Internal gross lesions may help determine whether an infection is viral, bacterial, or parasitic. Clear, straw-colored fluid in the abdominal cavity usually indicates a viral infection but there are exceptions. Bloody, cloudy fluid and/or large white pustules or variable size granulomas on visceral organs usually indicate a bacterial infection. Some bacterial infections cause the visceral cavity to have an intense putrid odor even in fresh fish. The presence of small white or yellow uniform-sized cysts in internal organs are indicative of a metazoa parasitic worm larvae.

A uniform hyperemia or hemorrhage in the viscera is indicative of a viremia or bacterial septicemia in which case the spleen is usually dark red and enlarged and the liver may be friable, soft and pale or mottled with petechiae. The kidney is often swollen and soft. Intestines are usually devoid of food, may contain white or bloody mucoid material, and the intestinal wall is often flaccid and reddish. Blood in internal organs may appear brown due to methemoglobin anemia (brown blood) caused by nitrite toxicity.

Disease Diagnosis

Because only a small number of representative animals from a population are necropsied, it is essential that these fish represent the affected population. Moribund specimens exhibiting clinical signs are best for necropsy and yield the most dependable results. Fish that have been dead more than 1 hour should not be used for diagnosis unless they were put on ice immediately upon death. Dead fish that have lost normal skin color, have pale and soft gills, cloudy eyes, and an offensive odor have probably been dead too long. Upon death, parasites tend to leave the gills and skin, bacteria escape the gut and invade visceral organs and body cavities, and bacteria present in the water may invade the skin. Fish caught by angling are not suitable for diagnosis because they are generally healthy.

Disease diagnosis should include examination for ectoparasites, isolation of possible viruses and/or bacteria, and pathogen identification. If a pathogen is found on initial examination the process should be completed because multiple infections may be present and successful therapy will require that all pathogens be addressed.

Parasitic Diseases: Necropsy of diseased fish is not complete without considering parasitic pathogens (Thoesen 1994; Lasee 1995; Mitchum 1995; Noga 1996; Hoffman 1999). When examining living fish for ectoparasites it is better to kill the fish by pithing rather than an overdose of anesthetic that may affect the ectoparasites. Before disinfecting the fish's skin with alcohol for an aseptic necropsy, examination of the gills and skin should be made and material from them examined for parasites in wet-mounts under light microscopy at low and high magnification. Phase contrast microscopy and histological sectioning of tissues may also aid in detecting parasites.

Many metazoa parasites appear as small white, yellow, or black cysts in tissues and internal organs and may contain larval or adult nematodes, trematodes, or cestodes. These tissue dwelling parasites can be detected by dissecting cysts and examining them in wet mounts.

Viral Diseases: Detection and diagnosis of fish virus diseases require special expertise and equipment (Wolf 1988; Sanz and Coll 1992; Thoesen 1994). Detection methods include virus isolation in tissue culture, serological and molecular procedures, and in some cases electron microscopic examination. Biotechnical and molecular procedures such as immunofluorescence,

enzyme linked immunosorbent assay, and polymerase chain reaction are available for identification of many fish viral agents. However, when these methods are used for detection of covert viral infections they should be accompanied by confirmatory testing when validation procedures are available. Some viruses are known only via electron microscopy, but generally, virus isolation in tissue culture continues to be the best method for diagnosis followed by serological or molecular confirmation. Type of tissue used for virus assay is based on size and age of fish. Ovarian fluids are assayed when testing broodfish for virus during egg collection.

Viruses are not visible under light microscopy because they are generally less than 0.3 µm in size. Therefore, living cells grown *in vitro* are used to detect virus where infected cells will develop cytopathic effect (CPE) that is visible with light microscopy. Many permanent fish cell lines are available for virus diagnosis and research but cell lines derived from the same species, or a species phylogenetically close to the one being assayed, should be used when available (Table 5.1). However, many known fish viruses replicate in multiple cell lines.

Table 5.1. Viruses of warmwater and cool-water fishes and cell lines used for isolation.

Virus	Cell line
Channel catfish virus (CCV)	Channel catfish ovary (CCO) or Brown bullhead (BB)
Diffuse epidermal hyperplasia of walleye	Walleye ovary (WO) or Walleye embryo (We-2)
Discrete Epidermal hyperplasia of walleye	None
Koi herpesvirus (KHV)	Koi fin (KF-1)
Largemouth bass virus (LMBV)	Fathead minnow (FHM)
Lymphocystis	Bluegill fry (BF-2)
Spring viremia of carp (SVC)	Epithelioma papillosum of carp Rainbow trout gonad (RTG-2)
Walleye dermal sarcoma (WDS)	None
White sturgeon herpesvirus-1	White sturgeon skin (WSSK-1) and -2 (WSHV-1 and WSHV-2)
White sturgeon iridovirus (WSIV)	White sturgeon spleen (WSSV-2)

Bacterial Diseases: Diagnosis of most bacterial fish diseases is accomplished by isolating bacteria and identifying the organism biochemically, serologically or by molecular methods. However, some bacterial organisms are fastidious and difficult to isolate on culture media.

Scrapings should be taken from external lesions and examined microscopically for ectoparasites, bacteria, and fungi before they and skin are disinfected with alcohol or a hot scalpel prior to making an incision. Internal organs can be accessed through the abdominal cavity by disinfecting the skin surface and removing the muscle flap from the left side of the body cavity via three cuts: (1) medial from anterior of the rectum to the isthmus, (2) anterior of the rectum along the dorsal line of the coelomic cavity to the upper insertion of the gill cover, and (3) and behind the gill from the isthmus to above the gill cover. An alternative approach to accessing the kidney is to disinfect the dorsal area and cut through the back muscle and spinal column.

Most bacterial organisms that infect warmwater or cool-water fish grow on general laboratory media such as brain heart infusion, tryptocase soy, nutrient, or blood agar; but specialized media are required for isolating flavobacteria, flexibacteria, and mycobacteria. Incubation temperatures are not critical for most of these bacteria, but generally are cultured at 20 to 30°C.

Bacteria isolated from clinically diseased fish may be presumptively identified based on relatively few biophysical and biochemical tests using conventional bacteriological procedures and tube media (Plumb and Bowser 1983; Shotts and Teska 1989; Shotts 1994). Presumptive identification criteria include bacterial cell morphology, motility, Gram stain reaction, production of cytochrome oxidase, reaction on carbohydrates (primarily glucose); indole, gas, and hydrogen sulfide production; temperature sensitivity, and sensitivity to certain growth inhibitors (0/129 or Novobiocin). Dichotomous keys are helpful in identifying many fish pathogens to genus and by using a variety of additional tests they can be further classified to species (Shotts and Teska 1989; Shotts 1994).

A battery of conventional tube media tests can be used to identify bacteria but are labor intensive and may take several days. Numerous commercially prepared systems can be used to shorten identification time but these systems are not always accurate for fish pathogens. The most popular of these systems are API, Crystal, Biolog GN Microplate, Abbott Diagnostics, and Vitek Systems, all of which were originally developed for human or environmental microbiology and

attempts to adapt fish pathogens to them have not always been completely successful (Shotts and Teska 1989; Teska et al. 1989; Taylor 1995; Robohm 1997). Most fish pathogens do not occur in numerical data bases because these systems require an incubation temperature of 35 to 37°C, which is higher than that normally used for fish pathogens.

To hasten identification and increase accuracy, serology and biotechnical procedures are used to detect some pathogens in tissues or after bacteria have been isolated and cultured. Serological procedures utilize agglutination, direct and indirect fluorescent antibody (IFAT), several variations of enzyme linked immunosorbent assays (ELISA), radio labeling, and polymerase chain reactions (PCR) (Schill et al. 1989).

Summary

Recognition and diagnosis of infectious fish diseases require expertise in several areas. It is not sufficient for a diagnostician to only identify a disease agent but he/she must also understand the aquatic environment and its role in the occurrence of disease. Only by knowing and understanding the relationship between fish, pathogen, and environment can diseases be better diagnosed, controlled, and steps taken to prevent reoccurrence.

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Part II

Warmwater and Cool-water Fish Diseases

6. Channel Catfish Virus
7. Largemouth Bass Virus
8. Spring Viremia Of Carp
9. Koi Herpesvirus
10. Lymphocystis
11. Discrete Epidermal Hyperplasia of Walleye
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25. Bothriocephallosis
26. Other Parasites

Chapter 6: Channel Catfish Virus

Channel catfish virus disease (CCVD) is an acute, communicable infection of cultured fry and fingerling channel catfish, the etiological agent of which is channel catfish virus (CCV).

Signs of Disease

Infected fish swim erratically or convulsively, sometimes rotating about their longitudinal axis. Moribund fish hang with their head up at the surface or sink to the bottom, become quiescent, and respire weakly but rapidly prior to death. Clinical signs of CCVD include abdominal distention due to fluid in the body cavity, exophthalmia, pale gills, and hemorrhage at the base of fins and throughout the skin, particularly on the ventral surface (Fijan et al. 1970). The body cavity contains a clear, yellow fluid and the visceral organs are generally hyperemic although the liver and kidney may be pale. The spleen is generally dark red and enlarged; the stomach and intestine are void of food, but contain a mucoid secretion.

Diagnosis and Detection

Any time a sudden increase in morbidity occurs among young channel catfish during the summer, CCV should be suspected and fish necropsied for the virus. Homogenized and decontaminated whole fish and/or viscera from virus suspect populations are inoculated onto channel catfish ovary (CCO) cells or brown bullhead (BB) cell cultures (Table 5.1) (Bowser and Plumb 1980). Channel catfish virus cannot be isolated from decomposing infected fish; however, it survives for up to 14 days in viscera of whole iced fish, and 4 to 6 months in frozen fish. The virus replicates in the nucleus of CCO cells at 15 to 35°C with about 30°C being optimum. Initial CPE consists of foci of pyknotic cells that may be visible in 12 to 24 hours following incubation at 30°C. Foci of CPE coalesce into multinucleated syncytia that are connected to other foci or normal cells by protoplasmic bridges resembling irregular spokes of a wheel. Rounded cells form a loose network and individual cells are released from the substrate.

Channel catfish virus is a herpesvirus with *Herpesvirus ictaluri* suggested as the specific epithet (Wolf and Darlington 1971). It is enveloped, icosahedral, with a DNA genome, and a nucleocapsid diameter of 95 to 105 nm; enveloped virions measure of 175 to 200 nm. Positive CCV identification of isolated virus is made by neutralization using CCV antiserum produced in

rabbit, goat, or fish and by fluorescent antibody or PCR in cell culture and in fish tissue (Hedrick et al. 1987; Baek and Boyle 1996).

A lack of consistency in isolating CCV from possible carrier fish makes it difficult to determine a fish populations infection status. However, CCV antibody is detectable in channel catfish that have been exposed to the virus, thus, providing criteria for separating virus exposed from non-exposed fish (Plumb 1973; Amend and McDowell 1984). Neutralization of CCV with serum from adult channel catfish showed that 40% of tested brood populations in California were positive for CCV antibody but only about 30% of these populations produced CCVD infected offspring (Amend and McDowell 1984). Crawford et al. (1999) used ELISA to show that channel catfish become seropositive for CCV between 22 and 28 days after first exposure, further suggesting a possible procedure to screen large numbers of fish to determine prior exposure and potential for being CCV carriers.

Epizootiology

Channel catfish virus occurs in all southern states, and most other states where channel catfish are cultured; however, to date the virus has not been detected in wild or feral fish. Juvenile channel catfish are most susceptible to CCV but fingerling blue catfish and channel catfish X blue catfish hybrids are also susceptible by injection (Plumb and Chappel 1978).

Most clinical CCVD outbreaks occur during June through October when water temperatures exceed 25°C. Epizootics occur most frequently during years when water temperatures are high, in heavily stocked fingerling ponds, or following handling and/or transport. Fry and fingerling channel catfish transmit virus horizontally during an active infection via water or possibly cannibalizim. Fish less than 4 mo. old are most susceptible to CCV with younger fish suffering the highest mortality. Often CCVD occurs in conjunction with a secondary *Flavobacterium columnare* infection which may prolong the disease.

Incubation time between fish exposure to CCV and appearance of clinical signs and morbidity is inversely related to water temperature. At 30°C first deaths occur in about 48 hours and up to lOO% die within 6 days; at 20°C incubation time is 10 days and mortalities are less than 50% (Plumb and Gaines 1975). The kidney, liver, spleen, and intestine become active sites of virus replication 24 to 48 hours after infection. During periods of overt virus infection fish shed

virus into the water (Kancharla and Hanson 1996). As clinical disease abates and the mortality rate subsides, virus concentrations decrease and can no longer be isolated from surviving fish after deaths cease. However, on one occasion CCV was isolated from asymptomatic adult channel catfish during the winter when the fish's immunity was presumably low (Bowser et al. 1985).

Presumptive evidence suggests that CCV is vertically transmitted from parents to progeny (Wise et al. 1988). Channel catfish virus nucleic acid was detected in gonads of adult fish by FAT (Plumb et al. 1981) and a CCV specific DNA probe clearly demonstrated CCV nucleic acid in livers of adult channel catfish including those from which virus had been isolated (Wise and Boyle 1985). Baek and Boyle (1996) used a nested PCR procedure to detect CCV DNA in asymptomatic adult channel catfish while Gray et al. (1999) showed that juvenile channel catfish survivors of an experimental challenge had CCV DNA in leukocytes, brain, intestine, kidney, and liver 140 days after exposure to virus. These data confirm that surviving channel catfish do develop a latent CCV infection and may be a source of the virus.

Management

Because vertical virus transmission has not been conclusively demonstrated but strongly suspected, it is recommended that survivors of CCV epizootics not be stocked into non-infected waters or used as broodstock. However, positive identification of CCV carrier fish is not possible by isolation in cell culture. Serological or molecular techniques are also means for distinguishing and separating potential carrier from non-carrier populations, thereby providing a first line of defense against the virus.

Stressful environmental conditions and transportation for susceptible fish during warm summer months should be avoided. Bacterial infections, especially “columnaris”, should be treated promptly and aggressively.

Ponds from which diseased fish are removed should be drained and dried or disinfected with 40 - 100 mg/L of chlorine. Survivors of CCV epizootics may be grown to a marketable size, but it is recommended that they be segregated from ponds containing healthy, susceptible channel catfish.

Significance

Because of the susceptibility of young of the year fish, CCV poses a significant threat to cultured channel catfish. However, only 1 to 2% of total disease cases examined at fish disease diagnostic laboratories in southern United States involves CCVD. Thus, its overall effect on the channel catfish industry has not been great.

While there is always the possibility that CCVD could be transmitted to areas where the virus does not now occur, the potential for CCV to have an impact on the Great Lakes basin is minimal due to less than optimum water temperatures for the disease and limited culture of the species in the region. However, CCV could have a significant effect in channel catfish facilities which use artificially heated water; therefore, stocking the largest fingerlings possible (10 cm or greater) is recommended in these situations.

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Chapter 7: Largemouth Bass Virus

Largemouth bass virus (LMBV), the cause of largemouth bass virus disease (LMBVD), was the first systemic virus reported from any centrarchid. The initial isolate of LMBV came from adult largemouth bass in Santee Cooper Reservoir, South Carolina in 1995 (Plumb et al. 1996). However, evidence obtained by PCR indicates that an iridovirus isolated from largemouth bass in a central Florida lake in 1991 was identical to the virus from South Carolina (Grizzle et al. in review).

Signs of Disease

Adult largemouth bass with clinical LMBVD loose their equilibrium to varying degrees, may float at the surface and have swollen abdomens which are apparently due to enlarged swim bladders. Internally, infected fish often appear normal except that it may contain a brownish-yellow mucoid exudate the gas gland is hyperemic. Experimentally injected adult largemouth bass develop a transient, inflamed, necrotic skin lesion (0.5 X 1.0 cm) at the injection site. Virus injected juveniles become dark, display distended abdomens, a lesion develops at injection site, and fish begin to die in 3 days (Zilberg, et al. 2000). Juvenile fish swim in a corkscrew fashion and moribund fish become laterally recumbent before death. Internally, the liver is focally pale, the spleen bright red, and the intestine has a reddish color.

Diagnosis and Detection

Largemouth bass virus disease is diagnosed by isolation in FHM or BF-2 cells which have been inoculated with filtrates of homogenized internal organs (Table 5.1). For isolation purposes, LMBV survives for at least 155 days in frozen fish (Plumb and Zilberg 1999a). Focal CPE develops within 24 hours and consists of a few pyknotic cells that form circular, cell-free areas with rounded cells at the margins. As CPE progresses, additional cells become pyknotic, rounded, detached, and the entire cell sheet is eventually affected. Culture media from infected FHM cells may contain $10^{8.9}$ $TCID_{50}$/mL virus particles. Currently, PCR is used to confirm isolation of LMBV, however, the primers identify ranaviruses and are not specific for LMBV (Mao et al. 1999). When comparing the accuracy of LMBV isolation in cell culture to PCR, J. M. Grizzle (Department of Fisheries and Allied Aquacultures, Auburn University, Alabama, personal

communication) showed that a higher percentage of fish were LMBV positive by PCR than by cell culture isolation.

The unenveloped LMBV virion is icosahedral, cytoplasmic, and measures 132 to 145 nm in diameter; the enveloped virion measures about 174 nm. Immunofluorescence and PCR indicates that LMBV is similar to an iridovirus isolated from guppy and doctorfish (Hedrick and McDowell 1995). Biophysical, biochemical, and PCR characterization confirms that LMBV is a ranavirus within the *Iridoviridae* (Mao et al. 1999, Piaskoski et al. 1999).

Carrier fish are detected by isolating LMBV from asymptomatic fish during late spring through fall with peak virus titers occurring during summer. Virus is less likely to be isolated during winter.

Epizootiology

In a survey of 78 reservoirs in 8 southeastern states, LMBV was isolated from wild adult largemouth bass populations in 8 reservoirs in 3 states (Plumb et al. 1999). A subsequent survey by the U. S. Fish & Wildlife Service found LMBV infected largemouth bass in rivers and reservoirs in most southern states from Florida to Texas and north to Oklahoma and Missouri (N. Hile, U. S. Fish and Wildlife Service, Warm Springs, Georgia; personal communication). The first LMBV isolate in the northern United States was from largemouth bass in a lake on the Indiana-Michigan border, however, recent reports indicate that the virus is also present in several additional Indiana and Michigan lakes (J. Hnath, Michigan Department of Natural Resources, personal communication) and in two hatcheries and four reservoirs in Illinois (Tony Goldberg, College of Veterinary Medicine, University of Illinois, personal communication).

Largemouth bass are most susceptible to LMBV, but it has also been isolated from wild smallmouth bass, spotted bass, Suwanee bass, black and white crappie, redear sunfish, and bluegill (N. Hile, personal communication). More recently LMBV was isolated from chain pickerel (J. M. Grizzle, personal communication). The species diversity from which LMBV has been isolated suggests that the virus is not specific for largemouth bass**,** however, no mortalities have been reported in other species. Juvenile striped bass are also experimentally susceptible but to a lesser degree than largemouth bass (Plumb and Zilberg 1999a).

In southern United States all reported epizootics of LMBV have occurred during July through September in adult fish weighing from 1 to 5 kg. Mortality in wild populations is difficult to assess but in the original outbreak in a 12,000 ha reservoir in South Carolina about 1,000 dead adult fish were counted. In a Mississippi investigation of an LMBV associated epizootic approximately 3,000 dead adults were reported (Hanson et al. 2001a). Deaths in the Michigan and Indiana lakes were conservatively estimated at 200 to 400 per lake (J. Hnath, personal communication). Although the virus has been isolated from asymptomatic adult and juvenile largemouth bass in hatcheries no major epizootics have been reported in cultured fish.

Mortalities of largemouth bass have been associated with LMBV in wild populations, but the virus' pathogenic capability remains unclear. Several epizootics have occurred in which LMBV was concurrent with sufficient facultative bacteria and/or parasites to suggest that the virus may not have been the primary pathogen. Also, the role of environmental stressors in the epizootiology of LMBV is unknown, but could be significant.

Injection experiments with adult largemouth bass (0.5 to 1 kg) produced no mortality or adverse effects except for a transient skin lesion at the injection site (Plumb et al 1996). Nevertheless virus titers of $10^{2.3}$ to $10^{7.5}$ TCID50/g occurred in normal muscle and necrotic muscle at the injection site and in all internal organs for 28 days post infection. In infectivity studies of LMBV in juvenile largemouth bass, mortality as high as 100% resulted, whereas, only 20% mortality was observed in a cohabitation study (Plumb and Zilberg 1999b).

The fact that LMBV was isolated from asymptomatic fish in several reservoirs for a two-year period following LMBV associated mortalities suggests that the virus has an extended survival capability. In a study of 15 reservoirs in Mississippi by Hanson et al. (2001b) it was found that fish in 73% of these reservoirs had LMBV carrier fish in the spring compared to 27% the previous autumn. Percentage of LMBV positive fish in the reservoirs ranged from 0% to 86%. They concluded from this study that LMBV is sustainable in largemouth bass populations, incidence of infectivity increases over time, virus is passed from generation to generation and adult carrier fish are the presumed virus reservoir. Because LMBV has been isolated from gonadal tissue, vertical transmission is a likely route of infection . However, in studies by Woodland et al. (in press) fish captured from an LMBV-positive reservoir for broodstock were virus negative during the early spring in the hatchery where they were being held. Their offspring

also tested LMBV negative but it was suggest that the virus can be distributed to noninfected waters by stocking infected fish.

Management

The current approach to managing LMBV is to monitor fish populations and avoid using fish from virus positive populations for hatchery broodstock. Virological assays of potential broodstock should be done between late spring and early fall to determine LMBV carrier status. However, recent sampling data indicates that some populations have a very low incidence of LMBV positive fish which could be missed by conventional sampling methods (J. Grizzle, personal communication). As LMBV specific PCR primers are developed, molecular procedures may be employed to better indicate possible carrier populations. To prevent spread of the virus within the Great Lakes basin, no fish should be transferred from an LMBV positive body of water to one where the virus is not known to exist. It also seems prudent that all centrarchids imported into the basin come from inspected LMBV free sources. Fishermen and boaters should be encouraged to dry trailers, boats, live wells and other equipment between visits to different waters in the basin to prevent transfer of LMBV.

Significance

The significance of largemouth bass virus is not clear. Experimental transmission studies indicate that the virus may not be highly pathogenic, therefore, its role in fish kills is not conclusive. There are no reports of reoccurring epizootics in successive years in a single lake but anecdotal evidence indicates that largemouth bass fishing declines for 1 or 2 years following an LMBV epizootic and recovers thereafter. Species susceptible to LMBV are indigenous to the Great Lakes basin, hence, the virus could have a negative impact on the fishery resources if it were to become widespread in the region. In view of this, regulations are recommended regarding the introduction and transfer of LMBV infected largemouth bass into and within the Great Lakes basin.

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Chapter 8: Spring Viremia of Carp

Spring viremia of carp (SVC) is a subacute to chronic disease of cultured yearling, sub-adult, and adult common carp. The virus was first isolated in the former Yugoslavia from common carp with clinical signs of erythrodermatitis and infectious dropsy, both of which are bacterial diseases (Fijan et al. 1971).

Signs of Disease

Early in an SVC infection, fish are attracted to the water inlet and as infection advances they become moribund, respire slowly and lie on their side (Fijan et al. 1971). External clinical signs include darker pigmentation, enlarged abdomen, protruding eyes, an inflamed prolapsed anus, and pale gills with distinct petechiae. A generalized hyperemia is apparent in the viscera with peritonitis, enteritis, and hemorrhages in the kidney, liver, and air bladder. The liver is edematous with adhesions and hemorrhages in the muscle tissue. In small carp, the swim bladder is inflamed with focal hemorrhaging or petechiae.

Diagnosis and Detection

Spring viremia of carp virus (SVCV), *Rhabdovirus carpio* (family *Rhabdoviridae*), is a bullet shaped RNA virus which measures 70 X 180 nm (Ahne 1976). The most sensitive cell lines for virus isolation include fathead minnow (FHM), epithelioma papillosum of carp (EPC), or primary carp ovary cell cultures (Table 5.1). Cytopathic effect consists of degeneration and rounding of cells which detach from the substrate. Affected cells exhibit nuclear chromatin granulation, thickening and lysis of nuclear membrane, and cytoplasmic degeneration. Rainbow trout gonad (RTG-2) and bluegill fry (BF-2) cells are also susceptible, in which CPE apparently develops more slowly.

Rhabdovirus carpio is neutralized by specific antiserum, but some cross neutralization may occur with the pike fry rhabdovirus (PFRV) (Vestergaard-Jorgensen et al. 1989). The virus can also be detected in frozen liver, kidney and spleen tissues by either immunoperoxidase (ELISA), or fluorescent antibody (Way 1991).

Screening for possible carrier populations is done by virological assay of the kidney, spleen, gill, and brain (Anonymous 2000). Filtrates are inoculated onto FHM or EPC cells and

viral identification is confirmed by ELISA or FAT (Vestergaard-Jorgensen et al. 1989). Fish exposed to SVCV produce virus antibodies that can be detected by either ELISA or FAT and used to identify potential carrier fish (Dixon et al. 1994).

Epizootiology

To date SVCV occurs only in Europe, having been confirmed in at least 11 European countries (Wolf 1988). Species of fish naturally infected are common, crucian, bighead, silver, and grass carp. Even though the rhabdovirus isolated from (northern) pike fry (PFRV) is similar to SVCV it causes an entirely different disease syndrome. In pike fry the cranium becomes enlarged and in larger fingerlings the lateral musculature is hyperemic (de Kinkelin et al. 1973; Ahne 1986).

Spring viremia of carp occurs primarily in pond populations of yearling or older fish at water temperatures of 12 to 22°C, with an optimum of 16 to 17°C (Baudouy et al. 1980). Severity of SVC varies from site to site and from year to year on a given farm, but 70% mortality has been reported in some naturally infected yearling populations. Experimentally infected carp become resistant to repeated challenges with SVCV, suggesting an acquired immunity. Pike fry infected with the virus experience very high mortality.

Spring viremia of carp can be transmitted by waterborne exposure, cohabitation of infected and healthy fish, contaminated food organisms, and by intraperitoneal, intramuscular or intracranial injection. Incubation period for SVC varies from 6 to 60 days depending on water temperature and route of infection.

Shedding of *R. carpio* by adult carp occurs via fecal casts and feces. Virus may be present in ovarian fluid of a small number of fish but apparently does not occur in seminal fluid (Békési and Csontos 1985). Whether or not the virus resides within eggs is not known.

Management

Spring viremia of carp is best managed by detection and avoidance of SVCV carrier fish to prevent introduction and spread of virus. Fish that have been exposed to SVCV produce antibodies which confer resistance during subsequent exposures. Vaccination against SVCV is possible and some experimental work has been done in this area. Culture units containing infected

fish should be disinfected before new fish are introduced. In the event that SVCV and PFRV are actually the same, pike eggs should be disinfected with 25 mg/L of iodine for 30 seconds to inhibit virus transmission (Bootsma et al. 1975).

Significance

Spring viremia of carp is an important disease of cultured carp in Europe. However, overall disease impact has been offset by a mortality reduction in years following epizootics in a given year class of carp, probably due to an acquired immunity among disease survivors. Since SVCV per say has not been reported in North America it currently poses no threat to the Great Lakes basin. However, because of similarities between SVCV and PFRV, importation of carp and pike from Europe into the Great Lakes basin should be regulated.

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Chapter 9. Koi Herpesvirus

Koi herpesvirus (KHV) causes mass mortalities in adult koi carp (colored common carp). It has been reported in the northeastern Atlantic region of the United States and in Israel (Hedrick et al. 1999).

Signs of Disease

Externally, affected fish are pale and have irregularly colored gills with hyperplasia and necrosis. Internally, interstitial nephritis, splenitis, and enteritis are evident but inconsistent.

Diagnosis and Detection

Diagnosis of KHV is made by isolating virus in koi fin (KF-1) cells inoculated with tissue extracts from the gill, kidney, or spleen (Table 5.1). Cytopathic effect is characterized by severe vacuolation after 7 days of incubation at 20°C. Typical herpesvirus particles are present in branchial epithelial cells, hepatocytes and circulating leukocytes of infected fish. No specific procedures are available for detection of carrier fish.

Epizootiology

Since there have been only two reports of koi carp herpesvirus, one in Israel and one in North America, little is known about its epizootiology (Hedrick et al. 1999). However, high mortality of adult koi carp occurred in both instances. Exposure of adult koi carp to a KHV water bath or IP injection resulted in 80 to 100% mortality concurrent with virus isolation from gill tissue and all visceral organs. It appears that the koi carp herpesvirus from Israel and the United States are similar.

Management

Since no methods are available to detect KHV carrier fish no management procedures are currently available.

Significance

Because KHV has the potential to kill a high percentage of adult koi carp, and possibly common carp, this virus may have implications in the Great Lakes basin, particularly in ornamental ponds and aquaria. It could be considered as a candidate for listing as a restricted disease.

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Chapter 10. Lymphocystis

Lymphocystis, a disease which affects cells in the skin and fins of fish, is the oldest and perhaps best known of all viral fish diseases. Lymphocystis virus belongs to the family *Iridoviridae*. Its an icosahedral (hexagonal) particle with a diameter of 145 to 330 nm and possesses a double-stranded DNA genome (Robin and Bertholimue 1981). Recognized since 1874 and thought to be a viral disease as early as 1920 (Weissenberg 1965), its viral etiology, lymphocystis virus, was not actually proven until the early 1960's (Wolf 1962).

Signs of Disease:

Lymphocystis virus is manifested by hypertrophied cells in connective tissue beneath the epidermis. Lesions are large, oval to rounded, gray or whitish in color and may appear singly or in grape-like clusters easily seen without magnification. While lesions may develop at any location they are more prevalent on the head, lateral body surfaces, and at the base of fins but can also occur on the eye and gills. Lesions are rarely found in visceral organs. Lymphocystis lesions are differentiated from other neoplastic diseases of walleye by color, grape like clusters, and a more irregular size and shape (Table 10.1). There are no behavioral abnormalities or mortality associated with the disease.

Table 10.1. Comparison of virus-caused epidermal lesions on walleye and the prevalence of each type of disease on 25 fish examined from a single population in Alberta, Canada.

Disease	Number Positive	Lesion characteristic	Virus family	Size	Isolated
Lymphocystis	12	White, red large, spherical cells	Iridoviridae	260mm	Yes
Discreet epidermal hyperplasia of walleye	10	Clear, raised distinct margin	Retoviridae	120mm	No
Diffuse epidermal hyperplasia of walleye	4	Mucoid, indistinct, flat, translucent	Herpesviridae	100mm	Yes
Dermal sarcoma	7	Smooth, round	Retoviridae	135mm	No

Source: Yamamoto et al. (1985)

Diagnosis and Detection:

Overt signs of lymphocystis virus disease and histopathology are used for confirmation. Infected cells that measuring up to 2 mm in diameter are often 20 to 1000 times larger than normal 10 to 100 µm cells (Anders 1989). The cytoplasm of infected cells contains an enlarged, centrally located nucleus, and Feulgen positive, basophilic, ribbon shaped inclusions containing viral DNA. A hyaline capsule surrounds each cell which forms a matrix when cells are clustered. There are no available guide lines to identify asymptomatic carrier fish.

Lymphocystis virus requires a lengthy incubation period for isolation in cell culture, but it has been isolated in BF-2 cells and largemouth bass cell cultures (Table 5.1). Initial cytopathic effect appears in 8 to 10 days after inoculation and incubation at 25°C; enlarged cells in a hyaline capsule ranging from 40 to 90 µm float in the media after 2 weeks (Wolf et al. 1966).

Epizootiology:

Lymphocystis virus is geographically the most widely distributed fish virus and has the greatest diversity in fish species susceptibility. The disease occurs in cultured, wild, and aquarium fishes and in most freshwater and marine environments of the world (Wolf 1988; Anders 1989). Centrarchidae (sunfishes) and Percidae (perch) are the most frequently affected fresh water families, while salmonids and cyprinids do not appear to be susceptible.

Generally, lymphocystis is a chronic, benign disease that seldom results in morbidity or death, however, in some cases infections can reach 100% in crowded populations. In intensive fish culture systems the unsightly lesions may result in economic loss. Lesions may become so large in the buccal cavity that fish die of starvation. Severely affected wild fish may experience weight loss and large skin or fin lesions can render smaller fish more vulnerable to gill nets. Lymphocystis lesions usually cover a small area of the skin or fins but experimental infections, or those that develop under confined conditions, tend to cover a larger portion of body surface.

Contraction of lymphocystis virus may be influenced by host species, fish density, environmental conditions, injury, pollution, or parasite load. Netting, tagging, etc. may increase susceptibility because lymphocystis lesions tend to develop where scales are disturbed or fins damaged (Clifford and Applegate 1970). In natural waters, the virus is presumably transmitted to healthy fish when ruptured cells release virus into the water. Injuries sustained during spawning

activities may also lead to a higher incidence of lymphocystis (Ryder 1961). In a spawning population of walleye in Alberta, Canada, 12 of 25 sampled fish had lymphocystis and some also had dermal sarcoma and diffused epidermal hyperplasia (Table 10.1) (Yamamoto et al. 1985). Pollution may contribute to a greater incidence of lymphocystis by adversely affecting a fish's immune and endocrine system which in turn allows for activation of latent viruses to produce skin tumors (Anders and Yoshimizu 1994). Bowser et al. (1999) infected 67 to 80% of naive walleye by waterborne exposure to lymphocystis virus and 50 to 74% of these fish developed both lymphocystis and walleye dermal sarcoma.

Incubation of lymphocystis disease depends upon host species, virus strain, and temperature. At 10 to 15°C, incubation may take up to 6 weeks for lesions to develop compared to 5 to 12 days at 20 to 25°C (Cook 1972). Lymphocystis virus from one fish species may not be infective to a different species.

Lymphocystis occurs in all sizes and ages of fish but a higher incidence of infection has occurred in smaller walleye (250 to 600g) than in medium (600 to 1200g) or large fish (Yamamoto et al. 1985). The infection rate in female walleye was reported to be three times greater than in males. The higher disease incidence in younger fish may be due to a lack of acquired immunity and a higher fish density.

Management

Avoidance and culling of infected fish is the only means of controlling lymphocystis. In ornamental fish, tumor bearing individuals should be removed from a population before cells rupture and virus is released into the water. Also in aquaria, replacing 50% of the water every 2 to 3 days will dilute the virus and reduce incidence of infection. Sanitation and disinfection of all equipment and holding tanks will help prevent virus transmission to non-infected populations.

Significance

Lymphocystis is enzootic to the Great Lakes basin, however, due to lack of mortality associated with the disease its significance is minimal other than the fact that infected fish are not aesthetically acceptable to fishermen and/or consumers.

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Chapter 11. Discrete Epidermal Hyperplasia of Walleye

Discrete epidermal hyperplasia of walleye is synonymous with walleye epidermal hyperplasia and epidermal hyperplasia (Wolf 1988). The disease was suspected to be a virus as early as 1965 but was not verified until Yamamoto et al. (1985a) described type-C retrovirus-particles in electron micrographs of skin lesions.

Signs of Disease

Discrete epidermal hyperplasia lesions are gently raised, translucent mucoid-like patches found on the skin and/or fins. Lesions may vary in diameter from a few millimeters to several centimeters but generally are 1-2 mm in height, appear singularly or in groups, and may cover large areas. Demarcation between normal epidermis and the tumor is sharply defined. Lesions on the epidermis are distinct from those associated with diffuse epidermal hyperplasia or dermal sarcoma of walleye, and lymphocystis (Table 10.l).

Diagnosis and Detection

Presumptive diagnosis of discrete epidermal hyperplasia is made by appearance of the skin lesions while histology and/or electron microscopy are required for confirmation. Lesions contain predominately cuboidal cells with mucous cells at the surface (Yamamoto et al. 1985a). The type-C retrovirus associated with lesions is pleomorphic, measures 120 nm in diameter, and is randomly distributed in microvilli-like extensions of the cell and at the cells periphery (Yamamoto et al. 1985b). The virus has not been isolated in tissue culture but LaPierre et al. (1998) described and sequenced the genes of two separate type-C retroviruses associated with discrete epidermal hyperplasia.

Knowing a fish populations history and actual observation of epidermal hyperplasia lesions are the only means of determining possible carriers.

Epizootiology

Discrete epidermal hyperplasia has been found only in adult walleye in Saskatchewan and Manitoba in Canada, and Oneida Lake in New York and appears to have little deleterious effect (Yamamoto et al. 1985a; Wolf 1988). One New York walleye population had 5% disease

incidence, whereas infection rates of 0 to 20% occurred in a survey of nine Canadian walleye populations in the spring but none were found in autumn (Yamamoto et al. 1985a). Some fish were infected with lymphocystis, discrete epidermal hyperplasia, and dermal sarcoma. Although the virus that causes discrete epidermal hyperplasia has not been isolated, but Bowser et al. (1998) transmitted the disease to 97% of naive age-0 walleye by injecting filtrates prepared from lesions. Infection was confirmed by PCR.

Management

Management of discrete epidermal hyperplasia of walleye should include avoidance, culling infected fish, and avoiding broodfish from infected populations.

Significance

Due to a lack of associated mortality, discrete epidermal hyperplasia of walleye has minimal significance, however, infected fish may be rejected by consumers.

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Chapter 12: Diffuse Epidermal Hyperplasia of Walleye

A herpesvirus *(Herpesvirus vitreum)* is the causative agent of diffuse epidermal hyperplasia in spawining walleye in Canada (Kelly et al. 1980).

Signs of Disease

Diffuse epidermal hyperplasia lesions, which resemble thick areas of slime on the skin, are actually flat, diffused translucent growths with soft, swollen underlying tissue (Yamamoto et al. 1985a). These lesions may measure several centimeters in diameter and spread laterally on the surface of the fish. Diffuse epidermal hyperplasia is not as obvious as lymphocystis or dermal sarcoma of walleye (Table 10.1).

Diagnosis and Detection

Characteristic lesions on the body surface provides presumptive diagnosis which should be followed by histological confirmation and virus isolation in cell culture. Cells in diffuse epidermal hyperplasia lesions are disorganized with slightly enlarged nuclei that contain granular inclusions. Electron microscopy shows typical herpesvirus particles within the nucleus and mature enveloped virions near the cells periphery. The virus has a DNA genome and measures 200 nm in diameter, presumably with an envelope (Kelly et al. 1983).

Herpesvirus vitrium replicates in cultured walleye ovary (W0), and walleye embryo (We-2) cells at temperatures of 4 to 15°C (Table 5.1). Cytopathic effect consists of syncytia followed by cell lysis. There are no procedures to identify carriers of diffuse epidermal hyperplasia of walleye other than the presence of lesions from which virus is isolated.

Epizootiology

In western Canada, diffuse epidermal hyperplasia of walleye occurs during spawning walleye in early spring but the transient lesions disappear by early summer. To date the disease has only been reported in wild walleye populations. Little is known about what effects the virus or its associated lesions have on infected fish, however, it was noted in 4 of 25 fish examined during spring (Table 10.1) (Yamamoto et al. 1985b).

Management

Avoid collecting eggs from walleye populations known to be infected with diffuse epidermal hyperplasia.

Significance

While diffuse epidermal hyperplasia of walleye does not result in mortality, it does affect aesthetic quality of the fish and their acceptance by the public.

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Chapter 13: Walleye Dermal Sarcoma

Walleye dermal sarcoma (WDS) is a benign tumor on the skin of walleye caused by a type-C retrovirus, walleye dermal sarcoma virus (WDSV).

Signs of Disease

Walleye dermal sarcoma is characterized by large vascularized tumors which are smooth, usually variably firm, and have a pinkish to white color (Table 10.1). The disease may be confused with lymphocystis but can be differentiated by its more rounded, smooth textured lesions.

Diagnosis and Detection

Walleye dermal sarcoma lesions are comprised of irregularly shaped tumors which contain normal size cells that are often arranged in whorls. Tumor size ranges from 1 to over 10 mm with a mean diameter of approximately 5 mm (Yamamoto et al. 1976). Although field observations may indicate the presence of dermal sarcoma, the only definitive way to identify the disease is by histology of tumor tissue and confirmation by electron microscopy examination of sediment pellets of homogenized and centrifuged tumors (Martineau et al. 1991). The RNA genomic type-C retrovirus has not been isolated in cell culture, but Martineau et al. (1992) did characterize its molecular structure. The reported size of WDSV varies in diameter from 90 nm to 135 nm.

Epizootiology

Dermal sarcoma lesions have been reported from adult walleye in Oneida Lake and Lake Champlain in New York, other waters in the Great Lakes region, and central and western Canada. Dermal sarcoma virus has not been reported in cultured walleye.

Experimental transmission of walleye dermal sarcoma to healthy 4 month-old walleye and sauger by intramuscular injection of homogenized tumors from adult fish was documented by Martineau et al. (1990). Tumors developed 4 months post infection in over 90% of both species and transmission was greater at 15°C than at 10 or 20°C (Holzschu et al. 1998). Walleye dermal sarcoma was transmitted by waterborne exposure to 71 and 89% of two groups of juvenile walleye and by injection to 82% of young walleye in the spring (Bowser et al. 1996; 1999). At

15°C, about 8 weeks incubation time was required for tumors to develop. Bowser and Wooster (1991) showed that tumors totally regressed in females but only partially regressed in male walleye during 18 weeks post tumor development when water temperature rose from 7 to 29°C. Most WDS tumors in wild walleye are confined to the epidermis but some invasive tumors did develop in experimentally infected fish (Earnest-Koons et al. 1996).

In some walleye populations, incidence of neoplastic lesions can be high and individual fish may be infected with more than one type of epidermal tumor (Yamamoto et al. 1985). In one population in Canada, 7 of 25 walleye examined had dermal sarcoma (Table 10.1). Bowser et al. (1988) noted that in spring up to 27% of adult walleye in some North American lakes are affected with dermal sarcoma and that the number of infected fish in Oneida Lake was higher in spring and fall than during summer. In a more extensive study Getchell et al. (2000) showed that adult walleye developed WDS with increasing frequency from age 3 to 6 years but declined thereafter. Also, fish that developed tumors in spring and fall one year had regressed tumors the following spring and disappeared by summer. Getchell et al. (2001) demonstrated that when walleye were exposed to WDS and challenged again 9 months later, the fish were significantly less likely to develop lesions following the second exposure which suggests that walleye can acquire an immunity to WDS.

Walleye dermal sarcoma virus RNA was found in fish injected with infectious material from spring tumors but was absent in fish injected with material from fall tumors indicating that WDS develops in the spring and infectious virus is no longer present as tumors regress in autumn (Poulet et al. 1995). According to Bowser et al. (1999) an increase in WDS infections during spring spawning is enhanced by high fish densities on spawning grounds and elevated virus concentrations in the water due to higher tumor incidence on spawning fish.

Management

Fish with walleye dermal sarcoma lesions should be avoided for potential broodstock.

Significance

Walleye dermal sarcoma is endemic to the Great Lakes basin but generally causes minimal problems. Its main significance is the unsightly tumors which appear seasonally thus rendering the fish undesirable to the public. To date none of the neoplastic diseases of walleye have been reported in cultured walleyes but this could change if their culture were to increase significantly.

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Chapter 14. White Sturgeon Herpesvirus

At least two herpesviruses have been isolated from white sturgeon; white sturgeon herpesvirus - l (WSHV-l) in 1989 (Hedrick et al. 1991a) and white sturgeon herpesvirus - 2 (WSHV-2) a year later (Hedrick and Wingfield 1992; Hedrick et al. 2001).

Signs of Disease

Fish less than 6 months old infected with WSHV-1 have no detectable external disease signs, are full bodied, and continue to feed until death. White sturgeon herpesvirus-2 occurs in older sturgeon and manifests itself as small white blisters which develop into open lesions on the body surface. These lesions are frequently infected with secondary bacteria and/or ectoprotozoa parasites. Internally, the stomach and intestines are filled with fluid but other tissues appear normal. Wild white sturgeon infected with WSHV-2 become listless and appear to stop eating.

Diagnosis and Detection

White sturgeon herpesvirus-1 and -2 are isolated in white sturgeon skin (WSSK-l) cells where syncytia develops in about 3 days post inoculation when incubated at 20°C (Hedrick et al. 1991a). Cytopathic effect of WSHV-2 at 5 to 25°C consists of grape-like clusters at the foci of plaques with heteromorphic and fused cells that eventually detach from the substrate. Mature WSHV particles are hexagonal, enveloped in cytoplasmic vacuoles, and possess all morphological herpesvirus characteristics. The virion nucleocapsid measures about 110 nm in diameter and 230 nm with an envelop. Confirmation of WSHV identity is by PCR, but antiserum neutralization can distinguish WSHV-l from WSHV-2.

Epizootiology

The initial outbreak of WSHV-1 occurred in California sturgeon that were less than 10 cm in length. Mortality reached 97% after these fish were moved into the laboratory; however, it is important to note that they were also infected with columnaris. Mortality from WSHV-1 is usually less than 50%. Experimental virus transmission from cell culture to juvenile fish resulted in a 35% mortality following immersion exposure (Hedrick et al. 1991a).

White sturgeon herpesvirus-2 was isolated from internal organs of adult fish and later from juvenile fish on commercial farms, and from wild sturgeon in Oregon which had been trapped in the Columbia River (Hedrick and Wingfield 1991b; Engelking and Kaufman 1996). Mortality in older fish infected with WSHV-2 is generally less than 10%. Experimentally, the shovelnose and pallid sturgeon were susceptible to WSHV-2 but other species were refractive.

Management

Current management strategies for controlling WSHV-1 and -2 are avoidance and inspection of potential carrier fish via cell culture assay. It is recommended that sturgeon infected with WSHV-2 be prophylactically treated with salt and parasiticides to reduce secondary infections in open ulcers.

Significance:

The significance of WSHV in the Great Lakes basin is unknown but because it is pathogenic to other sturgeon species it should be considered an Emergency pathogen. LaPatra et al. (1995) did, however, demonstrate that white sturgeon could be a vector of infectious hematopoietic necrosis virus, therefore, they should be handled accordingly.

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Chapter 15. White Sturgeon Iridovirus

White sturgeon iridovirus (WSIV) was first isolated in 1988 from cultured juvenile white sturgeon at several fish farms in northern California and other locations in the Pacific Northwest (Hedrick et al. 1990; 2001).

Signs of Disease

Diseased fish tend to go off feed, possibly due to infected olfactory tissue, appear weak and lethargic, experience weight loss, drop to the bottom, and die (Hedrick et al. 2001). Gills are pale, swollen, necrotic, and may have some petechia. Internally, there is no body fat, livers are pale, and the gut is empty.

Diagnosis and Detection

White sturgeon iridovirus has been diagnosed by electron microscopy and isolated in white sturgeon spleen (WSSV-2) cells which become enlarged and then slowly degenerate (Hedrick et al 1992). The cytoplasm of enlarged cells is blue to blue/pink with hematoxylin and eosin (H&E) stain and has translucent bar-like structures (Hedrick et al. 2001). Giemsa stained infected cells contain large cytoplasmic inclusions. Isolation requires 10 to 30 days for CPE to appear and replication occurs at 10, 15, and 20°C (optimum temperature) but not at 5 or 25°C. The icosahedral WSIV, which contains a DNA genome, measures about 290 nm in diameter.

Although isolation of WSIV takes a long time, and cell culture is not particularly dependable, this is the detection method currently being used. Presence of typically H&E stained cells in the gills are good indicators of infection and PCR also shows promise as a sensitive diagnostic test.

Epizootiology:

In Oregon, Idaho, and California farms WSIV has been isolated from juvenile white sturgeon ranging in length from 7 to 46 cm. The virus is highly virulent; one farm lost 95% of 200,000 juvenile sturgeon in 4 months when the water temperatures were about 15°C. Severity of disease may also be related to transmission from adult to progeny and stocking density. Lake sturgeon suffered low mortality following an experimental infection while most non-sturgeon fish

were resistant to the virus. According to Hedrick et al. (2001) WSIV has also been found in wild asymptomatic white sturgeon populations in the Pacific Northwest.

Management

There are currently no management strategies for controlling WSIV other than avoidance which can be accomplished using cell culture to check for presence of virus. The transmission of WSIV is such a concern to many aquaculturists in North America and Europe that it is listed as a Restricted agent for imports of white sturgeon (Hedrick et al. 2001).

Significance

Because of its pathogenic potential, WSIV is a serious threat to cultured white sturgeon and may also be detrimental to lake sturgeon in the Great Lakes basin, therefore, it should receive Emergency disease consideration.

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Chapter 16: Motile *Aeromonas* Septicemia

Motile Aeromonas septicemia (MAS) is associated with infections caused by motile members of the genus *Aeromonas*. Synonyms of MAS are hemorrhagic septicemia and infectious dropsy. *Aeromonas hydrophila, A. sobria* and *A. caviae* are the principal motile aeromonad species that affect fish (Austin and Austin 1987).

Signs of Disease

Clinical signs of MAS are varied and diverse. Motile *Aeromonas* septicemia infected fish loose their appetite, become lethargic, and swim lazily at the surface. Lesions occur on the fins, caudal peduncle, top of the head, and on dorsal, ventral, or lateral body surfaces. No external signs of MAS are specific but differences in disease manifestation can be noted between scaled and scaleless fish. In scaled fishes, lesions begin as hemorrhages at the scales base, the scales become raised, and are eventually lost; skin lesions develop a large red (inflamed) central area surrounded by whitish (necrotic) tissue; fins are frayed, hyperemic, and congested. The first sign of disease in scaleless fish is an irregularly shaped depigmentation of the skin that often progresses into necrosis, an ulcerated lesion, and exposed muscle.

Motile *Aeromonas* infected fish may have swollen, hemorrhaged, cloudy eyes; enlarged abdomen, pale gills indicative of anemia, and swollen musculature. Internal organs are friable, generally hyperemic, the kidney and spleen are swollen, and the liver is often mottled with hemorrhage that is interspersed with light areas. Fluid in the body cavity is bloody and cloudy. The intestine is flaccid, hyperemic, contains yellowish mucus, and is void of food.

Diagnosis and Detection

Diagnosis of MAS is not based solely on clinical signs because other bacteria (i.e. *Pseudomonas fluorescence* or *Flavobacterium columnare*) and protozoan parasites (i.e. *Epistylis* sp. and *Ichthyobodo* sp.) often produce similar lesions, and may be involved in co-infections with motile aeromonads. Primary isolation of motile aeromonads is made on either BHI agar or TSA and incubated at 25 to 30°C for 24 to 48 hours at which time entire, slightly convex, white to yellowish mucoid colonies are obvious. *Aeromonas hydrophila* can be isolated and presumptively

identified on Rimler-Shotts (RS) media where orange-yellow colonies form when incubated at 35°C (Shotts and Rimler 1973).

Presumptively the motile aeromonads are cytochrome oxidase positive, gram-negative, short, motile rods which ferment glucose. These bacteria measure about 0.8 X 1.5 µm, are polar flagellated, produce no soluble pigments, and are resistant to the vibriostat 0/129 and Novobiocin. Confirmatory identification of aeromonads is made using API-20 or other bacterial identification systems.

Detection of carrier fish is not practical because of the pervasive nature of facultative motile aeromonads and the wide range of host susceptibility.

Epizootiology:

Motile *Aeromonas* septicemia is a ubiquitous disease that affects fishes in warm, cool, and cold freshwater around the world. Although no fish species are known to be resistant or immune to motile aeromonads, channel catfish and other ictalurids, cyprinids, eels, centrarchids, esocids, percids, cichlids, and true basses are most frequently infected. Trout and salmon are also susceptible to MAS but usually only when water temperatures reach the upper tolerance limits of these fish. Some motile aeromonads can also produce localized infections or fatal septicemia in humans and other vertebrates (Goncalves et al. 1992).

The bacterial species responsible for MAS are facultative and commonly found in most surface waters, particularly in those with organic content such as warmwater culture ponds that receive large daily rations of manufactured feed.

Infections of motile *Aeromonas* are seasonal and peak during spring and fall in the southern United States. As one moves northward, infections tend to occur later in spring and earlier in the fall when water temperatures are 18 to 25°C. Low natural resistance of fish during spring probably contributes to greater susceptibility. Infections of *A. hydrophila* in fish are often associated with some type of predisposing stress such as temperature shock, low oxygen, high ammonia, and other adverse water quality problems, trauma from improper handling or hauling, and presence of other pathogens (Walters and Plumb 1980; Grizzle and Kiryu 1993). Even social stress (crowding) may increase susceptibility to *A. hydrophila* by elevating ventilation rate, glucose, and leukocyte volume (Peters et al. 1988). Although motile aeromonad infections in

most animals, including man, are usually secondary to other complicating conditions they occasionally occur as primary infections. In either case it is often the final insult that kills the fish.

Fish mortality associated with a motile *Aeromonas* infection is usually chronic in cultured and wild populations with indistinct peaks of deaths per day, however, cumulative mortality can be significant. Infections of *A. hydrophila* can be acute in very young fish and high mortality may result when a virulent strain is involved. A chronic infection can advance into acute disease when environmental or physical stressors are present.

Infections caused by motile aeromonads may be external, internal (systemic), or more commonly both. In external infections bacteria can be isolated from skin lesions but not from internal organs. Systemic infections are characterized by a septicemia and associated clinical signs, and the causative organism can easily be isolated from any internal organ as well as skin lesions.

Aeromonas hydrophila readily adapts to its environment, therefore, it exists in most natural freshwater ponds, streams, reservoirs, and bottom mud where it survives as a facultative organism utilizing any available organic material as a nutrient source. Different bodies of water, or watersheds, harbor unique strains of *A. hydrophila*. If fish which are resistant to a particular strain of the bacterium are moved to another body of water and exposed to a new *A. hydrophila* strain, for which they have no immunity, they may become infected as a result of injuries or stress encountered during handling and transport.

Management

Motile *Aeromonas* septicemia usually results from undue stress placed on fish, therefore, prevention through best management practices is important. Prophylactic treatments with salt or potassium permanganate after handling or during transport will help prevent many mechanically induced injuries from becoming infected. Although Terramycin, as a feed additive at a rate of 100 mg/kg of fish per day (2.5 to 3.75 g/45 kg) for 10 days, is approved for medicating MAS infected catfish it has minimal efficacy unless the disease is treated early. Infections which are confined to the skin, especially those of centrarchids in ponds in late winter and spring, can often be successfully treated with an indefinite application of potassium permanganate at 2-4 mg/L. There are no vaccines available for this disease.

Significance

Disagreement abounds among fish pathologists as to the significance of MAS. The frequency of its appearance cannot be disputed, but the fact that MAS is usually a secondary disease reduces its significance in many cases. However, when MAS, especially *A. hydrophila* and *A. sobria*, are present in an aquaculture environment accompanied by a high stress potential the disease cannot be ignored. Because motile aeromonads are present in surface waters throughout the Great Lakes basin, these pathogens cannot be regulated.

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Chapter 17: Atypical Nonmotile *Aeromonas salmonicida*

Atypical nonmotile *Aeromonas salmonicida* (*A. salmonicida achromogens*) causes "goldfish ulcer disease" (GUD) in goldfish in the United States (Elliott and Shotts 1980) and "carp erythrodermatitis" in common carp in Europe (Fijan and Petrinec 1973). The organism is also identical to *Hemophilus piscium*, the etiological agent of “ulcer disease” of brook trout (Paterson et al. 1980).

Signs of Disease

Goldfish infected with atypical *A. salmonicida* develop skin lesions and muscle ulcers. Lesions begin as small hemorrhages in scale pockets and slowly progress into necrotic ulcers in the epithelium. Hemorrhagic inflammation also occurs in the fins. Affected goldfish are anemic with pale gills, exhibit edema in the muscle, and scales are raised. Internally, infected fish have a general hemorrhagic appearance, pale liver, and inflamed intestine.

Diagnosis and Detection

Diagnosing atypical *A. salmonicida* infections is aided by clinical signs but caution must be taken because they are similar to those seen in MAS. The pathogen is difficult to isolate on BHI agar with inocula from necrotic musculature or hemorrhagic skin lesions, and seldom from the visceral organs of goldfish. Isolation can be enhanced by adding whole blood to the agar (Paterson et al. 1980). The organism is fastidious, especially on primary isolation, but growth capability improves upon repeated subcultivation. The organism produces slow growing, small pin-point, non-pigmented, cream colored colonies after 24 to 72 hours incubation at 20°C. Muscle lesions are often contaminated with other bacteria such as *A. hydrophila* which overgrow the atypical *A. salmonicida*.

Atypical *A. salmonicida* is a gram-negative, non-motile bacillus measuring about 0.5 µm by 0.7 to 1.4 µm. It produces no diffusible brown pigment in culture media below 25°C. The organism is either cytochrome oxidase positive, as are isolates from carp and goldfish, or cytochrome oxidase negative, as in the case of marine fishes (Chapman et al. 1991). Optimum growth temperature for the bacteria is 27°C does not grow at 37°C.

There are no procedures for detecting carrier fish of atypical nonmotile *Aeromonas salmonicida*.

Epizootiology

Atypical *A. salmonicida* affects cyprinids (goldfish, common carp, and golden shiners), several species of salmonids, eel (McCarthy 1975), northern pike (Wiklund 1990) and marine species from the North and Baltic Seas (Wiklund and Dalsgaard 1995). Goldfish ulcer disease has been reported from goldfish farms in several states in the southern, eastern, and midwestern United States.

The epizootiology of atypical *A. salmonicida* is similar in most warmwater or cool-water fish species in terms of seasonality, water temperature, age susceptibility, and mortality patterns. Goldfish ulcerative disease is a chronic condition which affects cultured fish that are primarily 1+ years old including adults. The disease usually occurs in early spring when adult goldfish are stocked into spawning ponds and water temperatures are 18 to 23°C. Loss of larger subadult and adult goldfish during the spring may reach 45 to 90%. Survivors of atypical *A. salmonicida* epizootics appear to have reduced egg yield. Saleable size goldfish (5 to 10 cm) become more susceptible as fish density, ectoparasite load, and environmental stressors increase. Other potential pathogenic bacteria such as *F. columnare* and *A. hydrophila* are often found in skin lesions associated with atypical *A. salmonicida*.

Management

Handling, skin injuries, and high fish densities during optimum disease periods should be kept at a minimum to avoid atypical *A. salmonicida* infections in goldfish and other susceptible cyprinid species. If brood goldfish are stocked in late fall or early winter it will eliminate handling when fish are most vulnerable. The disease responds poorly to antibiotics and no vaccines are available.

Significance

Goldfish ulcer disease is a significant health problem on some goldfish farms where it causes loss of yearling and adult fish as well as reduced egg production in adults. Unless there are goldfish farms in the Great Lakes basin GUD should pose no threat to the fishery resources.

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Chapter 18: Columnaris

Columnaris, an acute to chronic skin infection, is one of the oldest known bacterial fish diseases. The causative agent is currently named *Flavobacterium columnare* but was previously known as *Flexibacter columnaris,* and *Cytophaga columnaris*.

Signs of Disease

Clinical signs of columnaris are easily recognized and differ little between fish species. The disease generally begins as an external infection on the fins, body surface, or gills and the fins become frayed (necrotic) with grayish to white margins. Initial skin lesions appear as discrete bluish-gray areas associated with loss of metallic sheen. There is a loss of scales and necrotic lesions containing little mucus develop. Advanced skin lesions have mild inflammation and a yellowish appearance due to large numbers of bacteria. Gills are necrotic and white to brown in color. Columnaris may become systemic with little or no pathological change in internal organs, however, bacteria can be isolated from kidneys.

Diagnosis and Detection

Columnaris is normally diagnosed by the presence of typical lesions on the body, fins, and/or gills and by detection of long, slender rods that move by flexing or gliding bacteria in wet mounts of lesion material. These non-flagellated bacteria also form "hay stacks" or columns along tissue margins. Detection of *F. columnare* is enhanced by phase contrast microscopy.

A moist media with low nutrient and agar content, such as cytophaga (Ordal's) or Hsu-Shotts media, is required for isolation of *F. columnare* from skin lesions (Shotts 1991). The organism can also be isolated from kidney of some fish. *Flavobacterium columnare* incubated at 25 to 30°C for 48 hours produces thin, yellow, spreading, rhizoid, discrete colonies that adhere tightly to the media.

The genus *Flavobacterium* are gram-negative rods that measure about 2 to 10 µm by 0.5 µm; are motile by gliding, produce yellow colonies on agar, are chemoorganotrophs and facultative anaerobes, and decompose several polysaccharides but not cellulose (Bernardet et al. 1996). Griffin (1992) devised the following simplified method of presumptively identifying *F. columnare* from other yellow pigmented aquatic bacteria: (1) ability to grow in the presence of

neomycin sulfate and Polymyxin B; (2) thin, rhizoid, yellowish colonies; (3) ability to degrade gelatin; (4) bind congo red, and (5) production of chondroitin lyase.

Detection of carrier fish is not feasible because *F. columnare* is commonly found in nearly all natural waters; therefore, all freshwater fish are possible reservoirs for the bacterium.

Epizootiology:

Columnaris disease exists in fresh water throughout North America, and around the world. Channel catfish and other ictalurids are highly susceptible to the disease as are cyprinids, centrarchids, percids, tilapia and salmonids. No wild or cultured freshwater fishes, including ornamental fish in aquaria, are known to be totally resistant to columnaris and asymptomatic carriers occur throughout the environment. Transmission of columnaris is generally from fish to fish via water but numerous factors can affect its transmission and the appearance of clinical disease. Columnaris is occasionally found in wild fish populations but most often occurs in cultured fishes, especially juvenile channel catfish in ponds or tanks, tilapia in intensive culture or ponds, and bait minnow in holding tanks.

Columnaris disease can cause a primary infection without any significant predisposing stress to the host; however it more commonly develops following environmentally induced stress, rapid changes in water temperature; mechanical injury due to seining, handling or transportation; water quality (low dissolved or supersaturation of gasses such as oxygen, nitrogen, etc.); and other infectious agents. In many cases, the disease can rapidly become acute and result in high mortality.

Flexibacteria columnare infections can be chronic causing a gradual increase in mortality, but may appear suddenly and accelerates in a matter of days during optimum disease conditions. A 90% mortality in crowded tank held fingerling channel catfish and bait minnows is not uncommon. Losses in pond populations is usually lower, but 50% mortalities have occurred.

Columnaris is usually an external infection of the fins, gills, and body surface but can also be systemic. Of 53 cases involving *F. columnare* in channel catfish, 11% were external only, 17% were internal only, and 72% were a combination of the two (Hawke and Thune 1992). In cases where infections are both epidermal and systemic, it is believed that the epidermal infection has more pathological effect than does the systemic infection.

Generally fish are susceptible to columnaris at temperatures from 15 to 30°C with young fish being more severely affected than adults. While columnaris occurs in every month of the year it tends to be seasonal, especially in temperate climates where it peaks during March through April and again in autumn with decreased incidence in summer and winter.

From 1987 to 1989, columnaris was the most frequently reported infectious disease in the catfish industry accounting for 58% of all bacterial cases (Thune 1991). Columnaris often occurs as a secondary infection to external protozoan parasites or other bacteria. Of the 53 *F. columnare* infections studied by Hawke and Thune (1992), 87% involved more than one bacterial pathogen, predominantly *Edwardsiella ictaluri*. According to a USDA study 82% of infectious diseases in cultured channel catfish were a combination of *F. columnare* and *E. ictaluri* (Anonymous 1997).

Management

There are no fool-proof methods to prevent columnaris disease in warmwater or cool-water fish because of its prevalence and lack of host specificity. Therefore, the best management practice is to reduce stressors on fish by maintaining good environmental conditions, use proper handling and transport, avoid temperature shock and excessively high stocking densities, and use prophylactic treatments and chemotherapy as early as possible in the disease process. The most effective treatments for columnaris are potassium permanganate at 5 to 10 mg/L for 1 hour in tanks or an indefinite treatment of 3 to 5 mg/L in ponds.

Significance:

Columnaris has often been overlooked by fish pathologists because of its role as a secondary pathogen; however, its significance is elevated by its broad geographic range and extensive species susceptibility. In reality, columnaris is probably responsible for killing as many cultured fish annually, irrespective of species as any other bacterial organism.

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Chapter 19: Enteric Septicemia of Catfish

Enteric septicemia of catfish (ESC), caused by *Edwardsiella ictaluri*, was identified in 1976 (Hawke et al. 1981). It and columnaris are considered to be the two most important infectious diseases of cultured channel catfish.

Signs of Disease

Enteric septicemia is a chronic, to sub-acute disease in channel catfish with nearly definitive clinical signs. Diseased fish hang listlessly with a "head-up-tail-down" posture at the surface, spin in circles followed by morbidity, and death. Affected fish have pale gills, bulging eyes and occasionally enlarged abdomens. Small, circular, pale lesions of 1 to 3 mm in diameter that progress into inflamed cutaneous ulcers appear on the flanks and backs of infected fish. Chronically ill fish often have an elongated skull lesion at the insertion of the two frontal bones between the eyes, thus the name "hole-in-the-head" disease. Inflammation at the base of fins, under the jaw, and on the opercula and belly may be so intense that skin becomes bright red. The body cavity may contain a cloudy or bloody fluid and on rare occasions a clear yellow fluid; the kidney and spleen are swollen and the spleen is dark red; inflammation occurs in adipose tissue, peritoneum, and intestine; the liver is either pale or mottled.

Diagnosis and Detection

Generally, *E. ictaluri* is isolated from visceral organs of clinically infected fish on BHI agar or TSA where small punctate colonies form in 36 to 48 hours when incubated at 28 to 30°C. Less fastidious bacteria (i.e. motile aeromonads) may rapidly overgrow the *E. ictaluri*. On *Edwardsiella ictaluri* media (EIM), the pathogen produces clear greenish colonies with dark centers while growth of gram positive bacteria is inhibited (Shotts and Waltman 1990). Other gram negative fish pathogens will grow on EIM but can be separated by colony morphology and color: *E. tarda* colonies are medium size, have pale green margins and black centers; *A. hydrophila* colonies are large and light brown; and *Pseudomonas fluorescence* colonies are small and black with a pale halo.

Isolates of *E. ictaluri* have little biochemical diversity (Waltman et al. 1986). The organism is a gram negative rod (0.8 X 1 to 3 µm) that tends to lengthen in actively growing

cultures. The organism is cytochrome oxidase negative, weakly motile at 25 to 28°C, but nonmotile at 30°C or above and grows poorly or not at all at 37°C. At 20 to 30°C, *E. ictaluri* ferments and oxidizes glucose while producing gas but will not tolerate a NaCl concentration higher than 1.5% in the media. *Edwardsiella ictaluri* is easily separated from *E. tarda* by the formers indole negative reaction and lack of H_2S production on TSI. Isolation is the most reliable method of detecting *E. ictaluri*, but it can be serologically identified *in vitro* and *in vivo* by FAT or ELISA using polyclonal or monoclonal rabbit antiserum. There appears to be only one serological strain of *E. ictaluri* with no cross reaction to other aquatic bacteria.

There is currently no validated procedure to establish whether or not a specific population of channel catfish is free of the pathogen. However, isolation, PCR, ELISA, or FAT may be used in detecting potential carriers.

Epizootiology

Although the first *E. ictaluri* isolates were recorded in 1976 (Hawke et al. 1981), the organism was recently detected in a skull lesion of a 1969 formalin preserved channel catfish at the USDA National Research Center in Stuttgart, Arkansas (Mitchell and Goodwin 2000). In the United States *E. ictaluri* occurs in cultured channel catfish from Florida to Texas and north to Missouri and Kentucky (Plumb 1999). Rare infections of *E. ictaluri* have been reported in white and blue catfish with minor consequences as well as an occasional isolation from ornamental fish. *Edwardsiella ictaluri* infections were experimentally established in chinook salmon and rainbow trout at elevated water temperatures (Baxa et al. 1990), but most other fish species tested for susceptibility have been shown to be resistant.

When first described, *E. ictaluri* was thought to be an obligate pathogen that did not survive long outside the host (Hawke 1979), however, it is now known to survive in sterile pond bottom mud for extended periods of time and for a much shorter time in non-sterile water or mud.

Edwardsiella ictaluri remains in epizootic survivors for at least 6 months, thus becoming carriers. However, detection of carrier populations is less definitive because of the pathogens ability to survive in pond bottom mud. Because few catfish farms have closed water systems, and *E. ictaluri* is so widely spread throughout the channel catfish industry, it is difficult to identify populations which are totally free of the disease.

Transmission of *E. ictaluri* from adult channel catfish to offspring during spawning seems possible, but is as yet unproven. *Edwardsiella ictaluri* is easily transmitted horizontally (likely by contaminated feces) from infected fish to naive fish. The daily removal of dead fish from a culture unit is important during an ESC epizootic because greater concentrations of *E. ictaluri* are found in pond areas where carcasses accumulate than in areas of the same pond containing no carcasses (Earlix 1995). Taylor (1992) demonstrated the ability of *E. ictaluri* to survive in the intestinal tract of cormorants and herons, therefore, those and other piscivorous birds could serve as vectors for the pathogen. Enteric septicemia of catfish often occurs simultaneously with columnaris and one may exacerbate the effect of the other.

Although ESC has been diagnosed during every month of the year and in a wide range of water temperatures, it is considered a seasonal disease occurring primarily when temperatures range from 18 to 28°C in late spring to early summer and again in autumn; the optimum temperature being 20 to 25°C (Francis-Floyd et al. 1987). However, an increase in reported outbreaks during July and August and throughout the winter months may indicate an expanding temperature tolerance for the pathogen.

Mortality rate in *E. ictaluri* infected catfish varies from less than 10% to over 50%. The pathogen infects fingerlings as well as production-size fish in ponds, raceways, recirculating systems, and cages. Even though ESC can develop independent of extrinsic influences, adverse environmental circumstances can intensify disease severity. A correlation between confinement induced stresses (e.g. lowered environmental quality, handling, and hauling) and an increased susceptibility of channel catfish to ESC appears likely (Wise et al. 1993; Ciembar et al. 1995).

There is no indication that *E. ictaluri* possesses a health threat to human or aquatic animals other than fish because of temperature limitations under which it grows.

Management

Like most bacterial diseases of warmwater fish, ESC requires a holistic management approach. It is essential to maintain environmental quality through reasonable stocking densities, proper amounts of feed, and high water quality and judicious use of medications, and vaccination when possible. The only approved medication for ESC is Romet-30 in floating pellets fed at a rate of 100 mg/kg for 5 days. Because *E. ictaluri* infected fish stop feeding, medicated feed must

be offered as early as possible. However, cessation of feeding for several days, or intermittently when an epizootic occurs, is as effective as feeding Romet-30 (Wise and Johnson 1998).

Vaccination of cultured channel catfish against *E. ictaluri* is becoming a valuable management tool. To date, the most effective vaccine available is a modified (attenuated) preparation, Aquavac-ESC Vaccine, provided by Intervet, and applied by immersion to 10 to 30 day old fish (Klesius and Shoemaker 1999; Shoemaker et al. 1999). Protection lasts for over 4 months, appears to be protective against heterologous isolates, and produces relative percent survival of 50 to 95%.

Significance

Enteric septicemia of catfish is the most economically important infectious disease of cultured channel catfish in the United States, costing the industry millions of dollars annually in fish losses, prevention, and chemotherapy. The greatest threat from ESC to the Great Lakes basin is in raceway or recirculating channel catfish culture operations where artificially heated water is used and environmental quality is often marginal. Most of these types of operations maintain water temperatures in the mid 20°C range which is ideal for the disease. Although not a direct threat to the Great Lakes basin fishery resources, it is advisable that potential *E. ictaluri* carrier fish be regulated.

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Chapter 20: Edwardsiellosis

Edwardsiellosis, a subacute to chronic disease caused by *Edwardsiella tarda*, occasionally produces a septicemia in a variety of fish species.

Signs of Disease

Edwardsiella tarda infected channel catfish become moribund and develop small to large abscesses. These abscesses are depigmented, flat, or swollen areas in the lateral musculature which contain a bloody exudate, emitting a putrid odor when opened. The visceral cavity of infected fish is inflamed and will have an odor even in fresh fish. Clinical signs of *E. tarda* include protruding and opaque eyes, necrotic skin and muscle lesions, rectal protrusion, and abscesses in muscle and internal organs.

Diagnosis and Detection

Edwardsiella tarda is easily isolated on BHI agar or TSA from muscle lesions, and internal organs of infected fish and identified by conventional bacteriological or serological methods. Incubation at 26 to 30°C for 24 to 48 hours yields circular, convex, nearly transparent colonies approximately 0.5 mm in diameter. At 35°C *E. tarda* forms small, green colonies with black centers on EIM (Shotts and Teska 1989).

Edwardsiella tarda, an enteric bacterium, is a straight rod about 1 µm in diameter and 2 to 3 µm in length (Farmer and McWhorter 1984). It is motile, gram-negative, facultatively anaerobic, peritrichously flagellated, and cytochrome oxidase negative; produces indole in tryptone broth, H_2S on triple sugar iron slants, and gas and acid in glucose fermentation; tolerates up to 4% salt, and grows at 40°C (Waltman et al. 1986). Positive identification of *E. tarda* can be made by serum agglutination or fluorescent antibody; the antiserum does not cross react with *E. ictaluri*.

Detection of *E. tarda* carrier fish is not practical because of its wide dissemination in nature.

Epizootiology

Edwardsiella tarda is found in freshwater and marine environments throughout the world including North America (Plumb 1999). Channel catfish is the most commonly infected fish species in North America but largemouth bass, striped bass, tilapia, and others including chinook salmon in Oregon and brook trout in Quebec have been diagnosed with the disease. The pathogen also infects fish-eating birds, cattle, swine, reptiles, amphibians and humans (Hargraves and Lucey 1990).

Since *E. tarda* is commonly found in surface waters, pond water and mud, and the intestines of many aquatic animals it is a constant threat to fish. In one study it was isolated from 75% of water samples, 66% of mud samples and 100% of apparently healthy turtles, frogs and crayfish sampled from ponds used for catfish culture (Wyatt et a. 1979). Because *E. tarda* is a gut inhabitant of many aquatic animals, feces deposition in the water is the main source of infection.

An environmental stressor may not be essential for fish to become infected with *E. tarda*, however, high water temperatures, crowded conditions, high organic water content, and generally poor water quality can contribute to onset and severity of disease (Meyer and Bullock 1973). Broken or abraded skin appears to enhance opportunities for infection. Edwardsiellosis of catfish occurs most often in summer when water temperatures are near or above 30°C but the pathogen is not universally restricted by these temperatures. The disease seldom causes a major mortality in moderately stocked fish ponds where losses seldom exceed 5%; however, in crowded holding tanks during warm weather mortality can reach 50% (Meyer and Bullock 1973).

Edwardsiella tarda can be a health threat to humans where it usually manifests itself as gastroenteritis and diarrhea but can also cause meningitis, peritonitis with sepsis, cellulitis, hepatic abscess and typhoid like illness (Clarridge et al. 1980). The organism has also been associated with infected wounds caused by fish hooks or fish spines (Hargraves and Lucey 1990).

Management

Maintaining a good overall environment while providing good water quality and proper stocking densities is key to preventing *E. tarda* fish infections. Although orally applied Terramycin has been used to treat edwardsiellosis in channel catfish, there are no therapeutics or vaccines approved specifically for this disease in fish.

Significance

Generally, edwardsiellosis is not a high impact disease in aquaculture because of its infrequent occurrence. In the United Sates, unless channel catfish are crowded, the disease is of minor consequence. However, caution must always be taken when handling *E. tarda* infected fish. From a human health stand point, if fish in a processing line have subclinical *E. tarda* infections, the processing equipment can become contaminated and it must be cleaned and disinfected to prevent human exposure to the pathogen. Proper cooking will prevent ingestion of the organism and gastrointestinal problems.

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Chapter 21: Streptococcosis

Streptococcus spp. infections in fish date back to the mid-1950's and with intensive tilapia and striped bass culture they have become more prevalent. Hubert (1989) stated that "undoubtedly this disease (streptococcosis) represents a real danger to farmers (fish) engaged in warmwater aquaculture."

Signs of Disease

Streptococcosis can be sub-acute but more often is chronic. Infected tilapia are darkly pigmented, lethargic, exhibit erratic and/or spiral swimming, and bodies are curved; they have abdominal distension, and swollen, hemorrhaged, and opaque eyes; diffused hemorrhage in the operculum, skin, and base of fins; and a bloody mucoid fluid exudes from the anus. Internal gross pathology includes a bloody or gelatinous exudate in the abdominal cavity, pale liver, hyperemic digestive tract, and an enlarged, nearly black spleen. The lower gut is flaccid, hyperemic and contains a bloody fluid. Clinical signs of streptococcosis in other fish species are similar.

Diagnosis and Detection

Streptococcus spp. are isolated on Todd-Hewitt, BHI, or TSA media with 5% blood and 10 mg/L of colistin and nalidixic acid to discourage growth of other bacteria (Kitao et al. 1981). Yellowish to gray, translucent, round, slightly raised, punctate colonies appear in 24 to 48 hours at 20 to 30°C. Streptococci are gram positive, nonmotile, noncapsulated, and nonsporeformers that may be single, paired, or short chains of 2 to 6 cells. They may be - or ß- hemolytic or non-hemolytic. The presence of these cocci (sometimes ovoid) in smears from infected tissues or histological sections provides presumptive diagnosis.

Several species of *Streptococcus* have been isolated from tilapia and other fish, the most serious of which is *Streptococcus iniae*. This organism is ß hemolytic and its presumptive biochemical characteristics are: catalase negative, starch positive; fails to grow at pH 9, in 6.5% NaCl, or 40% bile esculin; and does not fit any Lancefield grouping. Other streptococci found in fish are a nonhemolytic *Streptococcus* sp. (Lancefield Group B), and or ß hemolytic *S. faecalis*, *S. faecium*, and *S. agalactia* (Lancefield Group D) (Austin and Austin 1987).

Currently there are no dependable methods for detecting possible *S. iniae* carrier fish or for determining whether or not a particular population is free of the pathogen.

Epizootiology

Intensively cultured tilapia (any species) are highly susceptible to streptococcus infections, but striped bass, striped bass X white bass hybrids, and some salmonids are also susceptible. Infections of *Streptococcus* have been reported in freshwater and marine fish from North America (Canada, Mexico and the United States), Central and South America and many other countries (Plumb 1997). A survey in the United States by Shoemaker et al. (2001) found *S. iniae* in 3.6% of tilapia, 4.8% of hybrid striped bass, and 0% of channel catfish farms. The pathogen was isolated from 3.8% of 970 tilapia and 7.3% of 415 hybrid striped bass. These data suggest a low incidence and prevalence of *S. iniae* in the United States.

Streptococcosis is seldom a problem in tilapia reared in a normal warmwater habitat. However, under stressful conditions due to low water temperatures, improper harvest or handling, poor water quality, high fish densities accompanied by high feeding rates (contributing to poor water quality) or ectroparasites tilapia can become more severely affected by streptococci (i.e. *S. iniae*) (Chang and Plumb 1996; Shoemaker et al. 2000).

In culture conditions mortality rates of 75% of infected tilapia have been reported but overall mortality of streptococcus infected fish may be low to high depending on environmental conditions and fish species. Infections of *Streptococcus* spp. can be complicated by opportunistic motile aeromonads and *E. tarda,* and the presence of parasites *Trichodina* and *Gyrodactylus* on the skin and gills. The removal of ectoparasites with formalin will often correct streptococcus infection thus indicating a parasitic influence on the bacterial infection.

Natural sources of a streptococcus organism may be mud, water, or carrier fish and transmission is thought to be by contact through water, or by feeding on diseased carcasses. In tilapia, nares are primary sites of an *S. iniae* infection which precedes a bacteremia (Evans et al. 2001). Although most streptococcal infections in tilapia have occurred in stressed fish, there have been reports of infection and mortality in unstressed tilapia, ornamental fish, and rainbow trout (Ferguson et al. 1994; Bowser et al. (1998). Because artificially heated recirculating systems for tilapia culture have expanded into temperate and colder climates it is increasingly difficult to

maintain a suitable environment; consequently infectious diseases such as streptococcosis have become more serious.

In 1995-96, there were reports of *Streptococcus iniae* infections being transmitted to humans from infected tilapia (Weinstein et al. 1997). These infections resulted from wounds incurred while handling or cleaning fish purchased from a "live" fish market. Most of these infections were confined to the wound site but one fatality, complicated by other health problems, caused major concern in the North American tilapia industry. However, Shoemaker et al. (2001) failed to "find any evidence to support the contention that *S. iniae* is a serious public health threat associated with commercially raised fish."

Management

The solution to streptococcal induced disease in tilapia and striped bass appears to be good management and vaccination (Plumb 1999). When managing streptococcus it is imperative to maintain moderate stocking densities, water temperatures above 27°C (80°F), oxygen concentrations above 5 mg/L, limit organic content in the water, and control ectoparasites. Orally applied antibiotics have generally been ineffectual in controlling the disease. Vaccination of tilapia against *S. iniae* by IP and IM injection of heterologous formalin killed preparations elicits a high degree of protection but additional work is required in this area (Klesius et al. 2000).

Significance

Expansion of intensive tilapia culture into temperate and cooler regions of North America has increased the significance of *Streptococcus*. Cage culture systems or those that depend upon the recirculating of artificially heated water, are particularly vulnerable to streptococcosis and could be a limiting factor in the expansion of intensive tilapia culture in North America. *Streptococcus iniae*'s potential as a human pathogen is uncertain but worthy of caution when handling infected fish. Because the effect of these bacteria on wild fish populations is minimal the overall impact of streptococcosis on the Great Lakes basin appears to be limited to intensive tilapia culture. Nevertheless, *S. iniae* should be considered as a "notable disease" in the Great Lakes basin.

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Chapter 22: Mycobacteriosis and Nocardiosis

Mycobacteriosis of fish is generally a chronic infection caused by members of the genus *Mycobacteria*; nocardiosis is caused by the genus *Nocardia*. Diseases due to these acid-fast staining bacteria, once collectively known as "fish tuberculosis", are more correctly called "fish mycobacteriosis" and "fish nocardiosis" respectively (Austin and Austin 1987). Diseases produced by these two genera of bacteria are similar, therefore, they are discussed simultaneously.

Signs of Disease

Mycobacterium marinum infected striped bass and their hybrids are lethargic, darkly pigmented, emaciated with sunken abdomens, and occasionally have ulcerations and hemorrhages in the skin (J. P. Hawke, personal communication). Other species infected with *M. marinum* are emaciated, have grayish irregular skin ulcers, deformed vertebrae and mandibles (particularly adult salmon), and swelling and/or loss of one or both eyes (Austin and Austin 1987). Nodular tubercles in the muscle appear externally as diffuse, light brown spots or swollen areas that can rupture. Scales become raised before sloughing and white streaks occur parallel to cartilaginous gill lamellae. Ornamental fish usually loose their bright coloration. Internal gross pathology includes a pale liver and an enlarged spleen and anterior kidney. Visceral organ surfaces are granular giving them a rough appearance.

Nocardia infected fish are sluggish or swim in a rapid tail chasing manner, and are emaciated, show abdominal distension, exhibit loss of scales, and one or both eyes are swollen and opaque. Multiple yellowish white nodules (granulomas), varying in size from 0.5 to 2.0 cm in diameter, are scattered throughout the muscle, gill, heart, liver, spleen, ovary, and mesenteries.

Diagnosis and Detection

Mycobacteriosis is caused by *M. marinum*, *M. fortuitum*, or *M. chelonei* and nocardiosis by *N. asteroides*, and *N. kampachi* (Van Duijn 1981; Arakawa and Fryer 1984). Presumptive diagnosis of mycobacteriosis is by detection of acid-fast bacteria in smears from nodules or histological sections of granulomas. These acid-fast (Ziehl-Neelsen) staining (red) organisms are gram positive, nonmotile, pleomorphic rods that measure 0.25 to 0.35 X 1.5 to 2.0 µm (Frerichs 1993). *Mycobacterium* spp. are more strongly acid-fast than *Nocardia* spp.

Since *Nocardia* and *Mycobacterium* cause similar gross pathology they are culture on Lowenstein-Jensen or Petriganis media supplemented with 5% blood, and sealed to retain moisture because of the lengthy incubation time required. Colonies of *M. marinum* form in 2 to 3 weeks when incubated at 25 to 30°C and can then be identified biochemicaly and biophysically (Hedrick et al. 1987). Colonies may be smooth or rough, moist or dry, raised or flat depending upon the media and age of culture. *Nocardia* spp. are long and branching rods in tissue sections or smears from granulomas. The more easily isolated *Nocardia* spp. produces irregular colonies that are rough; white, pinkish, orange, or yellow in color; and may require up to 21 days at 18 to 37°C to develop (Frerichs 1993).

There are no specific procedures for detecting *Mycobacterium* or *Nocardia* carrier fish, but many wild populations are thought to harbor a low incidence of these pathogens.

Epizootiology

Mycobacteriosis and nocardiosis occur primarily in marine fishes but are reported more frequently in freshwater. While these diseases may be serious in cultured cold-water, warmwater, and aquarium fish, they generally have mild consequences in wild populations. The disease has been documented in over 150 marine and freshwater fish species around the world and all teleosts should be considered as possible hosts (Post 1987).

Wile mycobacteriosis has become a serious disease in some intensive, recirculating striped bass (and hybrids) culture systems, it has been implicated in closing at least one intensive freshwater hybrid striped bass facility. The disease has not been a problem in pond cultured striped bass (Hawke 1996). The disease is usually chronic in intensive culture systems where an accumulation of bacteria may result in a serious subacute infection. Transmission of the pathogen occurs when infected fish shed bacteria into the water or when naive fish forage on infected carcasses. It is likely that wild fish harboring low level mycobacterial infections serve as a pathogen reservoir. Sakanari et al. (1983) found the prevalence of mycobacteriosis in wild marine striped bass populations to be 25 to 68% in California and 46% in Oregon. Months, or possibly years, may pass between natural exposure to the bacterium and clinical disease.

Morbidity due to mycobacteriosis in striped bass populations may be low at any given time but cumulative losses can be significant. Hedrick et al. (1987) reported that 50% of a yearling

striped bass population infected with *M. marinum* died within months of being stocked into an intensive culture system and a high percentage of the survivors became carriers. In closed recirculating systems where hybrid striped bass were experiencing chronic mortalities, 30 to 50% of randomly sampled fish had characteristic mycobacterial granulomas in internal organs (J. Newton, Auburn University, personal communication).

Mycobacterium marinum has the potential to cause skin infection in humans who come in contact with infected fish; therefore, caution should be exercised when handling suspect fish. Apparently, fish isolates are able to adapt somewhat to the higher body temperature of humans, especially the slightly cooler hands, wrists and forearms where these infections ususally occur. The infection will manifest itself as a hard, raised, calcified granuloma and on occasion, will cause an infection of the tendon sheaths, joints, and bone (Giavenni 1979; Wolinski 1992). The infection seldom becomes systemic unless an individual is otherwise debilitated or immunocompromized (Frerichs 1993).

Management

There are no therapeutic treatments for mycobacteriosis in fish, therefore, prevention by not feeding infected fish to naive fish and overall sanitation is important. Disinfection of a facility and equipment with chlorine between uses and prompt removal of moribund, dead, or live fish with clinical signs of disease will reduce the potential for transmission to non-infected fish. Workers should wear rubber gloves and disinfect hands after handling suspect fish.

Significance

In the southern and eastern United States the impact of *M. marinum* has been greatest in hybrid striped bass-white bass populations reared in recirculating freshwater systems and salt-water pen culture. Mycobacteriosis continues to be a chronic disease problem of ornamental fish in home and large public aquaria. Also, the potential for the organism to be transmitted from fish to humans makes it a more high profile disease. The impact of nocardiosis and mycobacteriosis will remain minimal in the Great Lakes basin unless highly susceptible species are reared in intensive closed culture systems.

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Chapter 23: Proliferative gill disease

Proliferative gill disease (PGD) of channel catfish, also known as "hamburger gill", is a major health problem on catfish farms where it can cause massive mortalities (Bowser et al. 1985; Bellerud et al. 1995). The disease is caused by the actinosporean, *Aurantiactinomyxon ictaluri* (Burtle et al. 1991).

Signs of Disease

Fish affected with PGD cease feeding and become lethargic. In early stages of infection the principal gross sign of disease is degeneration of gill filaments which become bent, swollen, and pale with red streaks.

Diagnosis and Detection

Proliferative gill disease is diagnosed by gross examination of the gills with confirmation by histopathology (Styer et al. 1991). The gills of PGD infected fish become intensely inflamed, swollen, and hyperplastic with necrotic epithelium and cartilage. Lamellae are fused and upon microscopic examination the gill tissue has granulomatous inflammation surrounding cysts which contain *A. ictaluri* spores. The cysts, which stain dark blue with hematoxylin and eosin, measure about 650 µm in diameter**.**

Identity of the causative agent of PGD remains confusing. Although *A. ictaluri* will be used here, according to PCR and gene sequencing the myxozoan parasite in the gills of channel catfish is actually *Henneguya exilis* (Pote et al. 2000).

There are no specific procedures for detecting subclinical infestations or carrier fish. However, Bellerud et al. (1995) found subclinical *A. ictaluri* infestations by histopathology in 12 of 14 ponds that were not experiencing overt PGD.

Epizootiology

Pond raised channel catfish of all sizes are the primary fish host of PGD in Southeastern United States and California (Thune 1994). Thiyagarajah (1993) found "PGD like" parasites in the gill of wild largemouth bass and bluegills in the Tennessee-Tombigbee Waterway in Mississippi.

Proliferative gill disease occurs in catfish culture ponds with earthen bottoms. Cysts containing *A. ictaluri* spores occur in the gills of channel catfish and are occasionally found in internal organ tissue (Burtle et al. 1991). Spores are shed from deteriorated gill tissue or dead fish into the water where they invade *Dero digitata*, an oligochaet in bottom mud. The spores invade the gut of the oligochaet where actinosporeans develop. The actinosporeans are shed from the worm into the water and again invade the fish's gill where they encyst. Although experimental transmission of PGD has been successful the complete life cycle is unclear but is thought to parallel that of *Myxobolus cerebralis* in salmonids.

The disease occurs primarily in spring and autumn at temperatures of 20 to 25°C but may occur at any temperature from 15 to 34°C (Bellerud et al. 1995). Mortality of PGD infected channel catfish can range from 1 to 90% depending upon fish size and water quality. Anecdotal observations indicate that newly built ponds have a higher frequency of PGD, but neither pond age nor bottom quality dictates whether or not PGD will occur. However, the prevalence of PGD in channel catfish appears to be more related to the diversity and abundance of oligochaetes found in dark, fine grained bottom mud than to any other environmental parameter (Styer et al. 1993; Bellerud et al. 1995).

Management

There is no specific management approach for prevention and/or control of PGD, however, maintaining high water quality during infections will reduce mortality. Most farmers try to market PGD affected fish as soon as possible.

Significance

Because little or no pond rearing of channel catfish occurs in the Great Lakes basin this parasite poses no threat to the area.

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Chapter 24: Other Protozoan Parasites

External protozoan parasites occur throughout wild and cultured warm- and cool-water fish populations in North America; and nearly all are enzootic in the Great Lakes basin (Rogers 1985; Hoffman 1999). While their presence and disease potential should not be trivialized they are often little more than a nuisance and only occasionally do they precipitate disease. However, ichthyophthiriasis ("ich" or "white spot"), caused by the ciliated protozoan *Ichthyophthirius multifiliis*, is an exception.

Fish with ichthyophthiriasis swim erratically or lethargically, gasp at the surface, loose their appetite, and die quickly. The parasite burrows into the epithelium of the skin and gill where it forms visible small (about 1 mm in diameter), discrete, white spots. During acute infection the skin and fins have enormous numbers of these spots and an excessive amount of mucus which gives the body surface a grayish color. Gills are also pale with hyperplasia and copious amounts of mucus.

Ichthyophthirius multifiliis is a ciliate that is visible with the unaided eye which provides presumptive diagnosis. The disease is confirmed by identifying adult and juvenile *I. multifiliis* cells in wet mounts of skin or gill scrapings using 100 or 400 power magnification (Ewing 1994). The adult trophont in the epithelium measures up to 1 mm in diameter, is uniformly ciliated on the cell membrane and contains a large "horseshoe" shaped macronucleus that is detectable only by staining. These adult cells are oval to rounded but continuously change shape while moving with the aid of the cilia. Juvenile *I. multifiliis* (tomonts) are smaller than adults, more "football" shaped, heavily ciliated, and swim more actively.

Essentially all fresh water fish species throughout the world are susceptible to ichthyophthiriasis, however, scaleless fish (i.e. catfish) are particularly susceptible. The parasite also causes serious disease in centrarchids, cyprinids, percids, salmonids, ciclids, and other fishes including aquarium species.

Ichthyophthirius multifiliis is an obligate pathogen with three life stages. It matures into a trophont in the fish's skin (the white spot), leaves the host as a tomont, and attaches to a substrate where it forms a cyst. Depending upon temperature up to 1000 small, oval, ciliated tomites may be produced per cyst overnight. After the cyst ruptures, free swimming tomites seek a new host that must be found within a certain time which is also temperature dependent (i.e. 36 h at 21°C).

At 20°C the time required to complete the life cycle is 5-7 days while about 40 days is required at 10°C (Ewing and Kocan 1986). The life cycle is generally disrupted at temperatures above 25°C and tomites die at 32°C.

Death rates during acute ichthyophthiriasis infection can be 50 to 90% depending upon fish species, age, immunity, water temperature, type of culture unit, fish density and water flow rate. The disease usually occurs during spring when water temperatures approach 18 to 20°C and continues until temperatures exceed the mid 20°C range. Because of warm water temperatures, few outbreaks occur during summer but may reoccur in autumn. However, warmwater and cool-water variants of *I. multifiliis* may also exist and this author has seen infected adult channel catfish under the ice on a pond in Wisconsin. Carrier fish are considered to be the reservoir for *I. multifiliis* because survival time is short in water. Wet seines, nets, boots, etc. can serve as short term sources of infection, therefore, they should be thoroughly dried between uses.

Other ectoprotozoan parasites infect and/or infest warmwater and cool-water fishes but none are discussed in detail because of their ubiquitous nature and wide host susceptibility (Mitchum 1995; Hoffman 1999). Some of these ectoprotozoa are obligate pathogens (i.e. *Ichthyobodo*, *Ambiphrya*, *Apiosoma*, and *Trichophrya*), while others (*Trichodina*, and *Epistylis*) are more saprophytic. All are detected by light microscopy. These ectoprotozoa commonly infect cultured and wild warmwater and coolwater fishes without causing disease. They frequently occur in low numbers on fish and only under certain conditions such as a poor environment, trauma or in conjunction with other disease pathogens do they cause disease.

Recently a microsporidia, *Heterosporis* sp., was discovered in the muscle of yellow perch in the Eagle River Chain of Lakes in Wisconsin and Leech Lake in Minnesota (Anonymous no date). This parasite was also found in walleye and norther pike in Minnessota and *Heterosporis* sp. has been reported in aquarium species in several countries in Europe and Asia (Hoffman 1999). *Heterosporis* sp. is not visible from casual observation and is found only when fish are cleaned. The parasite infects muscle cells which cause the flesh to have a "cooked" or "freezer burn" appearance. The parasite's life cycle is not completely known but as fish die and decompose, spores are released into the water and swallowed by other fish. Initial infections occur behind the head and then spread to the lateral musculature. The spores remain infective for a year or more in water but are susceptible to drying.

Controlling ectoprotozoa depends on the parasite. Drying or disinfecting seines, boots and other equipment between use will prevent transfer of *I. multifiliis* between culture units. Increasing water flow in tanks containing *I. multifiliis* infected fish will reduce transmission by preventing encystment on substrates and inhibit tomites from attaching to adults. If feasible, raising water temperatures above 25°C will inhibit completion of the parasites life cycle. Chemotherapy is effective on ichthyophthiriasis if applied before acute infection has developed. Copper sulfate at 0.5 to 3 mg/L will arrest a developing "ich" infection if fish are treated for 5 consecutive days but efficacy is dependent upon stage of infection, water quality and total suspended solids (Schlenk et al. 1998). Formalin at 25µL/L indefinitely in ponds or 1:6,000 for 1 hour in tanks is also somewhat effective but since these treatments only affect free living tomites repeated treatments are required. Survivors of ichthyophthiriasis are usually resistant to reinfection suggesting an acquired immunity.

Diseases resulting from other ectoprotozoa infestations are preventable with prophylactic treatment of formalin at 1:6000 for 1 hour in tanks or during handling. Overt infections are usually brought under control with formalin at 1:6000 for 1 h in tanks or 3-5 mg/L indefinitely in ponds; copper sulfate at 0.5 to 3 mg/L, or potassium permanganate at 3-5 mg/L may be used in ponds. There are no controls of *Heterosporis* sp., however, to inhibit its spread to non-contaminated lakes infected fish should be buried or burned. All equipment including boats should be thoroughly dried between use in different bodies of water; live wells, which are difficult to dry, should be disinfected with one cup of bleach in 5 gallons of water.

Of all the ectoprotozoan parasites, ichthyophthiriasis is one of the most devastating diseases of warmwater and cool-water fish and because of the wide temperature range in which this parasite occurs it could be a health threat to some fisheries resources in the Great Lakes basin. Other ectoprotozoan fish parasites are ubiquitous throughout the region, however, scrutiny for these parasites in existing populations, good hatchery hygiene, timely chemotherapy when applicable, and avoiding the movement of overtly infected fish will reduce the impact of these parasites. The full implication of *Heterosporis* sp. in yellow perch is not known, however, the culinary quality of infected fish is destroyed.

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Chapter 25: Bothriocephalosis

Bothriocephalosis (Asian tapeworm), caused by the intestinal tapeworm *Bothriocephalus acheilognathi,* has been known to be in the United States since the mid 1970's (Hoffman 1999).

Signs of Disease

Only heavily infected fry and small fingerlings show clinical signs of a *B. acheilognathi* infection. Fish hang listlessly near the pond edge, are emaciated with swelling in the anterior abdomen and in massive infestations scales are raised and muscle is edematous. Internally, the body cavity may be filled with a cloudy yellow fluid and the anterior intestine is enlarged, yellow to white in color, and the gut wall is thin and flaccid. Older infected fish show no ill effects of bothriocephalosis.

Diagnosis and Detection

Detection of the Asian tapeworm is made by examining squash preparations of material taken from the anterior portion of the gastrointestinal tract just posterior to the first bend (Mitchell 1994). The scolex (head) of the living tapeworm is shaped like an arrowhead during extension and contraction, a characteristic that is difficult to determine in frozen or preserved specimens. *Bothriocephalus acheilognathi* is completely and distinctly segmented and can reach a length of 50 cm but is usually less than 10 cm (Hoffman 1999). The scolex is flattened with two deep, elongated sucking grooves (bothria) and has no spines, suckers, tentacles, neck, or dorsal or ventral median furrow.

Carrier fish are identified by the presence of the parasite in the intestine. There are no inspection protocols for *B. acheilognathi* but squash preparations taken from the anterior intestine can be used to determine if worms are present (Mitchell 1989).

Epizootiology

Bothriocephalosis originated in Asia and has been spread throughout the world with distribution of grass carp. It has been reported in Mexico, British Columbia and throughout the southern United States, California, New Hampshire and New York (Hoffman 1999). In North America the most affected group of fishes are cyprinids, especially goldfish, grass and common

carp, golden shiners, and fathead minnows. The parasite may also infect percids and centrarchids but has not been reported in salmonids.

Eggs of *B. acheilognathi* are shed into the water via fish feces. A motile coracidium escapes from the egg and is eaten by copepods where larvae develop. When the copepod is in turn eaten by a juvenile fish the larval parasite is released into the anterior alimentary canal and attaches to the mucosal lining where the worm matures. Although death of infected fish as a direct result of bothriocephalosis is not common, up to 80% mortality among infected larval fish has been reported. The long term effects of *B. acheilognathi* are reduced growth, inability to withstand harvest, and increased prevalence of secondary infections of columnaris or motile aeromonads. The tapeworm appears to shorten the life span and stunt the growth of feral fish.

The Asian tapeworm prefers temperatures of 20-30°C with optimum maturation at or above 25°C. Speed of egg development, hatching and coracidium movement are maximized at 25-30°C with most worms being gravid in spring to late summer when there is a peak in the presence of susceptible juvenile fish.

Management

Bothriocephalus acheilognathi primarily infects juvenile cyprinids, therefore, reduction of copepods in the water by using a prophylactic treatment of Baytex or Trichlorophon prior to spawning or stocking will reduce infection rates and parasite intensity (Mitchell 1986). When fish parasitized with *B. acheilognathi* are treated with prazequantle at 0.6 - 0.75 mg/L for 24 hours 100% of the worms are removed from the intestines. However, none of these chemicals are registered by the FDA for fish.

Significance

According to Hoffman (1999) *B. acheilognathi* is a dangerous parasite and susceptible fish species do occur in the Great Lakes basin which is born out by the fact that the parasite was found in New York. However, because the parasite generally infects juvenile fish and an optimum temperature of 25 to 30°C is required for completion of its life cycle, the potential for this parasite to become a serious disease in the Great Lakes basin is minimal.

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Chapter 26: Other Parasites

A variety of helminthic worms infest the skin, muscle, and visceral organs of warmwater and cool-water fishes. The more common ones are monogenetic trematodes (*Dactylogyrus*, *Gyrodactylus*, etc.) on skin and gills; digenetic trematodes (yellow grub, *Clinostomum marginatum*; white grub, *Posthodiplostomum minimum*; black grub, *Uvulifer ambloplitis* in skin and visceral organs; *Diplostomulum spathaceum* in the eye); nematodes in muscle and visceral organs; and tapeworms in muscle, internal organs (larvae), and intestines (adults).

Pleurocercoids of the bass tapeworm (*Proteocephalus ambloplitis*) cause extensive fibrosis of the gonads of largemouth bass and sometimes renders them sterile (Mitchum 1995; Hoffman 1999). Low fecundity of hatchery broodstock can be avoided by periodically replacing older fish with young adults. Most of the helminths are ubiquitous and have complex life cycles involving small fish, snails, or copepods as intermediate hosts and fish or birds as the final host. These parasites can occasionally cause mortality in fish because of heavy infestations in the skin, muscle, or visceral organs and can also adversely affect the fish's aesthetic and/or culinary value.

Parasitic copepods found on the gills and skin of warmwater and cool-water fishes include *Lernaea* spp. and *Ergasilus* spp. both of which have a wide geographical range and a variety of fish hosts. The parasitic copepods and helminthic parasites are so wide spread and have such a diverse host infectivity that their regulatory control is impractical.

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Part III. Appendix

Appendix II. Common and scientific names of fishes.

Bighead carp *Aristichthys nobilis*

Black crappie *Pomoxis annularis*

Bluegill *Lepomis macrochirus*

Blue catfish *Ictalurus furcatus*

Brook trout *Salvelinus fontinali*s

Brown bullhead *Ameiurus nebulosus*

Channel catfish *Ictalurus punctatus*

Chinook salmon *Oncorhynchus tshawytscha*

Common carp (koi carp) *Cyprinus carpio*

Doctorfish *Labroides dimidatus*

Eel *Anguilla* spp.

Fathead minnow *Pimephales promelas*

Golden shiner *Notemigonus crysoleucas*

Goldfish (crucian carp) *Carassius auratus*

Grass carp *Ctenopharyngodon idella*

Guppy *Poecilia recticulata*

Lake sturgeon *Acipenser fulvescens*

Lake trout *Salvelinus namaycush*

Largemouth bass *Micropterus salmoides*

Northern pike *Esox lucius*

Pallid sturgeon *Scaphirhynchuys albas*

Rainbow trout *Oncorhynchus mykiss*

Redear sunfish *Lepomis microlophus*

Sauger *Stizostedion canadense*

Shovelnose sturgeon *Scaphirhynchuys platorynchus*

Silver carp *Hypophthalmichthys molitrix*

Striped bass *Morone saxatilis*

Smallmouth bass *Micropterus dolimieu*

Spotted bass *Micropterus punctulatus*

Suwanee bass *Micropterus notius*

Tilapia *Oreochromus* spp.

Walleye *Stizostedion vitreum*

White bass *Morone chrysops*

White catfish *Ameiurus catus*

White crappie *Pomoxis annularis*

White sturgeon *Acipenser transmontanus*

Yellow perch *Perca flavescens*

Appendix II

Recommendations for Amending the Great Lakes Fish Disease Control Program to Help Minimize the Risk of Introducing and Spreading Pathogens of Warmwater and Cool-water Fishes

Fish diseases may affect the propagation of warmwater and cool-water fish as well as wild fishery resources in the Great Lakes basin. Diseases of warmwater and cool-water fish present unique challenges when considering control programs to prevent or their introduction and/or spread in nature and aquaculture within a specific region. Many of these pathogens are wide spread and few are host specific, therefore, controlling their dissemination via regulations is often impractical and ineffective. Other than the viruses, many of these agents are non-obligate pathogens. Most warmwater and cool-water culture facilities lack closed water systems making it difficult to prevent wild fish which covertly carry disease agents from contaminated culture waters. Also, validated detection procedures to identify carrier populations of many of these pathogens are not available.

A significant emphasis has been placed on controlling the spread of infectious diseases of salmonids (Meyer 1983; Anonymous 1984; Hnath 1993; Horner and Eshenroder 1993; Thoesen 1994) but until recently little attention was paid to preventing the spread of warmwater and cool-water fish diseases. It has been perceived that these diseases are not geographically limited; they are inevitable, therefore not preventable; however, this perception is changing. The current document proposes amendments to the Great Lakes Fish Disease Control Policy and Model Program (Hnath 1993) to reduce the risk of introducing new diseases from enzootic areas and to limit the spread of infectious diseases of warmwater and cool-water fish within the Great Lakes basin.

The proposal is based on the aforementioned limitations and whether or not pathogens are obligate or non-obligate, occur in North America, endemic to the Great Lakes basin, and if introduced would the fishery resources of the basin be adversely affected.

Additional Fish Species Covered by the Model Program

Cultured and/or released fish species that should be added to the current list are:

Channel catfish - CC	Walleye - WE
Grass carp - GC	White sturgeon - WS
Largemouth bass - LMB	Tilapia - TP
Northern pike - NP	Yellow perch - WP

Additional Diseases Covered by the Model Program

Pathogens of warmwater and cool-water fishes that should be considered for addition to the Model Program are classified as: (1) *Emergency*, (2) *Restricted*, and (3) *Notable*.

Emergency Fish Pathogens

Emergency fish pathogens are virulent, have not been detected within waters of the Great Lakes basin, and their introduction could have a negative effect on the basin's fishery resources. Potential carrier fish from areas where Emergency diseases exist should be inspected and declared "specific disease free" (SPF) before being allowed to enter the Great Lakes basin. When an Emergency pathogen is confirmed in any fish stock under propagation in the Great Lakes basin, immediate steps should be taken to eradicate the agent from the facility and adjacent water as authorized by the member agency with jurisdiction.

Spring viremia of carp virus, *Rhabdovirus carpio*, SVCV, SVCVD

Spring viremia of carp is known only in Europe where it is considered a serious disease in cultured common carp. Because pike fry rhabdovirus is closely related to SVCV, and may be identical, no common carp, northern pike or their eggs, from any European source should be imported into the Great Lakes basin unless said source has been appropriately inspected. Common carp are seldom cultured in North America, but SVCV could adversely affect northern pike populations. Methods of inspection for SVCV are available by isolation in cell culture.

White Sturgeon Herpesvirus, WSHV, WSHVD

Two strains of white sturgeon herpesvirus, WSHV-1 and WSHV-2, occur in juvenile and adult cultured and wild white sturgeon in California and other areas in the Pacific northwest where they cause moderate to high mortality in cultured fish. Other species of sturgeon are also susceptible to WSHV. Detection of WSHV is by isolation in cell culture.

White Sturgeon Iridovirus Virus, WSIV, WSID

White sturgeon iridovirus is known only in the Pacific Northwest United States where it is pathogenic to cultured and wild white sturgeon. This virus is also at least mildly pathogenic to lake sturgeon and could affect fishery resources in the Great Lakes basin. Inspection procedures for WSIV involve isolation in cell culture.

Restricted Fish Pathogens

Restricted pathogens are those currently enzootic within the Great Lakes basin, but their known geographic range is limited. Appropriate action should be taken to further reduce the range of these pathogens because if spread throughout the region they could adversely affect cultured and wild fisheries resources. These pathogens should be considered as additions to the list of diseases for which fish are inspected prior to transfer to other waters from outside and within the Great Lakes basin.

Largemouth bass virus, LMBV, LMBVD

Largemouth bass virus has been reported in wild largemouth bass in many reservoirs and several hatcheries in southern United States and lakes and hatcheries within the Great Lakes basin. While the pathogenicity of LMBV is not clear, it has been implicated in several epizootics, therefore, it is prudent to reduce the possibility of further dissemination of the virus. Cell culture and molecular techniques are available for detection.

Streptococcosis, *Streptococcus iniae*, Bacterium, STP, SI

Streptococcus iniae has become a significant pathogen in intensive tilapia culture, especially recirculating systems, where artificially heated water is used to maintain elevated temperatures. Under adverse conditions the disease can be devastating to tilapia and this pathogen is also capable of infecting other fish species as well as humans. Limiting its spread is important; however, isolation of the bacterium, which is not 100% accurate is the only means currently available for detecting carrier fish.

Yellow Perch Microsporidian, *Heterosporis* sp., YPM, YPH

The yellow perch microsporidian, *Heterosporis* sp. is known to occur in a limited number of yellow perch populations in Wisconsin and Minnesota lakes. Although this parasite apparently does not kill fish it does make them unacceptable to the public. Detection is by examining fresh muscle material and histopathology.

Asian Tapeworm, *Bothriocephalus achielognathi*, ATW

The Asian tapeworm, *Bothriocephalus achielognathi*, is found throughout the world and there has been one report of it in New York. This parasite primarily affects very young cyprinids which may suffer high mortality but causes little problem in older fish. The effect of this parasite on the fishery resources of the Great Lakes basin should not be great. Detection is by tapeworm identification in wet mounts of material from the anterior intestine.

Notable Fish Pathogens

Notable fish pathogens include those of warmwater fishes that occur in warmer climates of North America and could be introduced into the Great Lakes basin via fish importations. However, the possibility of these pathogens becoming established and adversely affecting fishery resources in the basin is minimal due to environmental and/or biological limitations. These diseases would be known through diagnostic records.

Channel Catfish Virus, *Herpesvirus ictaluri*, CCV, CCVD

Channel catfish virus infects young of the year channel catfish during warm summer months. The possibility of this disease causing adverse effects in the Great Lakes basin is minimal

because of environmental requirements. However, if very young CCV infected channel catfish were stocked into a recirculating system with water temperatures above 25°C, a high mortality would likely occur. Detection of carrier fish by virus isolation is not dependable, therefore, detection of potential carrier fish is by PCR.

Enteric Septicemia of Catfish, *Edwardsiella ictaluri*, ESC

Enteric septicemia of catfish is almost exclusively a disease of cultured channel catfish occurring in pond, cage, and recirculating culture systems. Epizootic survivors carry *Edwardsiella ictaluri* which can be detected by isolation. Due to its extensive presence in the catfish industry fish without some exposure to *E. ictaluri* are difficult to find.

Antibiotic Resistant Motile Aeromonads, *Aeromonas* spp., MAS; and Columnaris, *Flavobacterium columnare*

Isolates of antibiotic resistant motile aeromonads, columnaris, and other bacteria have become more prevalent during the last 20 years and has hampered chemotherapeutic control of target diseases. Using diagnostic records it would be useful to know where and how often these resistant strains occur and when appropriate steps should be taken to restrict their dissemination.

Proliferative Gill Disease, *Aurantiactinomyxon ictaluri*, Haplosporidian, PGD

Proliferative gill disease affects the gills of cultured catfish throughout southern United States. The parasite has a complex life cycle involving bottom dwelling oligochaets but the possibility of this parasite occurring and/or becoming established in the Great Lakes basin is minimal. Detection is by histological examination of gill tissue.

<u>Inspection Procedures and Methods of Diagnosis</u>

It is suggested that the procedures for inspection of hatcheries and diagnosis of overt diseases follow those of the *Great Lakes Fish Disease Control Policy and Model Program* (Hnath 1993); *Blue Book: Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens* (Thoesen 1994) (Currently under revision); or the Canadian *Fish Health Protection Regulations Manual of Compliance* (Anonymous 1984). A new document is

in preparation entitled *Field and Laboratory Inspection Procedures for the Detection of Certain Pathogens for the Movement of Aquatic Animals* that is designed to be applicable and acceptable to state, federal, and international regulatory agencies for export/import of fish. The document is result of a collaborative effort by the Fish Health Section of the American Fisheries Society, the U.S. Fish and Wildlife Service and APHIS. The current Fish-Disease Inspection Report should be amended to include Emergency and Restricted diseases.

Hatchery Classification

Warmwater and cool-water fish hatcheries and other fish sources should be classified along the same guidelines as in *Great Lakes Fish Disease Control Policy and Model Program* (Hnath 1993) to include Emergency and Restricted diseases listed herein, and the Hatchery Classification Report in Annex VI be amended to accommodate warmwater and cool-water fish diseases.

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