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ROUTINE FISH DISEASE MONITORING

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The purpose of a fish disease monitoring program is to obtain information relative to the health status of stocks of fish, and the suitability of conditions under which fish are maintained at a production installation. A well-designed monitoring program should enhance the efficiency of hatchery operations and minimize the impact of fish diseases by providing the following:

1. Information need to plan a fish health program.
2. A quick response to disease outbreaks (diagnosis, therapy, prevention).
3. Reduction of the mortality rate in infected lots of fish by providing diagnosis and subsequent therapy. Food conversion ratios should improve in response to lowered mortality.
4. Information as to whether or not a disease problem is attributable to poor management or is primarily due to the presence of a fish pathogen.
5. Incentives for the adoption of measures to control or prevent the introduction of fish disease agents from outside sources, via the transfer of fish or fish eggs or exposure to other contaminated sources.
6. Enhanced market opportunities by the provision of stock that is free, or relatively free, of specific fish disease agents. The producer can, therefore, supply markets located in geographical areas protected by fish disease regulations.

PERSONNEL AND EQUIPMENT REQUIRED

Ideally, every hatchery should have at least one person trained in basic fish disease diagnosis, therapy, control, and prevention, and in the collection. preservation, and shipment of specimens for laboratory analysis.

At the present time, a number of short courses (1 to 2 wk duration) oriented toward the basics of fish disease problems are conducted by federal, state, and university departments. Attendance at such courses will provide basic fish disease information that will be a valuable asset in planning for and conducting a fish health program.

The procedures involved in the diagnosis of many important fish diseases are neither complicated nor time consuming. The presence of bacterial gill disease, perhaps the most common and debilitating infection of hatchery fish, can usually be detected by an individual with a minimum of instruction and access to a suitable microscope. Prompt, on-site identification of a fish disease permits the immediate application of chemotherapy or remedial measures to control or eradicate the disease. As a result, losses are kept to a minimum.

The following equipment is sufficient for diagnosing most disease situations in a hatchery:

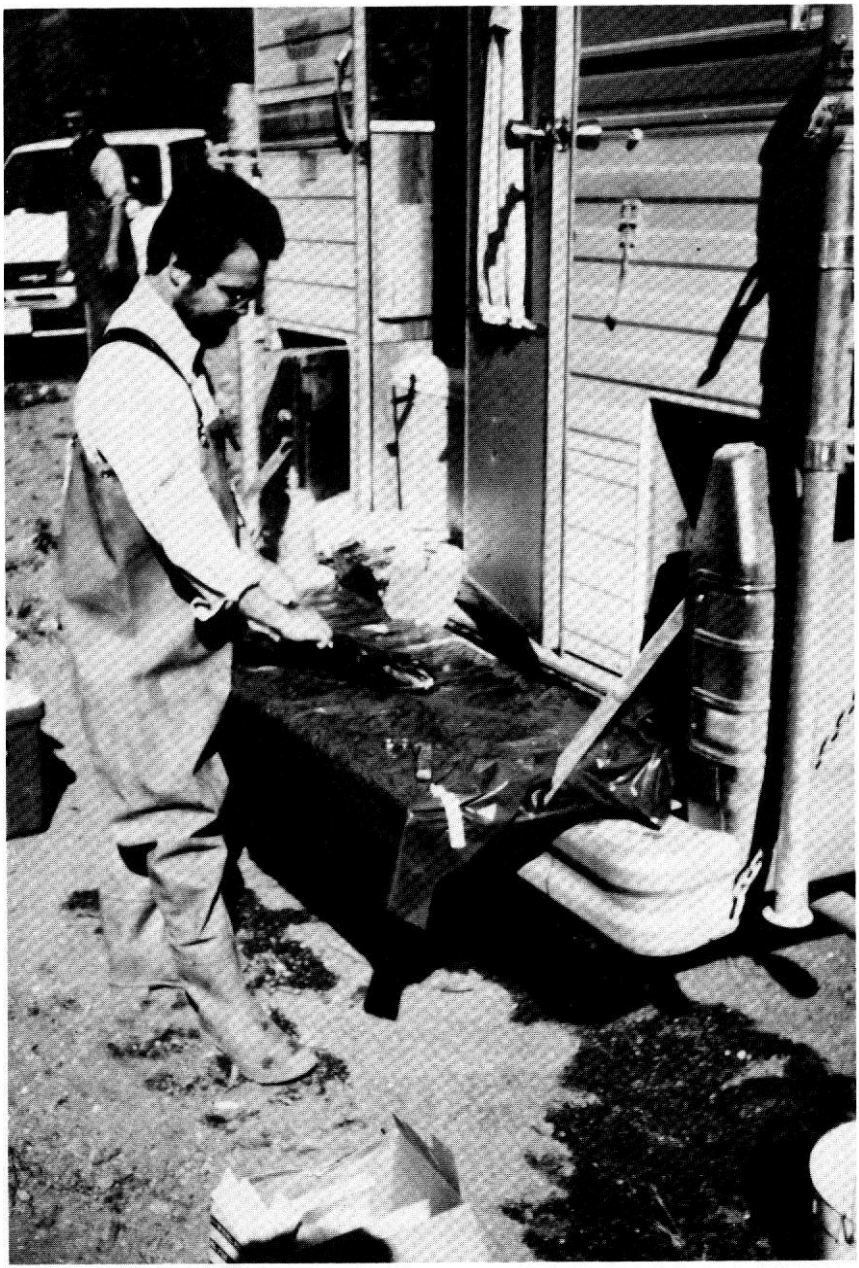
1. Compound microscope (binocular, oil immersion capability, 1000x magnification).
2. Set of dissecting tools.
3. Set of selected bacteriological stains.
4. Microscope slides and cover glasses.
5. Selected bacteriological media.
6. pH meter.
7. Oxygen meter or kit.
8. Bunsen burner or propane torch.
9. Bacteriological inoculating loop.
10. Media sterilizer (pressure cooker or autoclave).

PROCEDURES

Many fish disease problems are seasonal in nature due to factors such as water temperature, spawning, stress, and other conditions. Accordingly, a fish disease monitoring program should encompass the entire year in order to provide information relative to the status of fish health under all phases of production.

Ideally, a hatchery should be inspected by a qualified biologist for the presence of fish disease agents at least twice a year. The survey should be conducted using procedures that will detect the presence of parasitic, viral, and bacterial pathogens. All lots of fish present at the time of the survey should be included. A lot of fish is generally defined as a group of fish of the same age, derived from the same brood stock, and held in a common water supply.

Following determination of the number and location of lots present, a decision must be made concerning the number of fish to be sampled. Considering that a survey is usually conducted to detect the presence of fish disease organisms (in asymptomatic carrier fish) as well as the actual presence of diseased fish, the number of specimens required for sampling is based on a postulated 5% incidence and a 95% probability that carriers or infected specimens will be detected. In general, a 60-fish sample per lot will be required. However, in some situations, such as the presence of valuable brood stock, or other circumstances, it may be necessary to reduce the sample size for particular lots. The usual practice is that the individual in charge of the survey determines the number of specimens required.



A mobile fish disease laboratory for inspecting fish in a multi-hatchery program (British Columbia Min. of Env.)

To be meaningful, a complete survey must be supervised by a qualified biologist who has the expertise and access to laboratory facilities for processing collected samples. The individual conducting the survey must cooperate with personnel of the involved hatchery in preplanning and in conducting the survey. Depending on circumstances, surveys may be conducted for the presence of specific fish disease organisms only. If conducted according to established procedures, a disease survey should provide all necessary information concerning the present disease status of the production unit surveyed.

MONITORING

One way to develop information on the current fish disease status at a hatchery is to implement a disease monitoring program. A monitoring program should be so scheduled that specimens will be examined at various intervals throughout the year to ensure that fish will be examined under all production conditions and at all ages. A monitoring program can be conducted either by on-site examinations by hatchery personnel or by prior arrangements with a selected laboratory, or a combination of both. Live or preserved specimens, slides of specific tissues, and media inoculated from specific organs can be delivered or forwarded to the laboratory for subsequent examination and evaluation.

Ideally, sample specimens or material should be collected and examined during every month of the year. Care should be exercised in the selection of specimens for examination. In situations where a disease problem is suspect, only those specimens exhibiting symptoms of distress should be selected. Live moribund specimens are preferred but, if necessary, freshly-dead specimens may be collected.

When it is necessary to send specimens away for examination and circumstances prohibit live delivery, specimens may be forwarded by preserving them on wet ice, but not frozen. Specimens for parasitology examinations may be preserved in a 5-10% formalin solution. If the specimens are longer than 7.5 cm (3 in) a" incision should be made in the body wall to allow the preservative to reach the internal organs. Specimens should be completely immersed for 24 h in the preservative mixture, using a ratio of at least five volumes of preservative per volume of fish. Preserved samples can then be shipped in plastic bags containing just enough preservative to keep them moist. If possible, include solid materials that collected in the bottom of the original vessel in which fish were preserved. Glass containers should not be employed for shipment because of possible breakage and the hazardous nature of the preservative.

Slides of selected tissues and organs can be made on-site by merely smearing the material on a slide. Slides can then be stained and examined on-site or forwarded for processing and examination. Bacteriological media can be inoculated with material from various organs by employing aseptic techniques. The media may then be incubated on-site for subsequent examination or forwarded to a laboratory for evaluation.

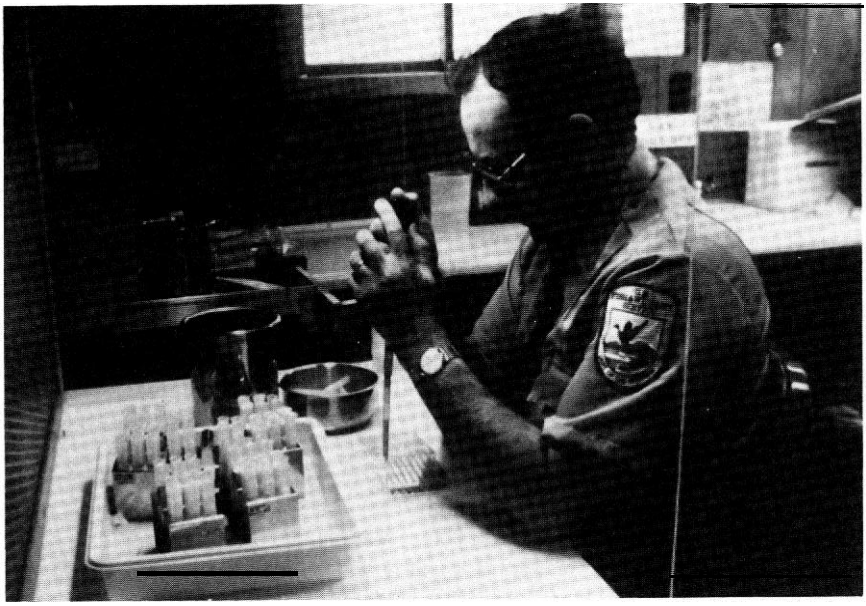
The presence of many common internal and external parasites can be determined on-site if a suitable microscope is available. Wet mounts of gill material, body and fin scrapings, or pieces of internal organs are made by placing a drop of water on a microscope slide, adding the excised material to the slide,

and placing a cover glass over the material. The material may then be examined by microscope at various magnifications.

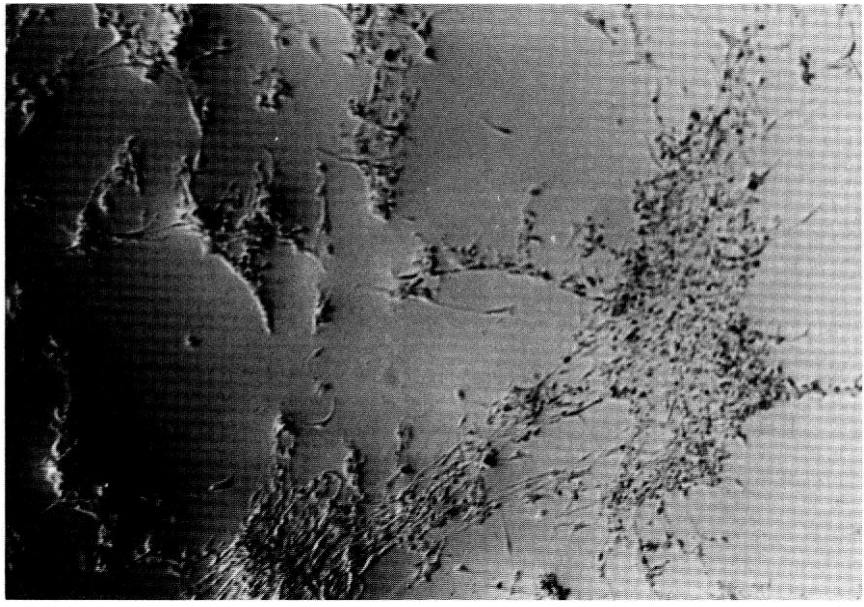
The presence of bacterial gill disease can be detected by a microscopic examination of wet mounts of gill tissue. Suspect gill tissues also should be smeared on a microscope slide, stained with a simple stain, and examined for the presence of the causative myxobacteria.

The presence of a systemic bacterial infection can often be determined by microscopic examination of stained slides prepared from excised material removed from various organs of suspect specimens. The presence of bacterial kidney disease (Renibacterium salmoninarum), a Gram-postive bacterium, can be detected and identified by this method. The presence of a Gram-negative bacterium such as ***Aeromonas*** salmonicida, the causative agent of furunculosis, can also be detected by this method, but no positive diagnosis can be made. Serologic methods are required for the positive identification of most Gram-negative bacteria. Fluorescent antibody techniques have been developed that help determine the presence and identity of bacterial organisms. However, such techniques are usually utilized only in well-equipped laboratories. For the confirmation of the presence of specific organisms, a complete bacteriological examination is still necessary.

The presence and identification of viral agents or viral diseases must be determined at a laboratory that maintains fish tissue cultures and specific antisera. Station personnel can collect the needed specimens and forward them to a laboratory for testing. Whenever specimens are to be submitted for viral testing, the diagnostic laboratory should be contacted inadvance for instructions on sample collection and shipment.



Good laboratory techmque, modem equipment, and sound technical training are required for the accurate diagnosis of fish diseases. This fish health specialist is preparing sampies Lo check for the presence of fish virus. (U.S. Fish and Wildl. Serv.)



Tissue vultures are used to check for the presence of fish viruses. The viruses destroy the normal continuous sheet of cells. This photo shows the extensive damage (cytopathic effect) caused by IPN virus (U.S. Fish and Wild,. Serv.)

Certain clinical signs may suggest the presence of viral disease in a particular lot of fish. In situations where an active viral epizootic is suspected, only relatively few specimens (15-20) will be required for examination. The specimens selected for analysis should be moribund and exhibit typical symptoms associated with the suspected disease. In viral infections of fish, usually only small size (2.5-7.5 cm) fish are involved; therefore, whole specimens can be forwarded for examination. Whole fish or kidney and spleen tissues should be packaged in sealed plastic bags, placed on wet ice immediately following collection, and promptly forwarded to the diagnostic laboratory. It is imperative that collected samples be kept on ice prior to testing so careful planning and timiig is required in order to expedite shipment delivery and to assure that samples arrive in satisfactory condition.

In addition to the careful selection of specimens or tissues, any pertinent information such as clinical signs, abnormal behavior, stress factors, lot number, species, mortality rates, water temperatures, loading densities, age, and water chemistry data should be included when shipping materials as they can aid diagnosticians in assessing the disease situation.

When information is desired as to whether or not viral agents are present, all lots maintained at the hatchery must be surveyed. Testing techniques are highly sensitive and can usually detect the presence of viral agents in carrier fish even though there are many situations where viral agents are present but do not give rise to disease. The usual survey procedure involves selecting 60 fish from each lot on hand. The fish are anesthetized and killed, and the kidneys and spleen excised. Excised material from five specimens is pooled in a plastic tube containing normal saline solution. Thus. there will be 12 tubes representing the

material excised from one lot. In situations where it is necessary to use 60 fish from a lot, e.g. valuable broodstock and selected stocks of fish, or where it is not practical to sacrifice 60 specimens, ovarian fluid samples may be collected for survey purposes. This practice should be used only when necessary because ovarian fluid is not as reliable a source of viral agents as the kidneys or spleen. The decision about number of specimens to be sampled to assure that adequate and reliable information is obained is generally left to the judgment of the individual conducting the survey. Certification requirements may dictate the minimum numbers of fish allowed during testing.

NUTRITIONAL DISORDERS

Nutritional disorders, in general, are not considered to be disease brob-lems. However, nutrition plays an important role in the prevention of disease by providing essential nutrients. Nutritional disorders can result in significant losses of fish stock, can be the cause of poor conversion rates, and can have a profound effect upon a station's production capability and quality of the fish produced. Certain parameters provide useful information for monitoring or evaluating the condition of fish stocks in relation to their diet. Parameters such as eye condition, excess fat, body configuration, anemia, sluggishness, abnormal coloration, fin erosion, nervousness, pale liver coloration, and poor conversion rates are valuable indicators in the surveillance for nutritional disorders.

Problems of a nutritional nature do not, for the most part, lend themselves to easy solution and may require an in-depth investigation. In the event that nutritional disorders are suspected and the immediate cause of factors involved cannot be readily determined, it is advisable to consult trained individuals or laboratories familiar with fish pathology and fish nutrition. The resolution of nutritional problems often involves diet tests or trials, designed to provide specific information and may require several years before results can be fully evaluated. Depending upon the nature of the problem, the cooperation of fish food manufacturers, biologists, and laboratories may be required to determine the nature of the problem. In many instances, a histological examination of vital organs is necessary for obtaining information concerning the nature and origin of observed tissue changes.

ENVIRONMENTAL CONDITIONS

Environmental conditions under which stocks are maintained can have a profound effect on the well-being of fish. A complete monitoring program should include tests to provide information concerning characteristics of the environment. Routine tests for oxygen, ammonia, nitrogen and other dissolved gases, temperature, and pH will provide necessary and valuable information that may prevent unfavorable environmental conditions. Additional testing may be required in problem situations.

Monitoring environmental conditions can alert hatchery personnel to the presence of stress factors that may cause mortality or give rise to serious disease problems. Water chemistry kits that employ "cook book" techniques will provide relatively accurate information. Such kits permit hatchery personnel to conduct on-site tests that will provide data concerning critical environmental conditions.

An instrument known as the Weiss gas saturometer can be employed to determine whether or not gas supersaturation exists. Water supplies that are supersaturated can give rise to a relatively common condition known as "gas bubble disease". The condition can arise when dissolved gas pressures exceed atmosopheric pressures. When a state of supersaturation exists, gas bubbles form in fish tissues causing conditions which are similar to the "bends" in deep sea diving. The Weiss saturometer measures the sum total of all dissolved gases and provides useful information within minutes. The instrument is portable, designed for field use, and is simple to operate. It can detect low levels of supersaturation which subject fish to stress but do not elicit overt signs of distress. The instrument is also useful for determing whether or not remedial measures to alleviate or reduce gas pressures are actually effective.

RECORD KEEPING

Record keeping is an integral part of any monitoring program and must include data concerning current and past information relative to the status of fish health at the facility and the conditions under which fish stocks are were maintained. The availability of good records provides a valuable reference source for future investigations concerning the health status and well being of fish stocks, and provides data that can be used to evaluate and enhance the efficiency of management procedures. Tbe following data should be included:

1. Diet (amount, size, type, source of food fed each lot or holding unit).
2. Mortality (number daily for each holding unit).
3. Therapy (chemicals and drugs employed, dosage rate, duration of treatment, results).
4. Environment (water volumes, flow rates, water chemistry data, loading densities, etc.).
5. Conversion rates used.
6. Notes concerning observations relative to abnormal behavior, presence of disease, nutritional disorders, stress factors, and other pertinent factors.

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