Strategies for Fish Health Management of Tank-Raised Walleye

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Introduction

No drugs or chemicals are registered by FDA (Food and Drug Administration) for use in the fish health management of walleye. Although salt and other low-regulatory priority drugs may be used, strategies to reduce stress must be employed for an effective fish health program. This paper will describe techniques that are used at Jake Wolf Fish Hatchery, Topeka, Illinois to minimize stress.

Methods

Pond culture

At Jake Wolf Fish Hatchery, we incubate walleye eggs at 53°F (12°C). Ten to 14 d before stocking, plastic-lined ponds are fertilized with organic fertilizers to achieve a Secchi disk reading of about 12 in (30 cm) when the fry are stocked. Fry are stocked into ponds at 100,000/acre (247,700/ha) when the fry are 4–5 d old. If the fry are stocked at that time, there will be sufficient zooplankton to grow them to 1.25–2 in (32–50 mm) in 30–40 d. If the zooplankton production increases dramatically, the water clears due to the grazing of zooplankton on algae, and Secchi disk readings increase to 7.5 ft (2.3 m), which is the pond bottom. At 30 40 d, careful monitoring of water clarity is necessary. The Secchi Qsk reading will begin to decrease as the algal populations recover, because the fish are cropping the zooplankton. When the Secchi disk reading reaches about 2 ft (0.6 m), the fish are harvested. If this scenario occurs, a harvest of >70,000 fingerlings/acre (>172,970/ha) may be realized.

The first stressor to intensive culture of walleye is poor condition of the fingerlings when they are harvested from the pond. If harvest is delayed, the fish may exhaust their food supply and stop growing. If the Secchi disk reading reaches 1 ft (30 cm), which means that the zooplankton population is exhausted, the fingerlings will begin to starve. Fingerlings harvested in poor condition that are brought inside and trained on pelleted diets will quickly succumb to columnaris *(Flexibacter columnaris)* or they will not accept formulated feed. If the harvest is timed correctly, 70–75% of the fingerlings will accept the pelleted diet.

Another stressor is pH shock. When the Secchi disc measurement in the ponds before harvest is <2 ft (30 cm), pond pH is probably high, and there is a possibility of pH shock while moving the fish from pond to tanks. At the Jake Wolf Hatchery, the pH at harvest may be 10 in the pond, 7.8 in the hatchery building, which is supplied with ground (well) water. Draining the pond from the bottom will draw down the high-pH top surface water onto the fingerlings being harvested. Flushing the pond kettle with fresh water will relieve this problem.

Tank culture

Timing of harvest and movement of fingerlings from the ponds to the hatchery is critical because of columnaris Qsease, a stress-mediated disease, that can result in mass mortality. Facilities for tank culture must be ready when the fingerlings are harvested. Feed must be presented to them immediately or they will never commence feeding. If there is any delay, major outbreaks of columnaris will occur. As the diet training period begins, columnaris will kill the non-feeders within 10 d, regardless of chemotherapy.

At Jake Wolf Fish Hatchery, our best success at reducing stress is achieved using single-pass heated (68–70°F, 20–21°C) well water. If well water is not available, we use filtered pond water, but Qsease problems develop because of its poorer quality and higher temperature.

Gas pressure, measured as **AP,** in the training tanks is another stressor. Gas supersaturation in our hatchery

was subtle and episodic. Typically, ΔP was low (4–5 mm Hg) but it often spiked briefly enough to stress the fish. We noted a reduction in outbreaks of columnaris occurred at the Jake Wolf Hatchery after we installed packed column degassers on each culture unit.

In our early efforts to convert pond-raised fingelings to formulated feed, we used 1,100-gal (4,164-L) rectangular tanks. These tanks were unsuitable because of poor flow characteristics; dead spots in the tank corner; and accumulations of uneaten food, feces, and ammonia. We observed the first case of gill rot fungus (*Branchiomyces sanguinis*) in walleye in North America due to the degraded environment.

Initially, we fed the fish every 5 min at a total of 100% body weight/d, with continuous illumination. After feeding for 3 d, the tanks contained a large mass of uneaten food. When we switched to circular tanks, their self cleaning capabilities quickly improved environmental conditions. Circular tanks seem preferable to rectangular tanks, because it allows the fish to swim continuously without encountering a barrier. The result is a calmer, less stressed fish. Feeder placement can be more easily optimized, and the circular motion of the water tends to keep feed particles suspended much longer than those in rectangular tanks, giving the fish several chances at striking at a food particle before it drops to the bottom. In our trials, with Biodiet or Biotrainer feeds (Bioproducts, Inc., Warrenton, OR) were readily accepted and trained the fish to accept pelleted feed. In some years, we were able to train 95% of the fish to accept a pelleted diet.

Careful phasing in changes in feed size is critical. If particle size is changed too soon, some fish may starve because their mouths are too small to ingest the larger pellet. If the feed size is not increased soon enough, food may be wasted and growth rate will be reduced.

Walleye are skittish and are spooked by movement above them. Walleye do not adjust as well as other cultured species to activities of hatchery workers. This problem can be reduced by lighting the interior of the culture tank and darkening the room or by covering the tank on the side where human activity occurs.

Fish density is another factor which can contribute to occurrence of diseases. We keep the density (lbs/ft^3 or kg/m^3) at no more than 0.9 lb/ft^3 (14 kg/m^3). When density exceeds 0.2 lb/ft^3 (3.2 kg/m^3) early in the training period, the fish in a tank are divided into two lots. Loading (lb/gpm of flow, g/Lpm), should not exceed 1.24 lb/gpm (149 g/Lpm) early in the cycle, but as fish increase in size, loading must be reduced to 0.5 to 0.7 lb/gpm (60-84 g/p/m).

Disease treatment

Strict attention to the density and loading requirements has prevented most disease outbreaks in our facility. Inevitably however, in spite of all these efforts, columnaris, fungus, or parasite problems can occur.

As a treatment for columnaris, we use a salt (NaCl) bath at 2% for as long as the fingerlings will tolerate it. This may be combined with lowering the temperature out of the optimal range for columnaris. If done promptly, these treatments have stopped columnaris outbreaks.

Although it has been used effectively in the past, formalin is not currently labeled as a fungicide or parasiticide for any life stage of walleye. As a result, we used hydrogen peroxide (H_2O_2) as a chemotherapeutant for the first time in 1994. FDA has declared the use of H_2O_2 as a fungicide to be of low regulatory priority. No Investigational New Animal Drug Application must be filed for its use, provided that it is used within guidelines. It is a low regulatory compound because its breakdown products are oxygen and water. Trials by Richardson and Colesante (1995) indicated that 15-minute exposure of walleye eggs to 500 ppm H_2O_2 were nearly as effective as 250 ppm formalin as a fungicide on walleye eggs, but clumping was noted in eggs treated with H_2O_2, leading to some loss of otherwise healthy eggs. They also noted that these clumps of dead eggs sometimes floated and plugged the outlet of the jar. Small eggs included a tendency to form bubbles on the egg and float the entire mass out of the hatching jar. This may be overcome by temporarily transferring the eggs to a bucket for treatment. Hydrogen peroxide does have some promise as a fungicide for eggs, it may be necessary if formalin does not receive the hoped for label extension by FDA for use on walleye. However, fingerling walleye are very sensitive and will tolerate less H_2O_2 than other species (Clayton and Summerfelt 1995). At Jake Wolf Hatchery, at 118 ppm total alkalinity, pH 7.8, and 68°F(20°C), healthy 3-in (76-mm)

fingerlings seem to tolerate a treatment of 50–100 ppm for up to 1 h.

Conclusion

stress is a fish health factor that contributes to occurrence of disease. Stress must minimizedd to achieve successful habituation of walleye to formulated feed. Most stress is related to techniques that are controlled by the aquaculturist. Our experience at Jake Wolf Hatchery demonstrates that successful pond culture of a 1.5–2.0 in (3.8–5.0 cm) fingerling and subsequent success of training these fingerlings to formulated feed are strongly influenced by the degree to which we control the stress factors.

References

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Disease Investigation of Tank-Reared Triploid Walleye

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Introduction

This report describes an investigation of a disease outbreak in an experimental group of 1,400, 11-month-old (150–200 mm) triploid walleye. They were used in an experiment by the University of Wisconsin-Madison Aquaculture Program (UWAP) at their campus facility. A disease investigation was required to mitigate the mortalities and to investigate whether the mortalities were related to husbandry practices, facility design, or to experimental treatment. Information on the cause of the mortality was needed for design of future research and to optimize fish health conditions in the facility. The information is presented here as a contribution to commercial production of walleye.

Methods

The triploid walleye that were the focus of this report were housed in a 5,000-gal (18,925 L) circular fiberglass tank. The experimental tank was supplied with tempered municipal well water presumed to be pathogen-free. The temperature had been held at 70°F (21°C) for the previous 75-d, and the flow was 15–25 gpm (57–95 Lpm). The inflow produced a circular flow current in the tank. The fish originated from eggs collected from wild diploid walleye broodstock. The eggs were treated with pressure-shock to induce triploidy. Triploidy was determined by flow cytometry. Their first month post-hatch was spent in a fertilized 0.25 acre (0.10 ha) earthen rearing pond at the Lake Mills aquaculture facility of the UWAP. When fish were 2.5–3 in (40–50 mm) total length they were harvested using a light-harvest technique. The fingerlings were transferred to circular fiberglass tanks when they were trained to accept a commercial salmon starter feed. They were subsequently fed commercial dry trout feed of appropriate pellet size.

When fish reached 4-inch (100-mm) they were divided into two treatment groups. One group of 300 fish remained in the Lake Mills facility and were housed in 60-gal (227 L) and 200-gal (757 L) circular fiberglass tanks. The other group consisted of 1,450 fish that were transported to and housed at the UWAP campus facility in a 5,000-gal (18,927 L) circular fiberglass tank. This later group was subjected to similar water quality and nutritional parameters as the group at the Lake Mills facility.

Prior to the disease outbreak in the UWAP site, the triploid walleye were growing rapidly, and were feedmg aggressively. Mortality in both groups of triploid walleye was <0.01%/d. Two d prior to the disease outbreak, the campus triploid walleye were confined, anesthetized (~50 mg/L Finquel), captured, and weighed and measured. Their tank was partially drained and the fish were collected using a seine. The weighing and measuring procedure lasted 3-h. Coincidentally, less than 24-h after the procedure, a mechanical failure resulted in a drop in tank temperature from 70–59°F (21–15°C). The day after the temperature decline, the fish fed poorly, appeared lethargic, and mortality increased to **3%** of the population on March 16, 1994. Thirty-six h after the procedure water temperature was returned to 70˚F (21˚C).

Potential sources of horizontal disease transmission were investigated. No new arrivals of fish to the campus facility had occurred in the previous 4 months. Six and 11 months earlier, columnaris disease had occurred in rainbow trout, and *Edwardsiella tarda* infection in channel catfish held in the facility. However, these disease outbreaks occurred in tanks in rooms other than the one that housed the triploid walleye.

The potential for transmission of infectious agents from contact with contaminated equipment was believed to be minimal due to hygiene practices. However, dip nets used to collect, or transfer fish were shared among groups of experimental species. These nets were disinfected in 500-ppm chloramine-T solution, and

rinsed in water, between use with different species and tanks. It was assumed that the chloramine-T disinfection of nets effectively destroyed most potential infectious agents. The net used to capture the triploid walleye for the weighing and measuring procedure was housed in dry storage for 6 months prior to its use. It was used to seine diploid and triploid walleye and yellow perch fingerlings from earthen ponds at the Lake Mills facility. The net contained dried pond mud.

In the tank, fish were generally lethargic, swimming primarily in the lower one third of the tank, and they refused to feed. No abnormal appearance or odor of the water was detected. Due to the flow-through design of the tanks and the time elapse since the weighing and measuring procedure, measurements of water quality parameters associated with accumulation of fish metabolic wastes was not pursued. Additional complicated and expensive water analysis was reserved pending the results of less expensive, rapid necropsy results.

Twelve moribund fish were collected for necropsy examination. They were not emaciated, which was evidence for an acute rather than a chronic disease problem. Gross abnormalities on external exam included: generalized pale appearance (12/12), hemorrhage at base of tail (12/12), anal fins (12/12), mandible and gill operculum (4/12), and diffuse yellow discoloration of ventral abdomen (12/12). All fish had fraying on most of their fins. A gross exam of internal organs revealed no abnormalities, other than absence of food material in the digestive tract.

Skin scrapings, fin clips, and wet mounts of gills arches from the sampled moribund and dead fish from the campus tanks were examined microscopically. The skin scrapings, and fin clips revealed parasitic, fungal, and bacterial agents. Numerous actively motile monogenetic trematode parasites with no eye spots, and two large hooks were evident on skin scrapings and fin clips of all moribund fish examined. Fungal organisms with non-septate filamentous hyphae were readily evident, particularly on the slun scrapings. Long filamentous gram-negative bacteria often in clusters were occasionally noted, particularly on the skin scrapings. The presumptive a diagnosis was severe gyrodactyliasis, moderate saprolegniasis, and a few fish with columnaris disease. None of the dead fish exhibited any external parasitic, fungal, or bacterial pathogens. A comparable investigation was performed on the Lake Mills fish and no abnormalities were found.

Appropriate samples of fish from the campus tank were collected to attempt to rule-out numerous bacterial and viral agents as contributory causes to systemic disease. Sterile hdney samples were collected for bacteriological isolation and identification. The samples were submitted to a private veterinary laboratory experienced in the isolation of fish pathogens. No bacteria were isolated from the samples submitted. Kidney and spleen samples were collected from 60 moribund and apparently healthy fish and submitted for virology testing at a federal fish health lab. The virology results were also negative.

It was determined that the first pathogen that had to be controlled was the ectoparasite, because it was considered the major underlying agent initiating damage to the shn, and it could be treated relatively easily. A formalin treatment at 167 ppm for 60 min in a static bath was attempted. Formalin is not approved for use in walleyes, although it is available by Investigational New Animal Drug (INAD), or extra-label veterinary prescription. Thirteen fish were transferred from the affected tank into a 227-L (60-gal) trial treatment tank and provided with supplementary aeration with airstones throughout the treatment period. All 13 fish died during the treatment trial. Our next alternative trial treatment was a 2.0% concentrated salt bath for 60 min. No mortalities resulted from the trial treatment, therefore we proceeded to treat the entire group of fish. The standpipe was lowered to achieve a practical volume. Supplementary aeration was provided with airstones that maintained dissolved oxygen levels of 6.6–8.6 ppm during treatment. The treatment was repeated the following day, and again 2 d later. The fish showed evidence of irritability and loss of equilibrium during treatment, however no mortalities occurred during treatment, and mortality rates declined with each subsequent treatment.

A sample of the treated fish were examined 14-d after the last treatment. There was no microscopic evidence of parasitic, bacterial, or fungal organisms. The fish recovered, regained vigorous appetites, and grew well. The fish were re-examined 90- later in preparation for 44-h truck transport to Washington state. This exam, did not indicate any parasitic, bacterial, or fungal organisms. Although there is stress associated with crowding

and transport, only one mortality occurred during transport, supporting the perceived benefit of eliminating treatable pathogen prior to exposure of the fish to this stress.

Conclusion

T h e findings of our investigation support the need to pursue existing principals of fish health, and employ a systematic, practical approach to disease diagnosis and treatment. The findings support the need to examine moribund opposed to dead fish for necropsy examination. Also, it is obviously beneficial to perform a trial treatment on a dispensable sample of the affected population before treating the entire population. Contrary to common assumption, ectoparasites can be a significant problem in tank-reared fish. The origin of the ectoparasites is speculative. The possibilities include: subclinical infestation since pond rearing stage, introduction by contaminated equipment, transmission from subclinically fish housed in other tanks in the campus facility. In this case, stress seemed to play a crucial role in precipitating clinical disease in seemingly healthy fish. Fish should be examined for fish pathogens prior to introduction into new rearing units, or exposure to stressful procedures. Fish pathogens can be opportunistic. There was no evidence to indicate that a disease outbreak of this nature is particular to tank-reared triploid walleye. Rather it supports the importance of decontaminating fish prior to introduction into indoor tank facilities. Diseased fish can be effectively treated, returned to vigorous health, and subjected to stressful procedures, such as transport, without significant mortalities.

Chemicals and Drugs

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Regulations and policies for walleye culture

In the United States, the application of chemicals to any organism such as walleye or to their environment is regulated by the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM), the U.S. Environmental Protection Agency (EPA), or the Animal and Plant Health Inspection Service (APHIS). CVM controls the use of drugs (e.g., therapeutants and anesthetics), EPA controls the application of chemicals and pesticides in the environment, and APHIS regulates all veterinary biologics (vaccines, non-drug biological therapeutants, and diagnostic test kits). In cases involving water treatments to control pathogens or the organisms that can cause disease, the jurisdiction becomes unclear and has changed over time. Each agency has developed guidelines and policies to implement the laws for their respective fields of responsibility. A recent publication by the Joint Subcommittee on Aquaculture's Working Group on Quality Assurance in Aquaculture Production (Working Group) has summarized the federal regulations of aquaculture substances by CVM, EPA, and APHIS (Worlung Group 1992).

The most important law enacted by the U.S. Congress having a major impact on walleye culture is the Federal Food, Drug, and Cosmetic Act. Section 512 of the Act states that a drug shall be considered unsafe and subject to enforcement unless it has an approved new animal drug application (NADA) and that the intended use of a drug and its labeling conform to the approved application. The Act provides exemptions for the investigational use of unapproved drugs called "investigational new animal drug" (INAD) exemptions.

CVM has developed regulations and policies to assist with implementing the Act. These are regularly published in the Federal Register and codified in Title 21 of the Code of Federal Regulations. Examples of these rulings pertinent to walleye culture include regulations on applications for new animal drugs, investigational use of new animal drugs, minor use provisions, medicated feeds, and good laboratory practices for non-clinical laboratory studies. In addition, CVM continues to develop guidelines and policies that are not codified but are either on file at CVM or placed in the Compliance Policy Guide. Good examples of these provisions are "Extra-label use of new animal drugs in food-producing animals" (Compliance Policy Guide 7125.06), "Guidelines for the preparation of data to satisfy the requirements of Section 512 of the Act regarding minor use of animal drugs," and low regulatory priority (LRP) provisions.

Status of chemicals and drugs for walleye culture

Current information on federally approved uses (includmg unapproved drug provisions) of drugs, vaccines, and pesticides in aquaculture production and in natural aquatic sites is available in a recent publication by the Working Group (Working Group 1994). This publication will be kept up-to-date electronically by the U.S. Department of Agriculture's National Program Leader for Aquaculture with input from CVM, EPA, and APHIS. All aquaculture producers are responsible for using the products that not only are federally approved but that also meet state and local regulations. The proper use of the products listed in this publication is important for insuring their safety and effectiveness, and the reduction or prevention of drug overuse, possible undesirable side effects, and illegal residues. Not many drugs or chemicals are available to walleye producers, as is the case with many other fish species (Table 1). The only approved drug for use on walleye is Finquel, an anesthetic that has a 21-d withdrawal time.

The only vaccine commercially available for use on walleye is Autogenous Bacterin available from BioMed, Inc. and Aqua Health, Ltd. It is effective on unspecified bacterial diseases (Working Group 1994).

Table 1. FDA-approved new animal drugs (Working Group 1994)

Trade Name	NADA Number	Sponsor	Active Drug	Species	Uses
Finquel	42-427	Argent Chemical Laboratories, Inc.	Tricaine methanesulfonate	Ictaluridae, Salmonidae, Esocidae, and Percidae. (In other fish and cold-blooded animals, the drug should be limited to hatchery or laboratory use.)	Temporary immobilization (anesthetic)
Formalin-F	137-687	Natchez Animal Supply	Formalin	Trout, salmon, catfish, largemouth bass, and bluegill	Control of external protozoa and monogenetic trematodes.
				Salmon, trout, and esocid eggs	Control of fungi of the family Saprolegniacae.
Paracide-F	140-831	Argent Chemical Laboratories, Inc.	Formalin	Trout, salmon, catfish, largemouth bass, and bluegill	Control of external protozoa, monogenetic trematodes.
				Salmon, trout, and esocid eggs	Control of fungi of the family Saprolegniacae.
Parasite-S	140-989	Western Chemical Inc.	Formalin	Trout, salmon, catfish, largemouth bass, and bluegill	Control of external protozoa and monogenetic trematodes
				Salmon, trout, and esocid eggs	Control of fungi of the family Saprolegniacae
				Cultured penaeid shrimp	Control of external protozoan parasites
Romet-30	125-933	Hoffmann-LaRoche, Inc.	Sulfadimethoxine and ormetoprim	Catfish	Control of enteric septicemia
				Salmonids	Control of furunculosis
Sulfamerazine in Fish Grade'	033-950	American Cyanamid Company	Sulfamerazine	Rainbow trout, brook trout, and brown trout	Control of furunculosis
Terramycin for Fish	038-439	Pfizer, Inc.	Oxytetracycline	Catfish	Control of bacterial hemorrhagic septicemia and pseudomonas disease
				Lobster	Control of gaffkemia
				Salmonids	Control of ulcer disease, furunculosis, bacterial hemorrhagic septicemia, and pseudomonas disease
				Pacific Salmon	Marking of skeletal tissue

'According to sponsor, this product is not presently being distributed.

Chemicals that are legal to use in walleye culture under existing pesticide labels include certain algicides, herbicides, and fish toxicants. These products must be used according to label directions and restrictions, especially those that pertain to use when fish are present. The algicides include copper compounds and copper sulfate; fish toxicants include antimycin and rotenone; and herbicides include 2,4-D, acid blue and acid yellow combination, dichlobenil, diquat dibromide, endothall, fluridone, and glyphosate (Working Group 1994).

Because of the high cost, the large number of unap-proved drugs, and general lack of interest by the pharmaceutical industry, aquaculture groups had to define the most needed chemicals, prioritize them, and eliminate the least important drugs from consideration. Several groups (U.S. Fish and Wildlife Service, National Biological Service, certain states, and Fish Health Section of the American Fisheries Society) requested and received rulings from CVM that resulted in the declaration of 18 unapproved drugs as being "low regulatory priority," six drugs considered as not low regulatory priority (i.e., requiring INAD's), 21 drugs or family of drugs that are considered high regulatory priority (HRP) for enforcement action, two drugs with deferred regulatory action, and nine substances that did not meet the definition of a drug for their intended uses (Tables 2 and 3). The use of drugs considered as HRP must be covered by either INAD's or approved NADA's; CVM also determined that any nitrofuran (e.g., furazolidone), malachite green, and methylene blue would not be granted any INAD exemptions and that sulfonamides would not likely be granted INAD exemptions until the carcinogenicity issues have been clarified (Working Group 1994; Geyer 1993; Homaire 1994; CVM, personal communication).

The FDA is unlikely to object at present to the use of low regulatory priority substances (Table 2) if the following conditions are met:
1. The drugs are used for the prescribed indications, including species and life stage where specified.
2. The drugs are used at the prescribed dosages.
3. The drugs are used according to good manage-ment practices.
4. The product is of an appropriate grade for use in food animals.
5. An adverse effect on the environment is unlikely.

FDA's enforcement position on the use of these substances should be considered neither an approval nor an affirmation of their safety and effectiveness. Based on information available in the future, FDA may take a different position on their use.

Classification of substances as new animal drugs of low regulatory priority does not exempt facilities from complying with other federal, state, and local environ-mental requirements. For example, facilities using these substances would still be required to comply with National Pollutant Discharge Elimination System requirements.

The LRP drugs of greatest importance to walleye culture include hydrogen peroxide and sodium chloride (salt). Hydrogen peroxide has been used experimentally to control fungal infections in eggs (Colesante 1995). Although hydrogen peroxide has the potential as a parasiticide, it has been found to be extremely toxic to fingerling walleye at concentrations greater than 50 ppm in a one-hour static bath (R.D. Clayton and R.C. Summerfelt, Iowa State University, personal communi-cation). Salt is a proven parasiticide and also useful and allowed as an osmoregulatory aid under LRP status.

Copper sulfate and potassium permanganate have been deferred for regulatory action when they are used to control fungal and bacterial infections and external parasitic infestations on walleye. INAD's for both these compounds have been developed at the encouragement of CVM.

Chemicals used to regulate water pH are not considered drugs and therefore, CVM does not regulate them. These chemicals include calcium carbonate, calcium hydroxide, sodium hydroxide, and tris buffer. EPA or the states have primary jurisdiction over these com-pounds. Facilities need to comply with the rules developed by the agency responsible for administering the National Pollutant Discharge Elimination System (NPDES) permits.

Dimethylpolysiloxane, when used to reduce foam or scum, is classified as a non-drug by CVM. It is also under EPA jurisdiction, and facilities are required to contact the NPDES permitting agency prior to discharg-ing water containing this chemical.

Provisions for unaparoved drug use in walleye culture

FDA recognized that provisions were needed to allow the use of certain unapproved drugs in fish culture until data are developed for full approvals (i.e., NADAs). The first provision was the designation of drugs as LRP;

currently, 18 unapproved drugs are considered to be LRP aquaculture drugs. CVM will not object to the use of drugs that arc classified as LRP if they arc used under the conditions indicated, at the prescribed levels, according to good management practices, of an appropriate grade for use on food animals, and not

Table 2. Unapproved new animal drugs of low regulatory priority (LRP) for FDA[1] (Working Group 1994)

Common Name	Permitted Use
Acetic Acid	Used as a dip at a concentration of 1,000-2,000 mg/L for 1-10 minutes as a parasiticide for fish
Calcium chloride	Used to increase water calcium concentration to ensure proper egg hardening. Dosages used would be those necessary to raise calcium concentration to 10-20 mg/L calcium carbonate. Also used to increase water hardness up to 150 mg/L to aid in maintenance of osmotic balance in fish by preventing electrolyte loss.
Calcium oxide	Used as an external protozoacide for fingerling to adult fish at a concentration of 2,000 mg/L for 5 sec.
Carbon dioxide gas	Used for anesthetic purposes in cold- cool- and warmwater fish.
Fuller's earth	Used to reduce the adhesiveness of fish eggs in order to improve hatchability.
Garlic (whole)	Used for control of helminth and sea lice infestations in marine salmonids at all life stages.
Hydrogen peroxide	Used at 250-500 mg/L to control fungi on all species and at all life stages of fish, including eggs.
Ice	Used to reduce metabolic rate of fish during transport.
Magnesium sulfate (Epsom salts)	Used to treat external monogenetic trematode infestations and external crustacean infestations in fish at all life stages. Used in freshwater species. Fish are immersed in a solution of 30,000 mg/L magnesium sulfate and 7,000 mg/L sodium chloride for 5-10 min.
Onion (whole)	Used to treat external crustacean parasites and to deter sea lice from infesting external surface of fish at all life stages.
Papain	Used as a 0.2% solution in removing the gelatinous matrix of fish egg masses in order to improve hatchability and decrease the incidence of disease.
Potassium chloride	Used as an aid in osmoregulation to relieve stress and prevent shock. Dosages used would be those necessary to increase chloride ion concentration to 10-2,000 mg/L.
Povidone iodine compounds	Used as a fish egg disinfectant at rates of 50 mg/L for 30 min during water hardening and 100 mg/L solution for 10 min after water hardening.
Sodium bicarbonate (baking soda)	Used at 142-642 mg/L for 5 min as a means of introducing carbon dioxide into the water to anesthetize fish.
Sodium chloride (salt)	Used as a 0.5-1% solution for an indefinite period as an osmoregulatory aid for the relief of stress and prevention of shock. Used as a 3% solution for 10-30 min as a parasiticide.
Sodium sulfite	Used as a 15% solution for 5-8 min to treat eggs in order to improve hatchability.
Thiamine hydrochloride	Used to prevent or treat thiamine deficiency in salmonids. Eggs are immersed in an aqueous solution of up to 100 ppm for up to 4h during water hardening. Sac fry are immersed in an aqueous solution of up to 1,000 ppm for up to 1h.
Urea and tannic acid	Used to denature the adhesive component of fish eggs at concentrations of 15 g urea and 20 g NaCl/5 L of water for about 6 min, followed by a separate solution of 0.75 g tannic acid/5 L of water for an additional 6 min. These amounts will treat about 400,000 eggs.

likely to cause an adverse effect on the environment (Worlung Group 1994; Table 2).

The second provision for the use of unapproved drugs is somewhat limited, but it offers some relief. That provision is extra-label use. CVM will allow extra-label drug use if the health of the animals is threatened and if suffering or death would result from failure to treat the affected animals. The major provisions of extra-label use includes:

1. Only applies to drugs approved for other animal species;
2. Only available for practicing veterinarians;
3. A valid veterinarian-client-patient relationship exists;
4. There is no approved or effective approved drug available for the species in question;
5. The veterinarian assumes responsibility for efficacy, animal safety, and residue levels in edible tissues;
6. Not for use with drugs mixed in feeds;
7. Not for use with drugs that prevent disease;
8. Not for use with drugs that improve growth rates or enhance reproduction or fertility; and
9. Human drugs are eligible.

Thus, under these criteria, only formalin could be prescribed for use on walleye by practicing veterinarians. Until the passage of "The Animal Medicinal Drug Use Clarification of 1994" (Public Law 103-396), extra-label use of medicated feeds in aquaculture was considered by CVM to be low regulatory priority if the requirements of the Extra-label Use Compliance Policy Guide 7125.06 were met and as long as the feeds were formulated and labeled properly in accordance with medicated feed regulations. This meant that oxytetracycline medicated feed approved for use in catfish could be prescribed extra-labelly for walleye by a licensed veterinarian and still be consistent with the extra-label use policy guidelines. When CVM promulgates extra-label regulations under the 1994 law (required by October 1996), The discretionary extra-label use in feed may be eliminated or curtailed because of the law expressly prohibits extra-label use of feed use drugs (CVM, personal communication).

All the remaining aquaculture compounds considered to be drugs by CVM are expected to have approvals or to be used under provision of an INAD exemption (Geyer 1993). There are two types of INADs: standard and compassionate. The compassionate INADs are being granted to producer groups and agencies willing to accept the responsibility of administering these INAD's. Under a compassionate INAD exemption, data must be generated to support the approval of the drug; if it is not, the INAD will not be renewed (Geyer 1994). All chemicals defined as drugs that are either currently used or anticipated for emergency use must be covered under an INAD so that the need for emergency use is eliminated or at least greatly reduced. All users of these identified drugs, except researchers working on laboratory animals, are included under tlvs INAD process — public and private production facilities, fishery management units, and participants in fishng tournaments, etc. (Stefan 1994). This process is not a panacea, but it is the only means of using the needed drugs legally (Geyer 1993).

FWS recognized that, as a national resource agency, it needed to provide leadership in complying with CVM regulations. In October, 1992, FWS started the process by initiating a survey of the chemical and drug needs of national fish hatcheries and other fishery facilities. At a workshop on INAD's in January, 1993, it was determined which drugs and chemicals would: 1) have INADs developed or renewed; 2) be presented to FDA for an opinion on their status; 3) not require INAD; and 4) not be acted upon. When the chloramine-T INAD was completed for FWS by the newly created National Biological Service (NBS), a template was offered for others to develop an INAD on chloramine-T or other water treatment compounds.

As of September, 1994, CVM had permission to disclose 74 INAD permits that are in place for 26 aquaculture drugs. Twenty-two disclosable, aquaculture INAD's were granted by CVM for use on walleye and include chloramine-T, oxytetracycline, human chorionic gonadotropin, copper sulfate, a copper compound, formalin, and diquat dibromide. Updated lists of disclosable INAD's may be obtained from CVM.

Walleye producers are encouraged to enroll in the quality assurance program being developed by the National Aquaculture Association (Shepherdstown, WV). This program is important because it will improve production efficiency and provide the consumers with farm-raised products that are healthy and free of any harmful chemical residues (Worlung Group 1994).

Table 3. Status of aquaculture chemicals not considered low regulatory priority (LRP) drugs.

Substances-Uses	Drugs not LRP[1]	High Regulatory Priority'	Regulatory Action Deferred	Chemicals not Drugs[2]
Acriflavine - egg disinfectant; bactericide, fungicide, and parasiticide		X		
Benzalkonium chloride - bactericide	X			
Benzethonium chloride - bactericide	X			
Benzocaine - anesthetic		X		
Calcium carbonate - water pH and alkalinity control				X
Calcium hydroxide - water pH control				X
Carp pituitary extract - hormone		X		
Chloramphenicol - bactericide		X		
Copper sulfate - parasiticide, bactericide, and fungicide			X	
Cytochalasin B - oyster triploidy inducer		X		
Dimethylpolysiloxane - foam reducer				X
Epinephrine - clam and oyster metamorphosis inducer	X			
Erythromycin		X		
b-Estradiol - hormone		X		
Fluoroquinolones (e.g., enrofloxacin, sarafloxacin) - bactericides		X		
Human chorionic gonadotropin - hormone		X		
L-Dopa - clam and oyster metamorphosis inducer	X			
Luteinizing hormone - releasing hormone analog - hormone		X		
Malachite green - parasiticide and fungicide		X		
Methylene blue - parasiticide		X		
17-a-methyltestosterone - hormone		X		
Metomidate - anesthetic		X		
Nitrofurans (e.g., nitrofurazone, furazolidone, nifurpirinol) - bactericides		X		
Oxygen - maintain saturated dissolved oxygen conditions				X
Oxytetracycline - fish marker and bactericide		X		
Ozone - disinfectant and organic compound remover				X
Penicillin - control bacteria in algae fed to oyster larvae				X
Penicillin dihydrostreptomycin - bactericide		X		
Potassium permanganate - oxidizer, disinfectant, parasiticide, bactericide, and fungicide			X	
Quinaldine - anesthetic		X		
Quinolones (e.g., oxolinic acid, naladixic acid) - bactericides		X		
Salmon pituitary extract - hormone		X		
Sodium chlorite - bactericide and fungicide	X			
Sodium hydroxide - water pH control				X
Streptomycin - control bacteria in algae fed to oyster larvae				X
Sulfonamides (e.g., sulfamerazine, sulfadimethoxine) - bactericides		X		
Trichlorfon - parasiticide	X			
Tris buffer - water pH buffering				X

'Compassionate INAD's or NADA's are required for the use of these drugs; however, no compassionate INAD's will be issued for malachite green, methylene blue, chloramphenicol, or the sulfonamides.

[2]These chemicals are not considered drugs for the specific intended uses recommended.

Data generation for drug approvals for walleye culture

T h e INAD process will work only if INAD's lead to approved NADA's. The INAD's will allow the use of drugs on a provisional basis until all the data needed by FDA for new animal drug applications are generated. These data include target animal safety, residue, environmental, and mammalian safety studies. These data must be developed in a timely manner or the INAD's will be rescinded. These data (except field efficacy) must be generated according to good laboratory practices (GLP) provisions.

Data required for the approval of human chorionic gondatropin (HCG) as a hormone to regulate spawning is being funded the Striped Bass Technical Committee, American Fisheries Society, and implemented by Auburn University and Intervet, Inc. Currently, CVM has disclosed that at least 13 INAD's on HCG are in effect. The National Research Support Program Number 7 (formerly the IR-4 program) has projects underway for amoxicillin, chloramine-T, copper sulfate, formalin, and oxytetracycline.

Funding to support the generation of human food safety, analytical methods development, and environmental fate data for certain drugs for use on freshwater fish was recently obtained by a partnership with the International Association of Fish and Wildlife Agencies (IAFWA), on behalf of state agencies, NBS, and FWS (**NBS** 1994). Research to gain approvals or extensions of approvals for eight drugs identified as high priority by the states and to establish the crop grouping concept was initiated July 1, 1994 and will extend until June 30, 1999. During that 5-year period, at least 37 states will contribute a total of **$3.6** million, NBS's Fish Farming Experimental Laboratory, Stuttgart, AR will provide some base funding, and the Upper Mississippi Science Center (formerly the National Fisheries Research Center, La Crosse, WI) will provide $4 million toward: (1) development of data under GLP provisions to gain CVM approval of NADA's for use in aquaculture for certain essential drugs; and (2) development of research information to allow acceptance of a crop grouping concept by CVM. Data will be generated to allow CVM to assess whether a few selected fish species can be used as surrogates for all or most of the cultured freshwater fishes in the United States.

Walleye culture should benefit greatly from the IAFWA project. Walleye are included in the development of data toward: (1) an extension of formalin to control fungi on eggs, as well as expansion to control external parasites and fungi on fish themselves; (2) an extension of oxytetracycline in the feed additive formulation to control furunculosis, bacterial hemorrhagic septicemia, and pseudomonas disease, to mark fish, and an expansion to control flexibacteriosis; (3) a NADA for chloramine-T to control flexibacteriosis; (4) classification as LRP drugs or NADAs for copper sulfate and potassium permanganate to control external fungal and bacterial infections and external parasitic infestations; (5) a NADA for benzocaine as an anesthetic and sedative with a low withdrawal time to supplement the use of Finquel; and **(6)** delineation of safe and efficacious concentrations of hydrogen peroxide as a LRP fungicide for walleye in all life stages, includmg eggs, and potential expansion of the LRP drug status to control external parasitic infestations and fungal and bacterial infections. Sarafloxacin is one of the drugs included in the IAFWA project and is a potential candidate to control bacterial diseases in walleye; however, research on sarafloxacin will be delayed until new information becomes available on the fluoroquinolones. The U.S. Food and Drug Administration will determine the extent of their use in animal husbandry "in terms of both the benefits of these drugs to animals and the potential risks to humans with respect to the use of these drugs in animals, includmg further antimicrobial resistance in human pathogens" (Anon. 1994).

Walleye culture will benefit because walleye will be one of the four fish species used to test the crop grouping concept under the IAFWA project. Information will be generated on the pharmacokinetics, uptake, and elimination of drugs in walleye that will lead to their inclusion in NADAs, thus providing more treatment options for walleye culturists, researchers, and fishery managers.

Summary

w a l l e y e culturists have the legal use of a few chemicals and drugs at the present time. However, INAD's in effect on walleye could lead to NADA approvals that will increase options for controlling disease and improving production conditions. A major project through the IAFWA will provide the funding needed to

complete research on hydrogen peroxide and to develop **NADA's** on seven drugs important to the culture of walleye.

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Viruses Associated with Diseases of Walleye, Sauger, and Walleye x Sauger Hybrids

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(Note: All Figures appear on pages 136–138.)

Introduction

Scientific literature concerning viral diseases of young walleye, sauger, and walleye x sauger hybrids is basically nonexistent. In contrast, virus-associated disease in adult walleye and sauger is well documented, probably because the lesions associated with these virus infections are visually prominent (Table 1). Although superficial, the lesions are temporarily disfiguring and can affect the marketability of the fish. These viral infections are not associated with epizootic mortality; but, in some cases, losses can occur from secondary bacterial and fungal infection of abraded, dislodged, or sloughed lesions.

The four viral diseases of walleye are: lymphocystis, dermal sarcoma, diffuse epidermal hyperplasia, and discrete epidermal hyperplasia. Infectious pancreatic necrosis virus has also been isolated from walleye, but no signs of disease were evident. From sauger, only one virus-associated disease is documented, lymphocystis. From walleye x sauger hybrids, no virus-associated disease is reported.

The occurrence of viral disease in young walleye, sauger, and walleye x sauger hybrids and interrelationships between viral disease prevalence between younger and older fish need to be determined. Disease in adult fish could be a result of infection in early life, and conversely infections in adult fish could be transmitted to the young. In addition, anecdotal observations, at least for lymphocystis, suggest that increasing geographical dstribution of viral disease of these fishes could be a consequence of culture and stocking programs.

Lymphocystis

Descriptors	— disease:	lymphocystis
		lymphocystis disease
	— virus:	lymphocystis virus
		lymphocystis iridovirus

Walleye and sauger are among the more than 125 species of fish susceptible to lymphocystis, a viral disease characterized by profound cell enlargement (Amin 1979; Wolf 1988). The disease was first reported in the United States in walleye (Mavor and Feinberg 1918). Lymphocystis is recognized worldwide and occurs in warm, cool, and cold water species from freshwater, estuarine, and marine environments. Lymphocystis is a chronic, but seldom fatal, disease. The nodular lesions eventually rupture or slough and heal.

Clinical signs and histopathology

Fish with lymphocystis develop macroscopic, superficial nodules on fins (Figure 1), skin, and eyes and sometimes on internal organs and tissues (Templeman 1965; Dunbar and Wolf 1966; Dukes and Lawler 1975; Amin 1979; Wolf 1988). Nodules are cream to pink or gray, dependmg on the condtion of the overlying epithelium and on the degree of vascularity. The lesions are composed of one or more cells that increase $\geq 10^5$-fold in volume to become 0.3 to 2.0 mm in diameter (Figure 2).

Lymphocystis virus infection causes a unique cellular hypertrophy (Nigrelli and Ruggieri 1965; Dunbar and Wolf 1966; Lopez et al. 1969; Yamamoto et al. 1985a). The cytoplasm and nucleus become greatly enlarged as infection progresses (Figures 3 and 4); infected cells do not divide. Degenerative changes in the nucleus include condensation and fragmentation of chromatin and

Figure 1. Walleye showing lymphocystis lesions on the caudal fin. From Yamamoto et al. (1976). Reprinted with permission of National Research Council of Canada.
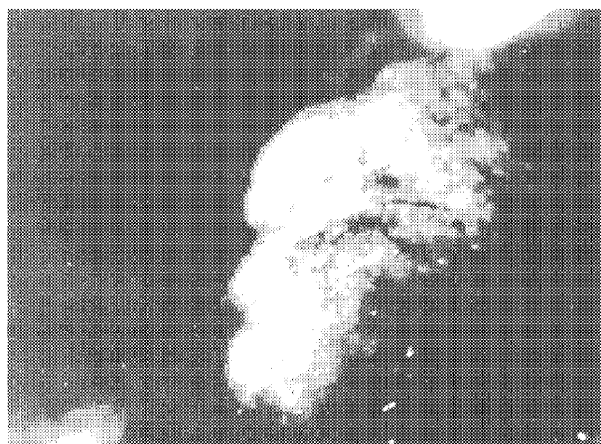
Figure 2. Aggregate of individual lymphocystis cells on ventral fin of a walleye. From Yamamoto et al. (1985a). Reprinted with permission of The Japanese Society of Fish Pathology.

Figure 3. Light micrograph of lymphocystis cell and surrounding tissue showing epthielial cell layer, the grossly hypertrophied lymphocystis cell, and a layer of hyaline substance surrounding the lymphocystis cell. From Yamamoto et al. (1976). Reprinted with permission of National Research Council of Canada.
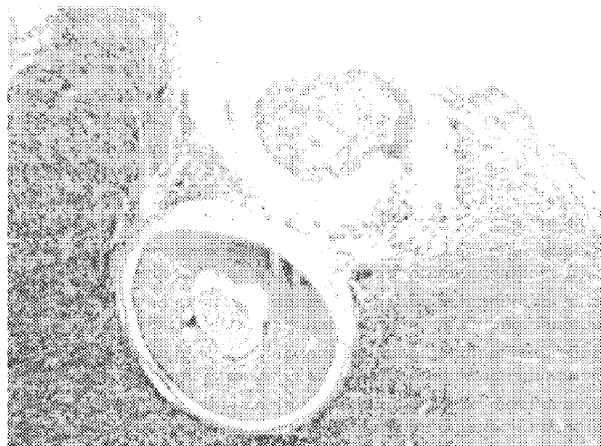
Figure 4. Light micrograph of walleye dermal sarcoma lesion in which two lymphocystis cells are embedded. From Yamamoto et al. (1976). Reprinted with permission of National Research Council of Canada.
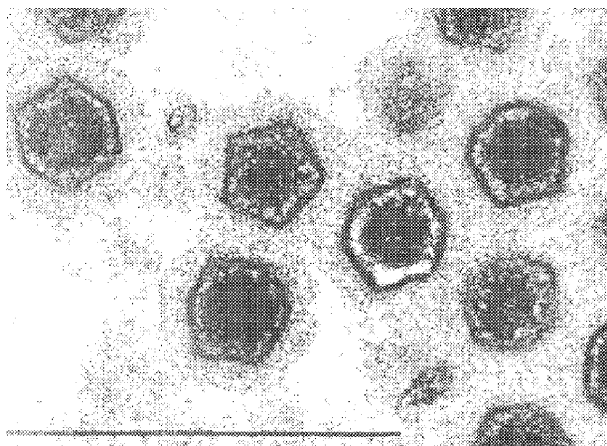
Figure 5. Electron micrograph of a thin section of a lymphocystis cell showing accumulation of lymphocystis virus in the cell cytoplasm. Line = 1 mm. From Yamamoto et al. (1976). Reprinted with permission of National Research Council of Canada.
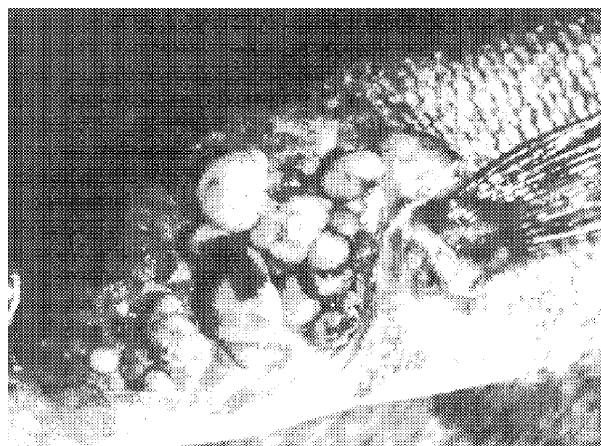
Figure 6. Dermal sarcoma nodules on the operculum of a walleye. From Yamamoto et al. (1985a). Reprinted with permission of The Japanese Society of Fish Pathology.
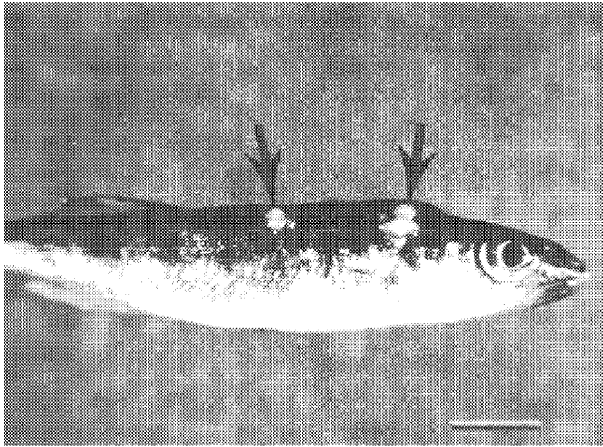
Figure 7. Fingerling walleye experimentally infected by intramuscular injection of cell-free filtrate of walleye dermal sarcoma tissue. Arrows indicate multiple dermal sarcomas on the skin. From Bowser et al. (1990). Reprinted with permission of the American Fisheries Society.
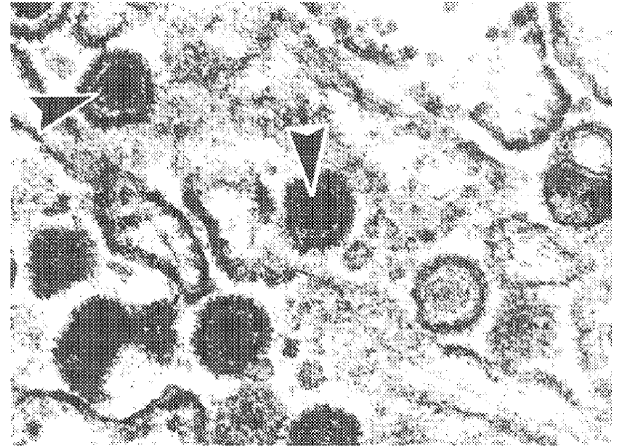
Figure 8. Electon micrograph of a thin section of a walleye dermal sarcoma cell showing enveloped retrovirus particles. Virus particles measure about 135 nm in diameter; arrows indicate the electron-dense, inner core of the virus particle. From Yamamoto et al. (1976). Reprinted with permission of National Research Council of Canada.
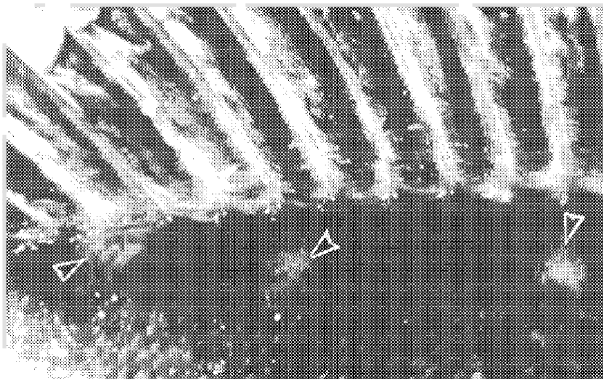
Figure 9. Areas of diffuse epidermal hyperplasia (arrows) on the dorsal skin surface of a walleye. From Yamamoto et al. (1985a). Reprinted with permission of The Japanese Society of Fish Pathology.
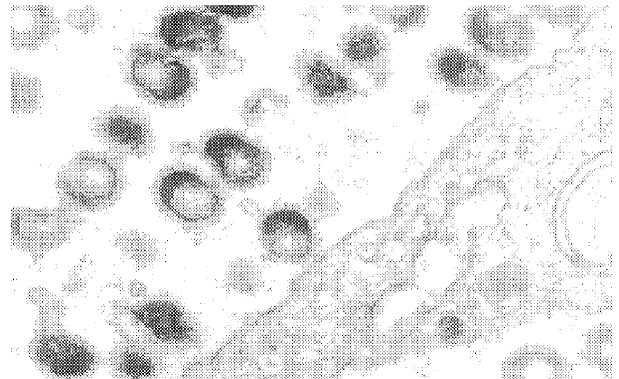
Figure 10. Electron micrograph of a thin section of walleye diffuse epidermal hyperplasia tissue showing enveloped herpesvirus particles in intercellular spaces. From Kelly et al. (1983). Reprinted with permission of Blackwell Scientific Publications.
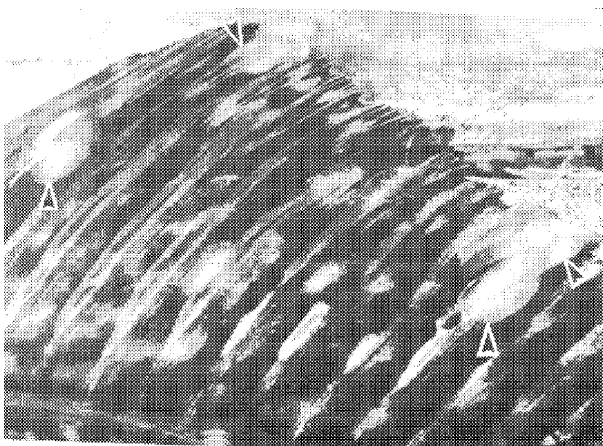
Figure 11. Areas of discrete epidermal hyperplasia (arrows) on the dorsal fin of a walleye. From Yamamoto et al. (1985b). Reprinted with permission of Blackwell Scientific Publications.
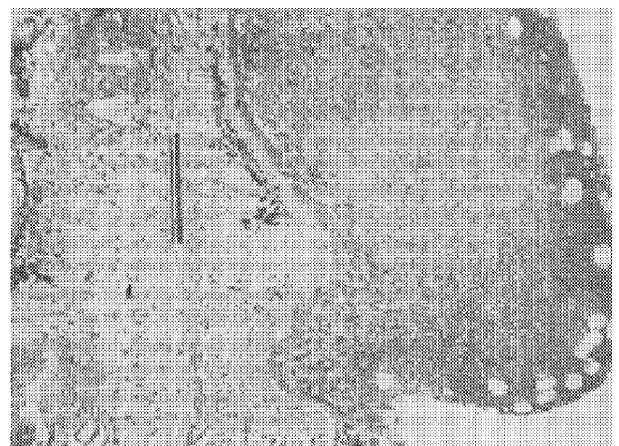
Figure 12. Light micrograph of walleye discrete epidermal hyperplasia tissue (right portion of the picture). Normal epidermis appears in the left portion of the picture (area of double bar). From Walker (1969).
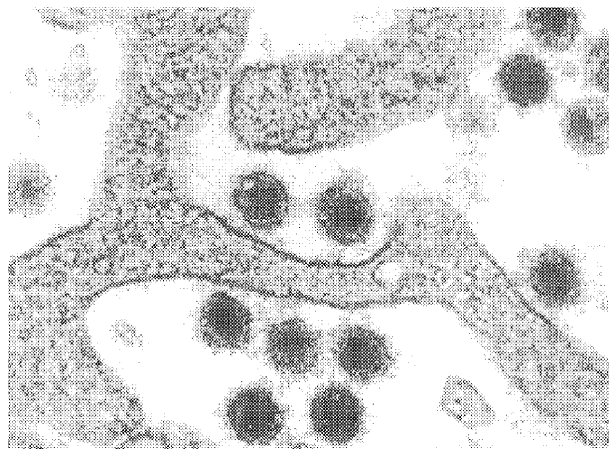
Figure 13. Electron micrograph of walleye discrete epidermal hyperplasia showing enveloped retrovirus particles in intercellular spaces. From Yamamoto et al. (1985b). Reprinted with permission of Blackwell Scientific Publications.

enlargement, distortion, and dissolution of the nucleoli. Within the expanding cytoplasm, Feulgen-positive inclusions develop that appear as web-like filaments or as dense vacuolated bodies. Web-like, filamentous inclusions are common in walleye and sauger (Figure 3) (Walker 1966). Associated with inclusions are lymphocystis virus particles (Figure 5). As the infected cell develops, a thick hyaline capsule forms at the periphery (Pritchard and Malsberger 1968; Howse and Christmas 1970). Proliferating fibroblasts isolate infected cells, and accumulating plasma cells, lymphocytes, macrophages, and polymorphonuclear leukocytes are evidence of host inflammatory response. Lesions eventually heal with little evidence of scar tissue.

Diagnosis and virus detection

Lymphocystis can occur in walleyes that exhibit clinical signs of dermal sarcoma, diffuse epidermal hyperplasia, and discrete epidermal hyperplasia (Figure 4; Table 1). Lymphocystis and walleye dermal sarcoma have similar macroscopic clinical signs (Figures 1 and 6). In addition, lymphocystis can be confused with superficial cysts of certain parasites (Wolf 1988). Definitive diagnosis requires histological examination (Figures 3 and 4).

Lymphocystis virus can be isolated from homogenates of lesion tissue. Cell cultures vary in susceptibility to individual isolates of lymphocystis virus, suggesting that there are intrinsic differences among virus isolates from different hosts (Wharton et al. 1977; Kelly et al. 1980a). Lymphocystis virus replication can occur in freshwater fish cell lines of centrarchid (BF-2, BF-W, and LBF-1), percid (WC-1), and sciaenid (SP-1) origin (Wolf et al. 1966; Midlige and Malsberger 1968; Wharton et al. 1977; Kelly et al. 1980a; Walker and Hill 1980; Berthiaume et al. 1984) and in a variety of cell lines derived from marine fish (Middlebrooks et al. 1979, 1980, 1981). The virus does not replicate in cyprinid (FHM), ictalurid (BB), or salmonid (RTG-2) cells (Wolf, 1988; McAllister, 1993a).

Electron micrographs of walleye lesions show enveloped lymphocystis virus particles that are 300-380 nm

Table 1. Clinical characteristics of virus-associated skin lesions of walleye and sauger.

Skin Lesion	Clinical Characteristics	Virus
Lymphocystis	Greatly enlarged, spherical cell; Lesion usually aggregate of cells; Texture—smooth, undulating; Color—cream to pink or gray.	Iridovirus
Walleye dermal sarcoma	Single or multiple nodules; Shape—dome to spherical; Texture—smooth to rough; Color—white to pink.	Retrovirus
Walleye diffuse epidermal hyperplasia	Laterally-spreading, indistinct; Shape—thin, flat, ill-defined; Texture—viscous slime, mucoid; Color—translucent.	Herpesvirus
Walleye discrete epidermal hyperplasia	Sharply delineated, firm, thick; Shape—dome plaque; Texture—smooth, undulating; Color—translucent, clear to gray.	Retrovirus

in diameter (Figure 5); the icosahedral viral capsid is −260 nm in diameter (Yamamoto et al. 1976, 1985a). Lymphocystis virus, an iridovirus, is remarkably stable. Significant levels of infectivity were recovered from lyophilized lesion homogenate that had been held for 13.5 years at 4°C, and residual infectivity was detected in dried lesion tissue held for 15 years at 4°C (Wolf et al. 1979). Lymphocystis virus is inactivated by exposure to lipid solvents (i.e., ether or chloroform), is progressively inactivated by sonication, but is stable to multiple cycles of freezing and thawing (Wolf 1988).

Transmission and epidemiology

Experimentally, lymphocystis can be transmitted by cohabitation, by exposure to water containing virus, by feeding excised lesion and lesion homogenate, and by applying lesion homogenate to gills and to scarified slun (Wolf 1962, 1988). The disease can also be experimentally transmitted by subcutaneous and intraperitoneal lesion implantation and by subdermal and intramuscular injection of lesion homogenate and medium from virus-infected cell cultures (Wolf 1962; Dunbar and Wolf 1966; Wolf et al. 1966, 1979). Lymphocystis can be experimentally transmitted with relative ease among species of a genus, but with difficulty among families. Such host specificity suggests the existence of strains of lymphocystis virus (Walker 1958; Wolf 1988).

Based on results of experimental challenges, lymphocystis virus is transmitted under natural conditions by exposure to waterborne virus or by ingestion of virus-infected cells (Stephens et al. 1970; Lawler et al. 1974). Mechanical injury to epithelial surfaces provides a portal of entry for waterborne virus. The stability of lymphocystis virus to drying and freezing and thawing suggests that the virus can remain infectious for an extended period (Wolf et al. 1979). Templeman (1965) reports that the numbers of lymphocystis lesions are greater in heavily parasitized fish, and Ryder (1961) suggests that social behavior is a factor in the transmission of lymphocystis. During spawning, behavioral-associated abrasion of the mucous layer protecting the skin, in conjunction with congregation of fish on spawning grounds, provides higher probability of contact with the virus. Amin (1979) reports that the prevalence of lymphocystis is related to the population density, and when a population is composed of a higher

percentage of larger, older fish, the prevalence of lymphocystis is higher.

In naturally infected walleye and sauger populations, lymphocystis occurs with equal frequency in both sexes and is observed throughout the year (Amin 1979; Bowser et al. 1988). Prevalence is higher in walleye than in sauger (Amin 1979). Survey data show that prevalence of lymphocystis in walleye varies with the season and approaches 38% in some populations. Prevalence is highest in early spring (water temperature = 0-5°C), is lowest from late spring to early autumn (water temperature = 10–25°C), and then increases significantly in late autumn (water temperature = 5–**10°C**) (Bowser et al. 1988). Host inflammatory response to lymphocystis lesions can occur throughout the year (Bowser et al. 1988). Experimentally, lymphocystis can occur in many species of any age, but natural infections of walleye and sauger have been reported only in older fish—an observation possibly skewed by sampling methodology (Amin 1979; Yamamoto et al. 1985b; Bowser et al. 1988).

Fishery management activities can affect the prevalence and geographcal distribution of lyrnphocystis. Scale sampling, fin clipping, tagging, and similar sampling and marking practices promote higher prevalence of lymphocystis. Clifford and Applegate (1970) reported lymphocystis occurred at higher prevalence in tagged (35.4%) compared to untagged (2.2%) walleye and found lesions were concentrated at the site of tag attachment. The occurrence of lymphocystis in areas previously free from the disease has been associated with stocking of hatchery-raised walleye, which were predominantly stocked as fingerlings (Amin 1979).

Industrial contaminants do not appear to be a contributing factor in increasing the prevalence or severity of viral skin lesions in walleyes. Smith et al. (1992) found no evidence that pollution affected the prevalence of one form of skin lesion (i.e., lymphocystis, dermal sarcoma, or discrete or diffuse epidermal hyperplasia) over another and that aromatic hydrocarbon contaminant levels were actually lower in walleye with lymphocystis.

Lymphocystis is a chronic, seldom fatal disease. Secondary bacterial and fungal infections can develop at sites of abraded or dislodged lesions, which can affect the health of a fish. Walleye with lymphocystis

weighed 5.5–6.5% less than fish of the same length that were lymphocystis-free (Hile 1954), and Ryder (1961) reported that fish with lymphocystis lesions are more easily captured.

Treatment and control

Lymphocystis occurs widely in nature, and there is no practical treatment for the disease.

Several approaches have been used to attempt prevention or control of lymphocystis in intensive culture systems. Fish should be raised in controlled water supplies (i.e., well water or spring water) because the virus can enter a production facility by way of open, untreated water supplies that contain infected fish. Further, raising fish in colder water can at least slow lesion development (Wolf 1988). Once lymphocystis is detected, removal of lesion-bearing fish is generally ineffective to control the infection.

The use of drugs or chemicals to control lymphocystis has not been extensively studied. Lopez et al. (1970) showed that the antineoplastic drug 6-mercaptopurine inhbited the appearance of virus-induced cytopathic effects in cell culture and controlled lymphocystis in fish.

Currently, no drug, chemical, vaccine, or pesticide is approved for use to treat lymphocystis in walleye, sauger, or walleye x sauger hybrids (Federal Joint Subcommittee on Aquaculture 1994).

Walleye dermal sarcoma

Descriptors	— disease:	walleye dermal sarcoma WDS
	— virus:	walleye dermal sarcoma retrovirus
		walleye dermal sarcoma virus
		WDS virus

Walleye dermal sarcoma is a slun tumor that is commonly seen in walleye from the Great Lakes regions of the United States and from the central and western regions of Canada (Walker 1958, 1961, 1969; Yamamoto et al. 1976; Bowser et al. 1988). Walleye dermal sarcoma is recognized as caused by a virus

because the tumor can be transmitted using cell-free filtrates from tumors and because retrovirus-like particles are associated with tumor tissue. The disease is principally associated with adults, but has been experimentally transmitted to fingerlings.

Clinical signs and histopathology

walleye dermal sarcoma is a surface lesion that consists of single or multiple dermal nodules covered by a thin layer of epidermis (Figures 6 and 7). Nodules occur predominantly on the trunk, but also occur on the head and fins. They are 1–10 mm in diameter, have a dome to spherical shape, and are smooth to rough in texture and firm in composition. Nodules are pink to white appearance, depending on the level of vascularity and the density of fibrous tissue. Abrasion or detachment of a tumor leaves an open, bleeding lesion. The nodules appear similar in gross morphology to lymphocystis (Figures 1 and 6), and lymphocystis cells can be found embedded in the sarcoma. However, the highly proliferative sarcoma cells can be readily distinguished microscopically from the hypertrophied lymphocystis cells (Figure 4).

The tumor is confined to the skin, and invasion of adjacent tissue or metastasis usually does not occur. Invasion of surrounding tissue has been seen in experimentally infected, fingerling walleye (Paul Bowser, personal communication). Dermal sarcoma cells are not enlarged, but they vary in morphology and are extensively conjoined by interdigitating cytoplasmic processes, collagen, and transverse capillaries (Yamamoto et al. 1985a; Martineau et al. 1990b). The cytoplasm varies in abundance and can be highly vesiculated. The size and shape of the nucleus varies, and can appear sharply marginated (Walker 1961, 1969; Yamamoto et al. 1976, 1985a). Tumor composition varies from a highly proliferative, irregularly arranged sarcoma to a densely whorled, well organized fibroma (Martineau et al. 1990b). Bowser et al. (1988) report that an inflammatory response to the tumor is temperature related, with significant response in spring and summer and little to no response in autumn. Martineau et al. (1990b) indicate that the density of lymphocyte infiltration between the tumor and adjacent dermis occurs with no significant seasonal variation. Dense lymphocyte infiltration of the tumor is associated with necrosis.

Diagnosis and virus detection

walleye dermal sarcoma often occurs in fish concurrently showing clinical signs of lymphocystis and diffuse and discrete epidermal hyperplasia. Walleye dermal sarcoma can be readily confused with lymphocystis because both conditions have similar macroscopic clinical signs (Table 1). Conclusive diagnosis requires histopathological examination.

A virus that causes this disease has not been isolated in cell culture. Polymerase chain reaction analysis of infected cell cultures suggests that the virus replicates in cultured cells without dramatic cytopathology (Paul Bowser, personal communication). Electron micrographs of tumor tissue show accumulations of retrovirus-like particles (Walker 1961, 1969; Yamamoto et al. 1976, 1985a; Martineau et al. 1990a). Virus particles are more prevalent in tumors occurring in the spring of the year (Paul Bowser, personal communication). The type C retrovirus-like virions are 90–135 nm in diameter and contain a centrally placed, electron dense nucleoid ≈ 75 nm in diameter (Figure 8). Variations in the overall diameter of the virion are apparently due to different methods of processing and measurement. Virus particles bud from cytoplasmic membranes and accumulate in intercellular spaces and cytoplasmic vesicles. The retrovirus (RNA genome) associated with walleye dermal sarcoma seems to be ultrastructurally different from retrovirus associated with walleye discrete epidermal hyperplasia (Figure 13) (Yamamoto et al. 1985b), and it is decidedly different from the iridovirus (DNA genome) that causes lymphocystis (Figure 5) (Yamamoto et al. 1976) and the herpesvirus (DNA genome) associated with walleye diffuse epidermal hyperplasia (Figure 10) (Kelly et al. 1983). No evidence of virus was seen in a cell line (WC-1) derived from walleye dermal sarcoma tissue (Kelly et al. 1980a).

The retrovirus etiology of walleye dermal sarcoma is supported by several observations. Retrovirus-like virions were purified from tumor homogenate, and reverse transcriptase activity was associated with the purified product (Martineau et al. 1991). Molecular cloning of purified virus showed the genome to be uniquely larger (13.2 kb) than other retroviruses (Martineau et al. 1992). A DNA species found only in tumor tissue hybridized with complementary DNA synthesized from retrovirus RNA purified from tumor homogenate (Martineau et al. 1991). Walleyes injected with cell-free tumor filtrates developed the tumor; whereas, those injected with ether-treated, cell-free tumor filtrate did not, which suggested that ether treatment disrupted the putative retrovirus lipid envelope inactivating viral infectivity (Bowser and Wooster 1994).

Transmission and epidemiology

walleye dermal sarcoma was experimentally transmitted to 3-month-old fingerlings by intramuscular injection of cell-free tumor filtrate (Figure 7) (Bowser et al. 1990; Martineau et al. 1990a; Bowser and Wooster 1994). Tumors were macroscopically evident 14 weeks after injection in fish held at 10°C and 8 weeks in fish held at 15°C, and were microscopically evident 4 weeks after injection in fish held at 20°C. Tumors developed indiscriminately on the trunk (150% of body surface), but also occurred on the head and fins. The tumors were most profound at or near the injection site. Water temperature affected the size and frequency of experimentally induced tumors. Tumors were larger and more numerous in fish held at 15°C and 20°C and were significantly smaller and fewer in number in fish held at 10°C (Bowser et al. 1990). Unfortunately, no study was performed to determine if tumor prevalence and development was affected by water temperatures >20°C.

Seasonal prevalence of the tumor is temperature related. Prevalence is high in early spring (water temperatures = 0–5°C), decreases in late spring (water temperature = 15–20°C), and is lowest in the summer (water temperatures = 20–25°C). The tumor regresses during the summer when water temperatures are >20°C (Bowser and Wooster 1991), but as water temperatures decline to ≤15°C in early autumn, tumor prevalence approaches the level seen in early spring. The temperature dependency of tumor prevalence may be related to host immune response (Bowser et al. 1988).

Walleye dermal sarcoma is routinely observed in adult fish and the prevalence can approach 29% of the population (Yamamoto et al. 1976; Bowser et al. 1988; Smith et al. 1992). The tumor often occurs in walleye that also show clinical signs of lymphocystis and diffuse and discrete epidermal hyperplasia (Yamamoto et al. 1985a, 1985b; Bowser et al. 1988; Smith et al. 1992). Dermal sarcoma is not routinely seen in young walleye, although Yamamoto et al. (1976) reported a

high prevalence of dermal sarcoma and lymphocystis in pre-spawning walleye (250-600 g).

Purported sex-related and age-related differences may reflect the seasonality of tumor prevalence, sampling methods, and sport fishmg pressure. Yamamoto et al. (1976) observed **a** greater prevalence of lymphocystis and walleye dermal sarcoma in females than in males and a greater prevalence in younger fish than in older fish. Bowser et al. (1988) reported the opposite results, i.e., prevalence was greater in males than females and greater in older than younger fish. Experimental transmission to fingerling walleye indicated that young fish were susceptible to infection and suggested that sexual maturity is not a prerequisite for development of the tumor. Extrapolation of results from experimental transmission suggests that young fish would be susceptible to infection under natural condtions, but no supporting data have been reported.

The mechanism for transmission in nature is unknown. Walker (1969) suggested that the dermal tumors were individually infected sites, with infection initiated as the result of abrasion. Martineau et al. (1990b) questioned the abrasion hypothesis and noted that the epithelium covering dermal sarcoma lesions was intact and showed no evidence of trauma. Furthermore, the results of experimental infections in which tumor development was remote from the site of injection suggested the virus was distributed systemically (Martineau et al. 1990a).

A growing body of evidence suggests that a variety of neoplasms are associated with aromatic hydrocarbon contaminants in water and sediments (Baumann et al. 1991). While industrial contaminants could play a role in increasing the prevalence or severity of viral slun lesions in walleye, a comparative assessment of aromatic hydrocarbon contaminant levels showed that contaminant levels were actually lower in walleye with the dermal sarcoma (Smith et al. 1992).

Thus, while the mechanism for transmission in nature remains undefined, susceptibility to and transmission of walleye dermal sarcoma could be affected by the interactions of a variety of factors, such as fish density and behavior, environmental and physiological stress factors, immune competence, and abundance and stability of the virus.

Treatment and control

No methods of treatment or control have been described for walleye dermal sarcoma. Holding fish at elevated water temperature (>20°C) seemingly pro-motes tumor regression (Bowser and Wooster 1991). Captive fish with abraded lesions or open wounds from sloughed or dislodged lesions are susceptible to secondary bacterial and fungal infections.

Currently, no drug, chemical, vaccine, or pesticide is approved for use to treat walleye dermal sarcoma (Federal Joint Subcommittee on Aquaculture 1994).

Walleye diffuse epidermal hyperplasia

Descriptors — disease: walleye diffuse epidermal hyperplasia
— virus: *Herpesvirus vitreum* percid herpesvirus 1 walleye herpesvirus

A skin lesion described as walleye diffuse epidermal hyperplasia is seen in walleye populations from Oneida Lake, New York and from Saskatchewan, Canada (Kelly et al. 1983; Yamamoto et al. 1985a, 1985b; Bowser et al. 1988). The condition probably occurs in walleye populations throughout North America (Yamamoto et al. 1985b; Smith et al. 1992). **A** herpesvirus, designated Herpesvirus vitreum, has been observed in and isolated from walleye dffuse epidermal hyperplasia tissue (Kelly et al. 1980b, 1983). The skin lesion does not cause mortality.

Clinical signs and histopathology

walleye diffuse epidermal hyperplasia is a flat, laterally spreading, indistinct cell proliferation (Yamamoto et al. 1985a). The lesion develops to several centimeters in diameter and resembles translucent, thickened slime (Figure 9). The normal epidermal structure is disorganized, and underlying tissue often appears mildly edematous. Cell nuclei are slightly enlarged, contain granular inclusions, and occasionally form syncytia. Gross examination indicates no hyperplastic involvement beyond the slun, suggesting no invasion of adjacent tissue or metastasis.

Diagnosis and virus detection

T h e walleye diffuse epidermal hyperplasia can occur in fish that also show clinical signs of lymphocystis, walleye dermal sarcoma, and walleye discrete epidermal hyperplasia, but the lesions can be distinguished by differential clinical signs (Table 1). The lesions of walleye dermal sarcoma and lymphocystis are nodular in appearance and clearly Qstinctive from the walleye epidermal hyperplasias (Figures 1, 2, 6, 9, and 11). The lesions of walleye diffuse epidermal hyperplasia are thin, flat, and ill-defined, and those of walleye discrete epidermal hyperplasia are dome-shaped, sharply delineated, and raised. Histological examination should be used to verify presumptive visual diagnosis (Figures 3, 4, and 12).

H. vitreum can be isolated from homogenates of diffuse epidermal hyperplasia tissue by using cell lines derived from walleye ovary (WO) and dermal sarcoma tumor (WC-1) and by using a cell line (We-2) derived from unspecified walleye tissue (Kelly et al. 1980a, 1980b, 1983). The virus replicates with distinctive syncytium-type cytopathology at 4°C and 15°C, but not at 20°C. The virus does not replicate in cell lines of cyprinid (FHM), ictalurid (BB), or salmonid (CHSE-214 and RTG-2) origin. At 15°C, syncytia began forming about 2 weeks after infection and subsequently lysed (Kelly et al. 1983). Replication of H. vitreum in cell culture was inhibited by DNA analogues (Kelly et al. 1983).

Electron micrographs of hyperplastic tissue and infected cell cultures show intracellular and extracellular accumulations of virus particles with the morphology and pattern of replication of a herpesvirus (Figure 10). Inclusions of viral precursor material are present in the cell nucleus and virions are seen budding from nuclear membranes. Mature, enveloped virions are 190–230 nm in diameter and contain a nucleocapsid about 100 nm in diameter (Kelly et al. 1983j.

Transmission and epidemiology

Although a herpesvirus can be isolated from walleye diffuse epidermal hyperplasia tissue, a causal relationship between virus infection and the disease has not been proven by experimental transmission.

Of the four virus-associated skin lesions of walleye, walleye Qffuse epidermal hyperplasia occurs at the lowest reported prevalence. Lesions are observed in early spring at a prevalence ≤4%, but are rarely seen from late spring through autumn (Yamamoto et al. 1985a, 1985b; Bowser et al. 1988). The seasonal appearance of the skin lesion seems to be a function of water temperature. Bowser et al. (1988) found that lesions were prevalent only when the water temperature was ≤5°C. The Qsease is observed annually in some populations, indicating the virus persists. Yamamoto et al. (1985a) suggested that winter temperature stress and physiological changes associated with spawning may activate latent infections. Smith et al. (1992) indicated that industrial pollution was not a factor in the prevalence of walleye epidermal lesions.

Walleye diffuse epidermal hyperplasia lesions are observed on older, sexually mature fish (Yamamoto et al. 1985b; Bowser et al. 1988). However, the purported age-related prevalence may be a function of sampling methodology.

Treatment and control

T h e diffuse epidermal hyperplasia occurs in feral, sexually-mature walleyes, and no method for treatment or control has been reported. The decreased prevalence of lesions at warmer water temperatures suggests that the disease be controlled in captive populations by raising fish at temperatures >5°C.

Currently, no drug, chemical, vaccine, or pesticide is approved for use to treat walleye diffuse epidermal hyperplasia (Federal Joint Subcommittee on Aquaculture 1994).

Walleye discrete epidermal hyperplasia

| Descriptors | — disease: | walleye discrete epidermal hyperplasia |
| | — virus: | walleye discrete epidermal hyperplasia retrovirus walleye epidermal hyperplasia retrovirus |

A skin lesion described as walleye discrete epidermal hyperplasia occurs with some frequency in walleye from Alberta, Manitoba, and Saskatchewan, Canada and from Oneida Lake, New York (Walker 1969; Yamamoto et al. 1985a, 1985b; Bowser et al. 1988). The condition probably occurs in walleye populations throughout

North America (Yamamoto et al. 1985b; Smith et al. 1992). Walleye discrete epidermal hyperplasia is thought to be caused by a virus, because retrovirus-like particles are seen in electron micrographs of lesion tissue.

Clinical signs and histopathology

w a l l e y e discrete epidermal hyperplasia is a firm, smooth lesion that appears as a translucent, clear to gray, sharply delineated, dome-shaped plaque (Figure 11). The lesions are 0.25–1.5 mm thick and several centimeters in diameter—lesions 7 cm in diameter have been reported. Multiple proliferations often coalesce. Lesions are common on the fins, but can occur on any body surface.

The uniformly homogenous, undifferentiated, hyperplastic cells are confined to the epidermis (Figure 12). Although roughly cuboidal in shape, affected cells have long cytoplasmic extensions that leave large spaces between individual cells. The lesion is not vascularized. Walleye discrete epidermal hyperplasia does not invade adjacent tissue or metastasize, nor is there evidence of necrosis of the dermal, subcutaneous, or muscle layers. Mucus cells are present at the periphery of the lesion, but are infrequent in the body of the hyperplasia.

Diagnosis and virus detection

w a l l e y e discrete epidermal hyperplasia can occur in fish that also show clinical signs of lymphocystis, walleye dermal sarcoma, and walleye diffuse epidermal hyperplasia. Diagnosis is based on clinical signs coupled with histopathological examination (Table 1). The gross appearance of discrete epidermal hyperplasia is distinct from the nodular lesions of lymphocystis (Figures 1 and 2) and walleye dermal sarcoma (Figures 6 and 7). Walleye discrete epidermal hyperplasia lesions are sharply delineated and raised (Figure 11) compared to the thinner, ill-defined lesions of walleye diffuse epidermal hyperplasia (Figure 9). Histological examination should be used verify presumptive visual diagnosis (Figures 3, 4, and 12).

Attempts to isolate virus in cell culture have not been successful (Yamamoto et al. 1985b). However, electron micrographs of walleye discrete epidermal hyperplasia show type C retrovirus-like particles 80-120 nm in diameter (Figure 13). The virions are enclosed in a peplomer laden envelope and contain an electron dense, centrally placed core (Walker 1969; Yamamoto et al. 1985a, 1985b). Virions bud from cell surface membranes, but have not been seen free in the cytoplasm or within vesicles. No virus-like particles have been observed in the dermis adjacent to the lesion or in normal epidermis.

Transmission and epidemiology

Experimental transmission of walleye discrete epidermal hyperplasia has not been reported, and little is known about the pathobiology of the disease.

The prevalence of walleye discrete epidermal hyperplasia varies; in some populations 220% of the fish are affected (Walker 1969; Yamamoto et al. 1985a, 1985b; Bowser et al. 1988). As with other walleye slun lesions, prevalence varies seasonally. The highest prevalence of walleye discrete epidermal hyperplasia occurs at colder water temperatures. Based on limited data, lesions were most prevalent in early spring (water temperature = 0-5°C), were not found in late spring, summer, or early autumn (water temperature = 15–25°C), but can occur at low prevalence in late autumn (water temperature = 5–10°C) (Yamamoto et al. 1985b; Bowser et al. 1988). Lesions are found only in older fish (Bowser et al. 1988). No lesions have been observed in fingerlings and juveniles. There are no reports documenting lesion regression or recovery, nor are there reports of secondary bacterial or fungal infections associated with abraded, dislodged, sloughed lesions.

Treatment and control

No methods of treatment or control have been described. The seasonal prevalence of the lesions suggests that culturing fish at water temperatures ≥15°C would inhibit development of walleye discrete epidermal hyperplasia.

Currently, no drug, chemical, vaccine, or pesticide is approved for use to treat walleye discrete epidermal hyperplasia (Federal Joint Subcommittee on Aquaculture 1994).

Infectious pancreatic necrosis virus

Descriptors — disease: no pathology reported in walleye

— virus: infectious pancreatic necrosis virus

aquatic birnavirus

Infectious pancreatic necrosis virus (IPNV) was isolated from adult walleye during a routine virological examination. The virus is one of the more ubiquitous of the fish viruses, and is known primarily for causing high levels of mortality in young-of-the-year salmonid fishes. IPNV is isolated with increasing frequency from non-salmonid fishes, mollusks, and crustaceans (Hill 1982; Wolf 1988; McAllister 1993b). Some of the IPNV's are initially isolated from diseased fish while others are recovered from apparently healthy wild and cultured species (McAllister and Owens 1995).

No signs of disease accompanied isolation of the virus from walleye. The diseased or disease-free status of the host of origin does not directly correlate with virulence of the virus isolate in other species. An IPNV isolate avirulent in one host may be highly virulent in another (McAllister and McAllister 1988). The virulence of IPNV isolates ranges from highly virulent to avirulent regardless of whether the IPNV's are from salmonid, nonsalmonid, or molluscan sources (McAllister and Owens 1995). The virus can be transmitted by waterborne exposure or by consuming virus-carrier fish.

In general, IPNV can be isolated from homogenates of internal organs and from surface mucus, feces, and sex products, using a variety of salmonid and non-salmonid cell lines (McAllister 1993b). IPNV is identified by serological reactivity with polyclonal or monoclonal antisera.

The IPNV-carrier status and variables of host specificity and virulence should be determined prior to the transfer of fish and invertebrate species from their established domains. Such transfers have the potential of exposing resident species to non-indigenous IPNV's, or conversely, the introduced species can be susceptible to strains of IPNV indigenous to the new habitat.

Currently, no drug, chemical, vaccine, or pesticide is approved for use to treat infectious pancreatic necrosis in any species (Federal Joint Subcommittee on Aquaculture 1994).

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Observed Diseases of Walleye in Minnesota

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Introduction

Minnesota has long been known as one of the largest producers of pond-reared (extensive culture) walleye in North America. In 1993, 510 million walleye eggs were taken to produce 355 million fry. A total of 2.4 million fingerlings were raised by extensive culture. Disease occurrence in the culture of fish, whether intensive or extensive, seems inevitable, and walleye are no exception. Remarkably, over the last five years the pathology laboratory received only 43 diagnostic cases for walleye, and fish health inspections were conducted on only nine lots of fish from private fish hatcheries. The present report describes the kinds of infectious diseases observed in these samples. The responsible pathogens can be grouped into viruses, bacteria, parasites, and mysterious maladies.

Bacterial diseases

Only two types of bacteria were observed in the nine lots of fish from private hatcheries.

Cytophaga (flexibacter) columnaris–"Columnaris" disease

Columnaris disease is caused by the bacterium *Cytophaga columnaris,* is responsible for the majority of infectious disease cases associated with walleye culture. This disease can be triggered by high density, high temperature, and mechanical damage from handling. If not treated promptly, mortality may reach 60–90%.

Clinical signs may or may not be evident depending on the virulence of the pathogen. Externally, pathological changes are seen on the body surface, gills or both. On scaled fish, lesions occur initially as grayish white cutaneous foci on the fins, head, and body. The lesion may grow and the slun in the affected area may become eroded, resulting in small ulcerations. On the gills, the gill tissue becomes pale and necrotic, but fusion of the lamellae docs not occur (Thoeson et. al. 1994). There have been instances where saddleback or a discoloration encircling the dorsal fin has also been evident.

Yersinia ruckeri–"Enteric Redmouth Disease"

Bacterial isolates from a routine inspection of walleye from a private grower in northwestern Minnesota revealed pure colonies of the bacterium *Yersinia ruckeri*, the pathogen responsible for enteric redmouth disease, a systemic bacterial disease primarily known for its occurrence in rainbow trout. This bacterium is a certifiable disease of salmon and trout but its effects arc relatively unknown to walleye. The bacterium may occur naturally in some areas and the disease can develop when susceptible fish are subjected to stress. There are no reports of this pathogen in natural outbreaks among free-ranging fish (Nelson 1989).

Parasitic diseases

Although a great variety of protozoan diseases occur, the most common are "Ich" and trichodinas.

Ichthyopthirius multifiliis–"Ich"

Ich is a large ciliated protozoan parasite responsible for major losses in the cultured catfish industry. It is a common parasite of warmwater fishes, but can also be a serious parasite of cool and coldwater fish. Ich can be identified microscopically by its horseshoe shaped nucleus and by the "white spot" appearance macroscopically. This parasite has no host specificity; both fish and amphibians can be affected. Ich is an obligate fish parasite that requires a host to survive; however, amphibians may be reservoirs of infection.

The parasite lives in, or under the epithelial cells of the host. The trophozite is visible on the body surface as white specks up to 1 mm in size. The trophozoite feeds on cells by digesting away the cell membrane. The adult stage, cannot reinfect a fish, but it secretes a

mucoid adhesive over its exterior surface that will adhere to anything. The nuclear material then forms a dense mass in its center and undergoes multiple fission. Up to 1,000 tiny new bodies are formed (tomites), each with enough genetic material to produce a clone. The tomites seek fish and penetrate the epithelium. Tomites must find a fish within about 24 hrs or they die. Tomites have a penetration gland at the anterior end that secretes a material which dissolves membranes and allows the tomite to enter the cell. Once inside the cell a cytosome ("cell mouth") appears as the parasite consumes the cell contents, and the parasite develops its distinctive horseshoe nucleus.

The life cycle of Ich is temperature dependent. At optimal temperatures of 69–73°F (20.5–22.7°C), the life cycle may take as few as 3–4 d. The life cycle requires 2 weeks at 59°F (15°C) and more than five weeks at 50°F (10°C). The disease is difficult to control because not all adults emerge at the same time. There are no treatments known that will kill trophozoites imbedded in the fish. Multiple treatments are required to gain control.

In Minnesota, epizootics of ich seem to be common in recirculating systems. These problems have been persistent and chronic. Water temperatures in intensive systems are usually near optimum for parasite reproduction and outbreaks have been devastating to walleye culture and production. Ich has been the most pervasive disease problem we have encountered in the intensive culture of walleye. Clinical signs of the disease include fish rubbing on surfaces, twitching, gathering at the water inlet, tight schooling, seeking cover, failure to feed, white pustules, frayed fins, petechiae on body surface, pale gills, low hematocrit, excess mucus, and mortality. The cause of death is usually attributed to osmoregulatory failure, complicated by tissue destruction, respiratory damage, and severe anemia.

Trichodina sp.
Trichodinids are saucer shaped protozoans with rows of cilia around the margin of the body. These protozoans live on the skin, fin, and gill tissues of fish and, when abundant, cause considerable irritation to the fish. It seems that the large adult stage is less pathogenic than the smaller juveniles. Reproduction is by simple fission. Because they are less likely to be imbedded in the flesh of their host, than ich, so a single treatment usually relieves the infection.

Clinical signs of the disease are failure to feed, tail chasing in small fingerlings, emaciation, pinheads, erosion of fins, excess mucus production, and mortality. Infections of *Trichodina spp.* may lead to secondary bacterial infections by opportunistic pathogens.

Viral diseases
A variety of skin lesions of viral origin have been found in fishes from Minnesota (Economon 1957). Some of the more conspicuous are tumorous growths, to which walleye seem to have a high susceptibility. Walleyes can be affected by various slun tumors, including lyrnphocystis, dermal fibroma, and epidermal hyperplasia. A different virus is associated with each of these surface lesions. These lesions are benign and are confined to the external surfaces of the body. They are cyclical in nature, with the relative frequency appearing to fluctuate in successive years. They are also seasonal with the incidence most obvious during the coldwater months (Wyatt 1981). A thorough description of these viral diseases of walleye are given by McAllister (1996).

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