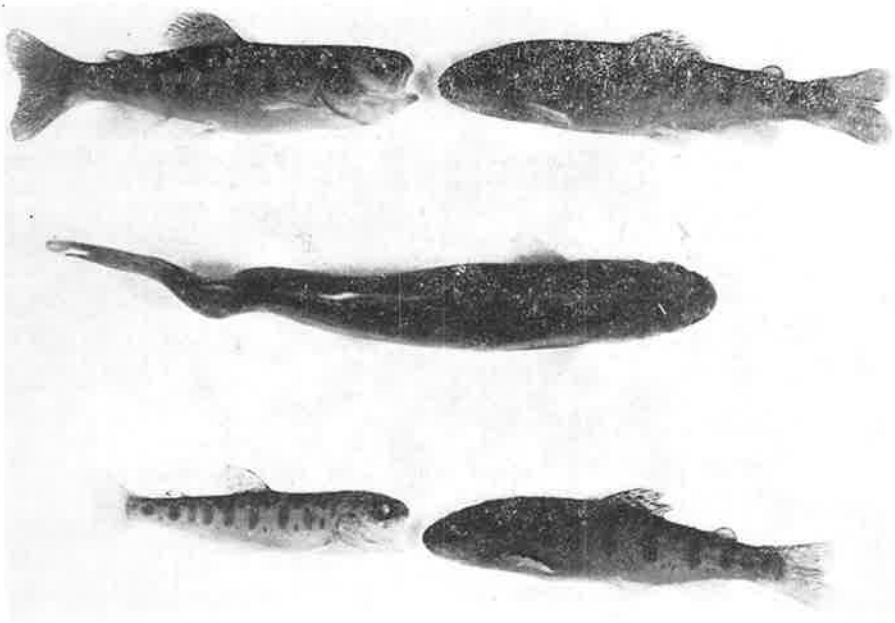

Submitting Fish for Disease Diagnosis

By P. M. Hine

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Copies are obtainable from the Editor, at the same address.

Cover: Rainbow trout (*Salmo gairdneri*) with whirling disease (*Myxosoma cerebralis*) (left top, middle, and left bottom) compared with uninfected fish on the right. National Publicity Studios photo.

1973

SUBMITTING FISH FOR DISEASE DIAGNOSIS

IN recent years there has been a considerable increase in the incidence of disease outbreaks reported in fish throughout Europe, Asia, and North America. This has been due to:

- Increased world communications, in both trade and the movements of people.
- The great expansion in the intensive culture of fishes, notably salmon, trout, catfish, and carp.
- The expansion of fish disease studies within the biological sciences, and the establishment of disease units by both Governments and universities overseas.

The establishment of disease study units has often followed serious epidemics such as the outbreak of U.D.N. (see glossary on page 9) in Britain in 1968. This has resulted in a general trend toward more legislation to control fish movements and to ensure adequate reporting procedures, etc., and some countries plan integrated disease control programmes.

New Zealand seems to be comparatively free of fish diseases, but this is largely because of the lack of research up to the present. The outbreak of whirling disease in Otago in 1971 shows that this country is not exempt from infectious fish diseases. The widespread outbreaks of viral diseases such as I.H.N. and I.P.N. in North America, U.D.N. and I.P.N. in Britain, and V.H.S. and I.P.N. in Europe, as well as the spread of bacterial and protozoan infections, increase the likelihood that these diseases will eventually appear in New Zealand.

To ensure that New Zealand remains fairly free of disease, and that any outbreak of a potentially harmful nature can be readily detected, the Fisheries Research Division of the Ministry of Agriculture and Fisheries is establishing a disease diagnostic unit in Wellington. This preliminary account of the proposed functions of the unit is intended for the guidance of fisheries officers and anglers. The aims of the service are:

1. Diagnosis and treatment of disease in hatcheries, farms, and holding tanks to permit the control or eradication of the infection. Sites of intensive culture or holding tanks will be given priority, as large numbers of fish are often involved, treatment may be feasible, and employment may be at stake. However, it is intended to provide an efficient service for all those concerned with fish and fisheries – acclimatisation societies, fisheries inspectors, and concerned laymen, and all outbreaks whether in culture or the wild will ultimately be investigated.
2. To examine all live imported fish suspected of carrying pathogenic or potentially pathogenic organisms to prevent the further introduction of exotic diseases. This will be carried out in association with the Port Agricultural Service and fisheries inspectors and be supported by stricter legislative controls on the importation of all live fish. It is hoped that better

disease control will be achieved with the co-operation of tropical fish importers, as a means of safeguarding New Zealand's fish stocks and reducing the incidence of disease in live fish sold for retail.

3. To investigate the occurrence and distribution of bacterial and parasitic infections already established within the country in order to contain these diseases and minimise the chances of their introduction into hatcheries and farms. At present there are no published studies on fish bacterial infections in New Zealand, and only just over one-quarter of fish species have ever been examined for parasites.

It is commonly thought that the culture of fishes encourages the spread of disease. Davis in his book "Culture and Diseases of Game Fish", published in its fifth printing in 1967, observed: "There is a widespread belief that the average hatchery is a hotbed of disease where ailing fish are the rule rather than the exception, and that, in contrast, wild fishes rarely fall victims to infectious diseases. . . . It is scarcely necessary to add that none of the diseases which make trouble for fish-culturists originated in hatcheries; all were introduced. Usually the diseases are brought in on wild fish, and it is fortunate indeed that only a small fraction of the diseases that occur on wild fishes have become established at hatcheries. It can be expected, however, that more of these diseases will from time to time appear among hatchery fish."

By studying the distribution of disease in New Zealand, we should be able to reduce the threat to cultured fish posed by disease in wild stocks and to protect wild stocks from new diseases and parasites imported with exotic fishes.

SUBMISSION OF SPECIMENS

Choice of Preservative or Fixative

The best method of preserving a dead fish depends on the nature of the organisms or factors causing death. Formalin-fixed tissues are ideal for histological studies of damage caused by worms, but useless for culturing bacterial infections. Therefore to choose the most suitable method of preservation the sender needs to have some idea of the cause of death before submission.

As a general guide the cause of death may be attributed to any of these five categories—environmental causes, microbial infections, parasitic infections, tumours, and unknown causes.

Environmental Causes

These include deaths caused by lack of oxygen, high temperatures, stress, poisons, pollutants, etc. Death usually occurs catastrophically over a short period and may involve large numbers of fish. In natural water systems large numbers of other animals may also be killed.

These deaths are obviously hard to diagnose from the distance of the laboratory and therefore it is especially important for senders to supply

as much detailed information as possible concerning these mortalities.

Though a water sample may be useful, only rudimentary analysis is possible, and it is not usually possible to measure dissolved oxygen. A quart water sample should be sent iced or with the addition of 1 to 5 cc of formalin. Fish should preferably be sent alive or moribund, but iced or frozen specimens are also useful.

Microbial Infections

These include infections caused by viruses, bacteria, Protozoa, and fungi. Infected fish may have internal or external lesions, surface areas of bleeding, swelling or boils, bleeding of the gills and vent, frayed or bleeding fins, soft rotting or erosion of large areas of the body, whitish blisters, swelling and rupture of internal organs, heavy bluish slime, or areas of cottonwool-like growths. Lesions may appear ulcerous, and swellings often contain oozing fluids.

In extremely virulent bacterial and some viral infections there may be few noticeable symptoms, and fish should be examined closely. Death may be intermittent, cyclic, constant, or increasing over a period of days or weeks. Fish should preferably be submitted live or moribund, but frozen or formalin-fixed specimens may be useful.

Parasitic Infections

Parasites may occur on the surface, for example, lice, some Protozoa, flukes, anchor worms, and leeches, or internally, for example, tapeworms, roundworms, and flukes. These organisms are usually visible to the naked eye, but some surface flukes and gill flukes are extremely small (less than 1 mm) and are difficult to detect. Death is usually intermittent or increases slowly over a period.

Individuals or groups of parasites should be submitted fixed in formalin; fish may be submitted live, iced, frozen, or formalin fixed.

Tumours

These are usually firm growths that appear slowly, show no sign of bleeding or having a fluid core, and do not often cause death unless at a critical site on the body. Their cause is often unknown and they are primarily of academic interest in relation to cancer research. Suspected tumours may be removed or submitted intact. Formalin fixation is preferable, and large tumours should be slit to enhance fixation.

Unknown Causes

These include deaths due to nutritional deficiencies, shock, congenital deformities, etc. Specimens should preferably be submitted or preserved by each of the four methods detailed below.

Methods of Preservation

Submission of Live or Moribund Fish

Live fish should be submitted whenever possible to facilitate easier diagnosis, permit first-hand observations on behaviour and the course of infection, and provide experimental fish for chemotherapeutic treatment. Live fry or fingerlings may be shipped over many hours in a thick polythene bag partially filled with ice-cold water.

Ten to 12 fry and 4 to 6 fingerlings or their equivalents may be

transported per ½ gallon of water provided that the water is thoroughly flushed with air or preferably oxygen before shipment and there is 3 to 5 times as much gas volume as water. Seal the bag and place it inside a second bag containing ice, seal this, and place it in a rigid container with bubbled polystyrene or newspaper as insulation.

Do not pressurise the bag, as the reduced air pressure in the cargo holds of high-flying aircraft may make the bag burst.

Iced Specimens

Freshly dead fish may be transported in ice in a thick polythene bag. This should be sealed and placed in a second bag for protection. Pack this in a rigid container insulated with cottonwool, newspaper, bubbled polystyrene, or similar materials.

This method is suitable only for specimens arriving in Wellington the same day as they are sent.

Frozen Specimens

If possible, freeze specimens in separate polythene bags and once frozen do not thaw and re-freeze. Pack the bags in ordinary ice or dry ice (solid carbon dioxide) in a polythene bag. Seal this bag if ordinary ice is used, but keep it in the open and ventilated to permit the escape of carbon dioxide gas if dry ice is used. Do not allow the dry ice or gas to come into direct contact with the specimens. Insulate and pack as before but with sufficient ventilation if dry ice is used.

Formalin-fixed Specimens

Place the specimen in 5 to 10 times the volume of 10 percent formalin made up with fresh water or sea water (depending on the origin of the specimen) in a glass or plastic container. Fish over 5 cm should be slit along the abdomen with a razor blade to allow penetration into the body cavity. Pad the container to avoid breakages. When submitting formalin-fixed material pay special attention to describing details of colour and texture of diseased areas, as formalin reduces colouring and hardens tissues.

Take care in handling formaldehyde: it is the active component of formalin, is an irritant to eyes, mucous membranes, and skin, and is poisonous if swallowed.

Pathological changes in diseased fish are best seen in comparison with healthy fish of about the same size from the same location. Therefore, if possible, a few healthy fish should be sent with the diseased specimens, but make sure that the two groups are kept separate and label them clearly.

If very large fish are involved and the disease symptoms appear to be localised, infected portions of the fish only may be forwarded, but details of the length (nose to tail fork or tip of tail), weight, and sex of the specimen should be included.

Before sending the specimens make sure a submission form or letter is included and write the nature of the contents clearly on the wrapping, for example, "Live fish—urgent". Address it as shown on the submission form and ring 556 169 Wellington, giving an indication of the contents, flight number, and time of arrival in Wellington.

If possible, try to avoid sending specimens near weekends unless the

disease problem is considered serious. Specimens that have been dead for more than 6 hours in warm weather or are partly decomposed are of little use for bacterial or any other examination.

Submission Form

Where possible standard submission forms should accompany all specimens to be examined. Both sides of one of these forms, which are available at no charge from the address on the form, are shown on pages 6 and 7. The forms have been designed primarily for the use of hatcheries, farms, and other intensive culture or holding establishments to provide maximum information not available from the examination of specimens. However, they may also be used for specimens collected from natural water systems by simply disregarding the sections relating to systems and management.

The tear-off portion at the bottom will be returned to the submitter, stating the diagnosis made and suggested treatment to be implemented. For fish submitted from natural water systems where treatment is impractical the diagnosis will be filled in and the treatment section used to outline possible origins and course of the disease and effects on the fish in the system. If a potentially serious disease is diagnosed or extensive treatment is needed, a covering letter will be included with the return slip, and in extreme cases staff will visit the area concerned.

The questions are fairly straightforward. "**No. submitted**" should be only approximate for large numbers. "**Verbal description of symptoms**" should contain as much information as possible and should include all noticeable symptoms as well as those specified in the sections below. The more information received the easier it is to make a diagnosis. "**Specific symptoms**" covers behavioural and physiological abnormalities and mortality patterns, none of which can be discerned from the specimens. Either the relevant sections can be ticked or remarks on the prevalence of the symptoms can be added.

"**Environmental conditions**" refers to the questions on water quality. If specific pH cannot be measured, an estimate of acidity or alkalinity would be useful. Similarly, a quantitative estimate of flow rate in cusecs is desirable, but a subjective estimate such as "fast", "stagnant", "moderate", etc., is sufficient.

Other questions relate to the possible origins of the disease and introduction and distribution within the culture establishment. If possible, a simple sketch of the establishment would aid in assessing the most suitable method of treatment.

TREATMENT

Treatment is practical in only artificial systems, though limited control can sometimes be achieved in natural systems. For successful disease control in any culture establishment there must be not only a diagnostic service recommending treatment, but the continual application of prophylactic measures to prevent disease.

Prophylaxis is synonymous with good management, and by applica-

Submitted by: MR J. H. EASTMAN

Address: WELLESLEY FISHERIES
568 TILGARD RD
CARWILLIN

Tel. No: CARWILLIN 7348

Specimen collected from: CREEK ABOVE TROUT HATCHERY PONDS Date: 14·2·73

Species: BROWN TROUT No. submitted: 3 Preservation: MORIBUND

OFFICE USE ONLY

Acc. No:

Diagnosis:

Treatment:

Verbal description of symptoms: DEAD TROUT WITH OPEN LESIONS HAVE BEEN SEEN IN THE CREEK OVER THE LAST 3 WEEKS. FIRST NOTICED WHEN FINE SPELL OF WEATHER BEGAN AND WATER LEVELS DROPPED. FISH SUBMITTED WERE SEEN SWIMMING LETHARGICALLY ON THEIR SIDES AND RUBBING AGAINST STONES. RED SWELLINGS AND LESIONS WERE NOTICED ON THEIR BACKS. RED SWELLINGS APPEAR TO DEVELOP INTO LESIONS.

SPECIFIC SYMPTOMS. STATE "YES" OR REMARK WHERE APPLICABLE

Behaviour		Physiology		Mortality Pattern	
Disorientation:	SWIMMING ON SIDE	Not feeding:		Catastrophic:	
Flashing:		Vomiting:		Continuous increasing:	APPARENTLY
Rubbing:	ON STONES	Discoloured faeces:		Continuous constant:	
Mouthing at surface:	OCCASIONAL	Gaping gills:		Intermittent:	
Circular movements:		Excess mucus:	YES	Cyclic:	
Jerky movements:		Sloughing mucus:	YES	Other:	
Other:		Other:	VENT INFLAMED		
				Approx. mortality rate:	—

OFFICE USE ONLY Our Reference Acc. No: B47/P79

Diagnosis: CHRONIC FURUNCULOSIS, A BACTERIAL DISEASE AGGRAVATED BY CROWDING, DAMAGE, AND WARM WATER. ALSO HEAVY WHITE SPOT INFECTION.

Treatment: TREATMENT OF THE BROWN TROUT IN THE CREEK IS NOT POSSIBLE. HOWEVER, WITH THE RETURN OF NORMAL TEMPERATURES AND WATER LEVELS, THE DISEASE SHOULD DISAPPEAR. DO NOT REMOVE OR TRANSPORT FISH OR WATER FROM THIS CREEK OR CATCHMENT.

The front of the submission form, issued to accompany specimens to be examined.

ENVIRONMENTAL CONDITIONS

Water: Temp. 19.5°C pH: SLIGHTLY ALKALINE Flow: VERY SLOW Source: LOCAL CATCHMENT

Any indications of poor water quality? NONE, BUT WATER LEVEL AND FLOW GREATLY REDUCED, TROUT RESTRICTED IN SHALLOW POOLS

Distribution of disease in system: ONLY 400 YDS OF CREEK OBSERVED, BUT DEAD TROUT SEEN OVER THE WHOLE OF THIS AREA.

Other fish in system: BULLIES AND A FEW INANGA SHOALS, LONG AND SHORT FINNED EELS.

Recent introduction into the system: NONE.

Origin of introductions: NOT APPLICABLE

Any recent changes in management? NONE APPLIED TO THE CREEK

Were the fish previously damaged? SOME FISH SHOW SIGNS OF DAMAGE FROM STRANDING

Time between first appearance of disease and death (days): THOUGHT TO BE ABOUT 8 DAYS

Have specimens previously been submitted? NO

What treatment, if any, was implemented? —

Remarks: AS THIS WATER IS THE ONLY POSSIBLE SOURCE SUPPLYING HATCHERY PONDS, IS IT POSSIBLE THIS DISEASE WILL APPEAR IN PONDS CONTAINING RAINBOW TROUT?

Send to: Dr P. M. Hine, Fisheries Research Division,

Ministry of Agriculture and Fisheries,

P.O. Box 19062, Wellington.

Mark specimens "AIRPORT ONLY" and telephone or telegram Wellington 556 169 notifying details of flight number and arrival time at Wellington.

Other measures: FURUNCULOSIS MIGHT WELL APPEAR IN RAINBOW TROUT PONDS, AND COULD CAUSE CONSIDERABLE MORTALITY. REDUCING WATER TEMPERATURES AND MINIMISING CROWDING AND DAMAGE WILL HELP PREVENT THIS. SEND ANY SUSPECT FISH IMMEDIATELY AND A COURSE OF TREATMENT WILL BE RECOMMENDED SHOULD THE DISEASE BE CONFIRMED.

24924A—50 pads/3/73 V.

The back of the submission form.

tion of good management disease can be virtually eliminated from culture tanks and ponds, and fish under treatment have optimum conditions for recovery. Overcrowding, warm water temperatures, stress, damage, and slow water flow all encourage the rapid spread of viral, bacterial, fungal, and parasitic infections once they are introduced into a system.

It is now considered that many healthy fish carry potentially pathogenic bacteria in their mucus, but as long as they remain healthy and in clean conditions they are able to resist infection. When they are damaged, under stress, or in conditions associated with bad management they become debilitated and succumb to initial bacterial and secondary fungal infections.

As well as good management there is a need to consider disease when designing hatcheries and farms. Circulatory systems should be designed to allow the isolation of infected tanks, which thus reduce the risk of spreading infection in contaminated water. Fry tanks and outside ponds should not be constructed of concrete or other porous materials which provide numerous crevices for microbial spores and are thus hard to disinfect. However, if it is necessary to use concrete, tanks should preferably be smoothly plastered and coated in epoxy resin paint before use.

The method of treatment recommended will largely depend on the factors or organisms causing debility or death. Environmental causes such as warm water temperatures and low oxygen concentrations can readily be overcome by increasing water flow rates. However, microbial diseases are not so easy to cure.

All viral infections, such as I.H.N., I.P.N., U.D.N., V.H.S., and cauliflower disease of eels are at present incurable, and should an outbreak occur, all infected fish or fish in contact with possibly contaminated water should be destroyed immediately and the hatchery, farm, or holding tanks closed down until they are thoroughly disinfected under supervision.

Many bacterial infections respond to treatment with antibiotics, and ectoparasites and internal parasites can also be eliminated by chemical treatment.

When restricted drugs or chemicals are necessary for treatment, a covering letter will be included. This should be presented to a veterinarian, who may issue a prescription for these substances.

Depending on the diagnosis, recommendations will be made about the most suitable methods of treatment and acceptable dosages.

GLOSSARY

Chemotherapy:	A course of treatment with chemicals or drugs.
Congenital:	Belonging to one at birth. The term is especially used for diseases and defects present at birth.
Debility:	A loss of health resulting in lowering of resistance to disease.
Diagnosis:	The determination of the cause of a disorder or disease.
Disease:	Any condition that reduces the ability of an organism to maintain itself or cope with changes in its environment.
Ectoparasitic:	Parasitic on the surface of a host. This includes parasites in orifices such as the nasal passages and buccal cavity, on the gills, or behind the operculum.
Epidemic:	Disease prevalent among a population or community at a particular time.
Exotic:	Introduced from abroad.
Histological studies:	Studies on the tissues of an organism by the examination of thin sections of the organism under a microscope.
I.H.N.:	Infectious haematopoetic necrosis — a viral disease prevalent in the American north-west.
Infection:	The introduction of a pathogenic organism into a susceptible host whether or not this causes overt disease.
I.P.N.:	Infectious pancreatic necrosis — a widespread viral disease.
Moribund:	In a dying state.
Parasite:	An animal or plant that spends at least part of its life in or on another organism and which may affect it deleteriously.
Pathogen:	An organism producing disease in a healthy animal. Some organisms such as the *Aeromonas/Pseudomonas* group of bacteria may be non-pathogenic, but become pathogenic under certain circumstances.
Pathology:	The study of the cause, course, and effects of disease.
Prophylaxis:	Preventive treatment of disease. A prophylactic measure is one designed to prevent the occurrence of disease.
Protozoa:	A group of unicellular organisms forming the lowest division of the animal kingdom.
V.H.S.:	Viral haemorrhagic septicaemia — a viral disease prevalent in mainland Europe.
Virus:	The smallest known group of infectious agents; they can multiply only within living cells.
U.D.N.:	Ulcerative dermal necrosis — a disease condition thought to be caused by a virus, though this has never been verified.

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