THE ICHTHYOGRAM

NEWSLETTER OF THE FISHERIES EXPERIMENT STATION
UTAH DIVISION OF WILDLIFE RESOURCES
VOLUME 2, NO. 1 WINTER 1991

FORT MORGAN LAB REOPENS - INSPECTIONS RESUME

After remaining closed for over a month due to budget games played by Congress and the White House, the USFWS laboratory at Ft. Morgan Colorado was reopened in November, just in time for the Fisheries Experiment Station personnel to schedule and complete inspections on all private aquaculture facilities which had made a request. The delay caused the postponement of several inspections for more than 30 days, but luckily the weather and spawning fish cooperated to permit complete inspections to be performed.

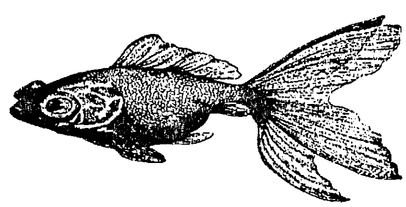
In the meantime, USFWS officials, acknowledging the valuable service offered by the laboratories, have promised budget increases of $390,000 and 3 additional employees. This would allow the overburdened lab to increase staff and meet the anticipated needs of the expanding aquaculture industry in this fiscal year. It remains to be seen however, if promises of increased financial support do indeed materialize. For the present, individual states are still being asked to purchase, prepare and mail all laboratory supplies required for fish inspection.

CRFWCFDS MEETS IN RENO

The annual meeting of the Colorado River Fish and Wildlife Council Fish Disease Subcommittee was held in November in Reno. A variety of items were discussed, including the current role and need for the organization. Other topics discussed were the problems and questions raised by bacterial kidney disease, problems associated with two annual inspections required by California, the need for standardization and national policy in regard to fish health and the number of wild fish to be required for fish health inspection.

In the end, it was decided that the Colorado River policy still serves a definite need, but that revisions reflecting the changing status of fish health within the drainage is necessary. It was also decided to write a letter to California expressing the concerns of neighboring states regarding the twice/year inspection requirement. Over vigorous objections, it was voted to recommend changing the minimum requirements for wild populations from 2% confidence level down to 5% (from 150 down to 60 fish). This is not expected to change Utah policy.

In this issue:



RESULTS OF FISH HEALTH SURVEYS

The beginning of the new year saw an end to several fish health surveys which have been conducted as an unofficial part of the normal annual inspections at private and state culture facilities in Utah for several years. These surveys were designed not to disadvantage any facility, but were performed to increase our awareness of the presence of several fish pathogens of varying degrees of importance. The results were confidential and not part of any official inspection, but the individual hatchery owners or managers were notified of the results for their facilities. The summary of results of individual surveys will be included in this and upcoming issues of the *Ichthyogram.*

Ichthyophonus hoferi

Ichthyophonus hoferi is the name of a systemic fungal pathogen of fish. This pathogen has been described in a number of fish species, both freshwater and marine. The life cycle of the organism appears to be rather complex, varying according to differing environmental conditions. Many organs have been demonstrated to harbor the parasite including the heart, liver, kidney, spleen, muscle, gills, spinal column and brain. It does seem clear that the organism can be spread, especially in culture facilities, through the consumption of the flesh and organs of dead fish. Consequently, large piscivorous fish such as brood fish, lake trout or Bear Lake cutthroat were more at risk. The overall incidence of this disease has gone down in recent years as a result of discontinuing the practice of feeding mortalities to other fish. There is no known effective treatment for this disease. Recently, California has initiated inspection of fish for *Ichthyophonus* at the border in order for commercial aquaculture producers to import live fish into the state.

Clinical signs of the disease vary depending on extent of the infection and organs involved. The primary pathologic sign in advanced cases is the appearance of grayish-white granulomatous nodules on the skin and also on the internal organs of the fish. Spinal deformities and proliferative gill changes have also been associated with this pathogen. Diagnosis is made on the basis of doubly refractive spherical spores, often detected from the kidney or directly from lesions.

During the four year life of this study, 33 locations were surveyed. This resulted in 3436 individual samples being taken, which represented an expenditure in excess of 1000 man hours. Of the 33 locations surveyed, 6 locations (18.2%) were positive for *Icthyophonus hoferi*. This translates to 13 positive lots of fish out of a total of 92 lots, or 14.1%. A positive sample was one in which 1 or more of the resting stages of *Ichthyophonus hoferi* were found. Positive samples were confirmed by histology and PAS staining when sufficient tissue was available.

Four distinct findings were noted when the results were finalized and the positives were separated out.

1. The first finding was that all the positive samples originated from private aquaculture facilities. Since the primary route of transmission is by ingestion of infected dead fish tissue, this could indicate improper management techniques. Private growers in Utah utilize earthen ponds and raceways to some extent and this could make the detection of mortalities as well as the disinfection of facilities much more difficult.

2. The next observation was that of the 6 species of fish sampled, all positive samples were rainbow trout. This reflects the fact that private growers in this state at the time of the survey were utilizing rainbow trout exclusively in their programs. Other species were examined at state facilities.

3. The third trend observed was that the number of positive lots decreased each year from 1987 through 1990 and the percentage of positive samples

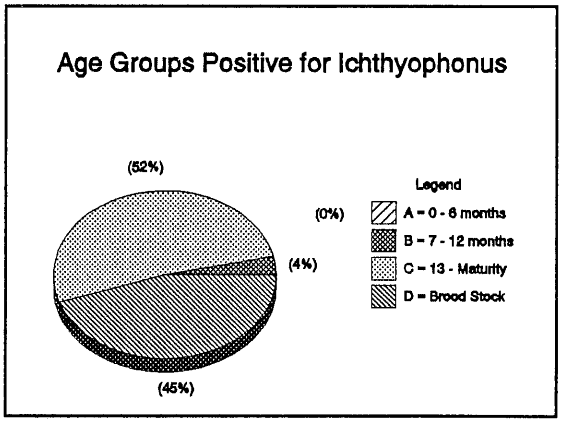


Figure 3

decreased each year from 1986 through 1990. No positive samples were detected in 1990. Improved management practices and increased awareness of the relationship between sanitation and fish health

(Survey on *Ichthyophonus*, cont'd)
on the part of fish culturists may account for this trend.

4. The last finding was that nearly all the positive lots were fish over 12 months of age (See Figure 3). This appears to support findings of other researchers that found older fish to be infected more often than younger fish. One possible explanation is that these fish may become more carnivorous as they mature and more prone to feed on mortalities or discarded fish wastes.

Since the number of positive lots has dropped to such a low level, it has been decided that further investigation in the form of a survey are unwarranted at this time. By continuing to promote the practice of prompt removal of mortalities and good sanitation this disease should remain at a low level among growers.

Correction

In the article on *Bacterial Gill Disease* which appeared in the Fall 1990 edition of the Icthyogram, one dosage for treatment was given incorrectly. Hyamine 3500 is available as a 50% solution instead of 10% as reported. Therefore, the treatment dose should be 4 mg per liter of water and not 20 mg. This equates to 2 mg per liter of the active ingredient.
Russell Lee

WATER QUALITY - AMMONIA

There are many factors associated with water which can affect the health, growth, and reproduction of fish. Some of the more important factors are temperature, oxygen, pH, and ammonia. Many of these not only affect the fish directly, but also interact with others to affect their final concentrations. Ammonia levels of the water are influenced by several other factors, such as temperature, pH, salinity, and feeding rates.

Fish have the ability to efficiently excrete their nitrogenous waste products in the form of ammonia. This occurs primarily across the gills with a small amount also being excreted in the feces. Ammonia is excreted primarily as the un-ionized form (NH_3). The breakdown of uneaten feed also adds to the total ammonia in the water.

In the aquatic environment ammonia exists in

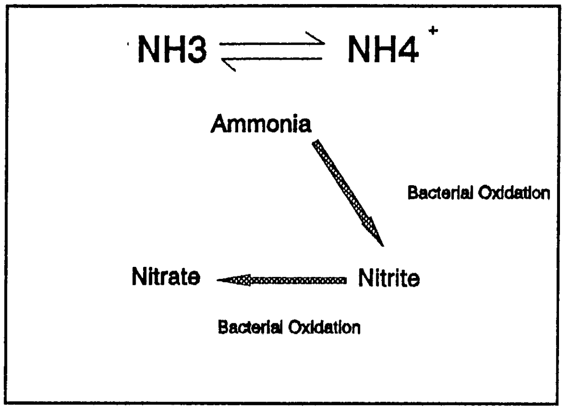


Figure 4

equilibrium between an ionized form (NH_4^+) and an un-ionized form (NH_3). While NH_4^+ is non-toxic, the NH_3 form is highly toxic to fish and is the form of major concern to culturists and fish health professionals. Rising temperature and pH causes the ammonia equilibrium to shift and results in more of the toxic (un-ionized) form being present. Levels of NH_3 below 0.02 mg/liter are recommended for optimum growth. Bacterial oxidation of ammonia results in the formation of nitrite (NO_2) which is also highly toxic to fish. Nitrite interferes with the oxygen carrying capacity of the blood and leads to a condition known as "brown blood disease". Further bacterial breakdown converts the nitrite to nitrate (NO_3) which is relatively non-toxic (See figure 4).

The primary effect of ammonia on the fish is gill irritation. This results in a thickening of the gill epithelium with a decrease in the ability to take up oxygen and excrete wastes. Chronic low level exposure to ammonia results in severe gill damage and according to several researchers, leads to poor growth rates, and an increased susceptibility to disease. With acute high level exposure, excretion of ammonia is reduced and the blood level increases. This results in kidney damage, central nervous system damage, electrolyte depletion and a reduction in energy metabolism with little obvious gill pathology.

Water ammonia levels can fluctuate dramatically during a 24 hour period depending on the feeding schedule and management functions being performed. Most excretion of ammonia takes place 4 - 6 hours after feeding and can raise the water ammonia level quite significantly. A multiple feeding schedule reduces these wide shifts and may also reduce feed waste. Sorting, grading and cleaning procedures also cause increased ammonia excretion due to the stress involved. Cleaning raceways and screens releases sediment which moves downstream and may cause mechanical irritation by accumulating on the gills and gill rakers (sestenosis).

Ammonia is generally not a problem with flow-through (single pass) raceway systems. However, if water is reused in any way, such as with raceways in series, then the ammonia levels can increase as the water travels down the line. The total ammonia content, un-ionized ammonia level, temperature, and pH should be monitored on a routine basis to prevent any adverse effects. Inexpensive and convenient water testing kits such as the Hach® kits are readily available. A multiple feeding schedule, demand feeders, increased aeration or increased water turnover rate may also reduce ammonia buildup. An effective biofilter system is necessary if water is recirculated.

Russell Lee

The Icthyogram
Newsletter of the Fisheries Experiment Station

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The Icthyogram is the newsletter of the Fisheries Experiment Station, Utah Division of Wildlife Resources. Comments and suggestions are welcome. Please address to: The Ichthyogram, 1465 W. 200. N, Logan UT 84321.

UPCOMING DATES OF INTEREST

February 4 - 15: Cold Water Fish Culture Course, Fisheries Experiment Station, Logan.

February 27 - March 1: Western Regional Aquaculture Expo, Reno, NV.

February 27 - March 1: Bonneville Chapter, American Fisheries Society, Wendover, NV.

STATUS OF THE HEALTH/CONDITION PROFILE

The Health/Condition Profile (HCP) has now been taught to over 700 people representing a variety of agencies and disciplines from 32 different states and the federal government. It has been received with considerable enthusiasm and is being used in varying degrees in many areas. It has been used in Utah for 20 years. The concept has developed considerably in that time. It has been taught around a 2 day workshop using a procedures manual, color atlas, a variety of hand-out materials, 35 mm slides, hands-on exercises, and a computer program named "*AUSUM*". It has now been published as part of the American Fisheries Society symposium No. 8 edited by Marshall Adams and entitled "Biological Indicators of Stress in Fish". The specific paper within that symposium is coauthored by Ron Goede and Bruce Barton and is entitled "Organismic Indices and an Autopsy-Based Assessment as Indicators of Health and Condition of Fish". It includes a review of organismic indices used in fisheries and introduces the Health/Condition Profile. It should be noted that during the delay from presentation to publication, the system developed further and now includes observations on the fins and opercles.

The procedure has been used on rainbow trout, brook trout, lake trout, brown trout, cutthroat trout, kokanee salmon, chinook salmon, silver salmon, largemouth bass, smallmouth bass, bluegill sunfish, redbreast sunfish, green sunfish, striped bass, yellow perch, walleye, gizzard shad, rainbow smelt, Utah chub, Utah sucker, american redhorse, common carp, channel catfish, red drum and freshwater drum. I am told that it has also been tried with squawfish, sturgeon, northern pike and muskellunge. Considering the wide range of uses it is likely that it is being used on other species as well and in applications not known to me.

Conceptually it has been sound and very adaptable. Some minor adjustments have had to be made in some of the criteria, but as long as the ranking is the same, a standard reference is still possible. The manner in which fish deposit fat varies considerably among the various families. The channel catfish has no pseudobranch, several species have no pyloric ceca. The liver in the common carp is difficult to evaluate. The black peritoneum in the

(Health Condition Profile - cont'd)
adult Utah chub can make observations difficult at times. The pseudobranch in the carp is difficult to observe and the channel catfish are likely to get blood out of the investigators before they give any up.

There has been a considerable effort to develop a standard reference procedure. There is a tendency to customize the procedure to fit specific situations. This is understandable but to do so is to risk loss of the standard. The broad use of the existing procedure provides support for the broad, adaptable application. It is being used in numerous facets of the U.S. Fish and Wildlife Service in a variety of ways. The TVA has expended considerable effort to monitor the quality of the largemouth bass in their many reservoirs. Missouri is beginning an attempt in their hatcheries and with free-ranging largemouth bass populations. It is being used in Utah in the context of quality control in their fish culture program and they are working toward integration of the management, special projects and fish culture efforts in this area to develop a major database which will track the HCP as a relative measure of quality. There is initial interest and application by agencies responsible for monitoring environmental quality. This application uses the HCP as an indicator of environmental quality. This may be a very important use in the future.

Through all of this activity it has been difficult to convince everyone that this is not a diagnostic procedure. It is an evaluation of *health* and *condition* of a fish population and is used to quantify departure from normal. The nature of the departure provides some diagnostic direction. The profile is compared to established norms and to previous profiles. Once a significant departure from normal is "flagged", a diagnostic procedure is initiated. This may require the technical effort of fish pathologists, toxicologists, etc. but often can be correlated to environmental changes, ecological perturbations, etc.

One of the most important features is that it can be taught to and conducted by the non-fish health specialists. It does not require expensive laboratory equipment and skills and highly specialized personnel. This means that it can be performed at the field level by a wide range of personnel. The HCP permits concentration of highly specialized procedures and personnel on the fish populations which show a significant departure from normal. These high tech programs then are not involved in extensive field monitoring and are used more efficiently. At the same time it provides good basic information on a population of fish for the field managers, research personnel and fish culturists.

There has been an effort in some circles to weight the various criteria. This has been brought on by the assumption that some departures from normal are more serious than others. They feel that a departure in a liver is more serious than a departure in an eye. This is not necessarily so and is a dangerous assumption to make. It must be remembered that this is a system of criteria. The more serious any one of the observed departures is the more apt you are to see departures in other criteria. The weighting, therefore, is internal and relative seriousness will be reflected in the final ranking. Once an individual observation is weighted it will be forever necessary to defend that decision.

The need for a final ranking or score is becoming increasingly evident. It is apparently needed as an aid in interpretation which would facilitate application. We are currently developing 2 more levels of interpretation to be included in the profile. There will be a tissue anomaly index, a blood anomaly index, a bioenergetic index and a visual parasite index generated from the data. A final health/condition index will be generated from these indices. After teaching this system so often to such a wide variety of people and discussing the system with so many people it is apparent that this addition is long overdue. All that remains is to complete the computer programming and test it.

The Health Condition Profile appears to have a bright future as a new and useful tool in fish culture, fisheries management and monitoring of environmental quality. The coming year will be very important as feed-back from the various users is received and evaluated.

Hatchery Cutthroat - Low "K" Factor

BACTERIAL KIDNEY DISEASE - A REVIEW

Perhaps the most controversial of current fish disease problems, bacterial kidney disease (also known as BKD, corynebacterial kidney disease, salmonid kidney disease, Dee disease) is a severe, slowly progressive disease of salmonid fishes. It can cause high mortality in both wild populations such as anadromous pacific salmon and Lake Michigan coho salmon as well as cultured fish. It has been observed only in salmonids. Pacific salmon, Atlantic salmon and brook trout are severely affected, brown trout and rainbow trout are less affected. This disease commonly occurs in a chronic form, and as such is seldom found in fish less than 6 months of age. Acute or subacute outbreaks can occur, especially in warmer water (13 -18^{o} C).

The causative agent of BKD is *Renibacterium salmoninarum*, a small Gram-positive diplobacillus bacterium. This organism is fastidious and very slow-growing, requiring 1-6 weeks for growth to appear when cultured. It requires specialized culture media, such as KDM-2 or charcoal agar. The organism does not survive long under natural conditions in water, but has been remained viable up to 21 days in sterilized river mud.

External signs of the disease might include exopthalmia (bulging of the eyes), small hemorrhages or lesions on the skin. Internal lesions might include swollen kidneys with white necrotic patches, fluid in the body cavity, enlarged spleen, pale liver, anemic appearance to viscera, hemorrhages on the body wall or white to yellow fluid-filled intestines.

Asymptomatic carriers and fish with subclinical infections are considered major reservoirs of infections. Both hatchery and wild fish are infected. Horizontal transmission (fish to fish) occurs, but vertical transmission (from parents to progeny via infected eggs) also occurs and is an important source of the pathogen. This may explain some cases in which the pathogen occurs in previously "clean" stocks.

Diagnosis

Presumptive diagnosis can be made on the basis of the clinical signs with the presence of characteristic diplobacillus bacteria. Definitive diagnosis can be made on the basis of techniques such as immunodiffusion, coagglutination, direct and indirect fluorescent antibody tests. Bacterial isolation alone is too inconsistent and time consuming for use in making a definitive diagnosis. Most fish health inspections make use of the direct or indirect fluorescent antibody tests. A new technique which has been used very successfully in diagnostic and research applications is the enzyme-linked immunosorbent assay (ELISA). This technique has been shown to be more sensitive and accurate than other current methods in the detection of the bacteria which causes the disease. It is not currently used as part of routine fish health inspections. Because of its increased sensitivity, it poses the difficult problem of penalizing state or private fish culturists who might show positive results while others subjected to less sensitive tests might be designated as free of the pathogen. It appears politics will have to catch up with technology, so that universal acceptance and adoption of ELISA will be necessary prior to its use in inspections. The Fisheries Experiment Station will be acquiring the equipment to perform ELISA techniques this year, but it is not clear when or if it will become a standard technique.

In reviewing diagnostic techniques, it is prudent to remind ourselves that all of the aforementioned procedures involve detection only of the pathogen, not the disease. On several occasions, the *R. salmoninarum* has been detected as part of a routine inspection, with no clinical signs or other indications that actual disease is occurring. The significance of detecting the pathogen in the absence of the disease is not clear. Until further research is available, most fish health professionals feel a conservative approach is mandated in our approach to these problems.

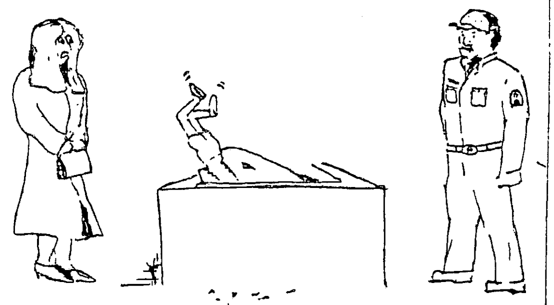
Control and Prevention

Avoidance of infected broodstock, infected eggs and a clean water source free of carriers are the best ways to prevent the occurrence of disease within a hatchery. The severity of an outbreak within a hatchery can be affected by several variables. Epizootics seem to be more severe in hatcheries in which the hardness of the water is very low. Experimental induction of the disease in hard water conditions in the Eastern U.S. has proven to be very difficult.

(Bacterial Kidney Disease - cont'd)

The effect of water temperature has not been clearly demonstrated, with different researchers reporting varying effects of temperature upon the severity and duration of the disease upon different species of salmonids. Diet appears to be a factor in the course of the disease, with some researchers reporting that high levels of corn gluten are associated with a more severe outbreaks. In one study, fish fed a diet with high levels of iodine and fluorine experienced a 3% infection rate vs. 38% in commercial feed controls. In addition, genetic manipulation of fish strains may alter the infection rates in hatcheries.

Management practices attempting to directly treat the eggs with various disinfectants such as povidone iodine or antibiotic solutions such as erythromycin have not proved successful. Eggs treated with erythromycin do not appear to maintain adequate levels of antibiotic and experienced a lower rate of egg-to-fry survival. Prophylactic injection of erythromycin into broodfish on a monthly basis prior to spawning resulted in therapeutic levels of the antibiotic in the eggs and may prove valuable in preventing vertical transmission. Researchers in the Northwest have been experimenting with use of ELISA techniques to detect and eliminate high titer broodstock from management programs.



I told him not to put his hand in the water!

Treatment

BKD is difficult to treat due to the tendency of the bacteria to sequester within the cell, beyond the reach of most antibiotics. The disease has a tendency to recur after the termination of antibiotic therapy. Antibiotics which have been used include sulfamethazine and erythromycin. Erythromycin is considered the drug of choice, although recent reports of drug resistance have been noted. It should be noted that erythromycin is not currently approved by the FDA for use in fish as a preventative or treatment at this time.

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