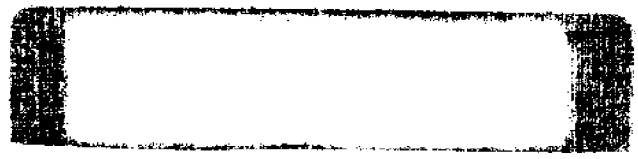# Proceedings from a conference on ease inspection and certification of fish and fish eggs

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acknowledgment

The OSU Sea Grant College Program attempts to foster discussion of important marine issues by publishing reports, sometimes dealing with controversial material. A balanced presentation is always attempted. When specific views are presented, they are those of the authors, not of the Sea Grant College Program, which does not take stands on issues.

related publications

DEVELOPMENT OF BACTERINS AND VACCINES FOR CONTROL OF INFECTIOUS DISEASES IN FISH. J.L. Fryer, et al. 1977 10 pp. $2.50 (ORESU-T-77-012)

Reviews the current status of immunization techniques to control certain infectious fish diseases. Areas covered include licensing and producing vaccines and bacterins; using bacterins and vaccines to control diseases in fishes cultured in fresh, salt, and warm water; testing and licensing of viral vaccines; and the future of immunization for controlling diseases in cultured fishes.

NETARTS BAY CHUM SALMON HATCHERY: AND EXPERIMENT IN OCEAN RANCHING. James Lannan. 1975. 28 pp. (ORESU-H-75-001)

Describes the construction and operation of the OSU Netarts Pilot Chum Salmon Hatchery. Gives special attention to low-cost, low maintenance designs for the weir, incubator and water system. The rationale for extensive mariculture (ocean ranching) is discussed.

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contents

INTRODUCTION

JOHN L. FRYER, Chairman, Department of
   Microbiology, Oregon State University

In recent years, there has been a marked
increase in the number of fish rearing facili-
ties in the United States. Fish culture has
largely been the responsibility of our fed-
eral and state conservation agencies, but
recently, particularly in the Pacific North-
west, an increasing number of private hatch-
eries devoted to the rearing of salmonid
fish have emerged. This has brought about
a massive movement of fish and eggs as these
operations seek to establish stocks, operate
their facilities, and dispose of their
product by release or sale. These develop-
ments have caused the establishment of both
federal and state regulations designed not
to prevent the shipment of fish and eggs,
but to control the spread of infectious
diseases. Of particular concern are the
so-called untreatable diseases such as those
caused by viruses, certain protozoa, and
drug-resistant bacteria.

The purpose of this conference is to bring
together fish culturists, fish health
specialists, and state and federal regulatory
biologists who are concerned with implement-
ing rules that govern transporting fish and
fish eggs. It is our hope that we can
review the rules and regulations concerning
inspection and certification of fish stocks
in a meaningful, understandable manner to
fish culturists. We also hope to explain,
in detail, the procedures required to appro-
priately inspect fish or eggs destined for
transport.

To our knowledge, this is the first meet-
ing of its type in the United States where
all three segments involved in this important
endeavor have been brought together. After
presenting the rules and regulations, as
well as the procedures involved in inspect-
ing and certifying fish stocks, we hope that
an open, free discussion will prevail that
will lead to a deeper understanding of the
problems involved and that the best course
of action to deal with them will emerge.

Part I Review of fish disease certification and inspection

REVIEW OF INTERNATIONAL REGULATIONS
CONCERNING FISH HEALTH

JOHN ROHOVEC, Assistant Professor, Department
of Microbiology, Oregon State University

Damage from communicable fish diseases,
whether endemic or imported, presents many
problems to private and governmental fish
culturists, and to those concerned with
maintaining wild fish stocks for commercial
and recreational use.

The damage, already severe in many coun-
tries, can easily increase with a growing
interest in aquaculture. The principal at-
tempts to control communicable fish diseases
have been through drug treatments after
recognizing the disease; preventive drug
applications or immunization; selective
breeding for disease resistance; disinfecting
water supplies and rearing facilities; and
import restrictions, which include require-
ments for disease-free certification and
prohibition of importing particular species.

Several international organizations, in-
cluding the International Office of Epizootics
(OIE), the European Inland Fisheries Advisory
Commission (EIFAC), and the Food and Agri-
culture Organization (FAO) of the United
Nations have separately and together studied
fish disease problems associated with inter-
national transfers of live fish and eggs
since 1960.

In 1968, under the auspices of the EIFAC/
FAO, laws and regulations governing the in-
ternational traffic of live fish and fish
eggs were surveyed. Although it is not
within the scope of this presentation to
describe each country's regulations, some
general comments will be made.

Questionnaires were sent to 117 countries
and representatives from 86 replied. Thirty-
eight of the responding countries had no
laws pertaining to fish health; however,
it was indicated that some of these countries
were, and are, in the process of formulating
regulations.

The legislation of the 48 countries exer-
cising some form of health control over
traffic in live fish and fish eggs takes

various forms. Generally, countries have legislation applicable to aspects of internal traffic, imports and/or exports. In most of the countries surveyed, the governmental department responsible for fisheries or agriculture regulates traffic in live fish or eggs. Of course, there are exceptions to this generality.

The nature of fish health or sanitary inspections exercised also varies. Inspections may only be visual, or may require disease-free certification of the fish or eggs; make sanitary and environmental requirements of the containers used for transport; and also demand quarantine measures.

Twelve countries surveyed apply quarantine measures. Quarantine periods range from 24 hours to 15 days, and are enforced in various situations.

Some countries totally or partially prohibit traffic in some or all species of fish. For example, Denmark prohibits all imports and applies a voluntary system of control over exports. Finland adopts a similar prohibition of imports with exceptions where justified. Other countries prohibit certain species. Ireland and the United Kingdom do not allow the import of live salmonids. New Zealand prohibits importing trout and salmon, and tropical fish that could breed in its territory.

While some countries that import and export fish have no regulations, at least one country which does not traffic in fish, Gabon, has laws to deal with that eventuality.

There is no uniformity regarding the nature of the controls, and also the extent that they are exercised. Relevant legislation may exist on the statute books, but may be partially implemented or not at all. Many cases exist where the legislation has not been conceived with controlling traffic of live fish and fish eggs in mind, but was originally intended to cover traffic in other animals and animal products. At best, such legislation stipulates control measures ill-adapted, or only partially applicable to fish.

In view of the disparity of regulations among countries surveyed, the EIFAC recommended that a uniform system of health control for international traffic of live fish and eggs be established. For practical reasons, it was advised that a solution be limited initially to European countries. The reasons for this were a similar degree

of economic development; a widespread practice in fish culture with similar species of fish; and a restricted land area with an already highly developed system of land, sea and air traffic control. In addition, most European countries belong to the EIFAC, and any solution agreed on could be implemented under the aegis of this commission.

Following this suggestion, numerous symposia have been held in Europe concerning this matter, and although no international regulations have been implemented to be followed by all European countries, advances in this direction have been made. At the outset, a consensus was required on certain basic points before any effective system of control could be designed and initiated. Some of these basic points included the species of fish to control, the diseases needing control, and the methods to diagnose diseases. It was suggested that any international control system should initially cover only live fish and fish eggs and exclude frozen fish and ornamentals, though leaving open the possibility of extending the system at a later date to cover them.

It appeared impractical that all international trade in fish and fish eggs infected by any disease be prohibited. Not all fish diseases are well enough known, and not all present the same dangers. Some diseases may concern one importing country and not another. However, there was basic agreement that the following six diseases should be regulated in Europe: viral hemorrhagic septicemia (VHS), infectious pancreatic necrosis (IPN), infectious hematopoietic necrosis (IHN), spring viremia of cyprinids (SVC), whirling disease and furunculosis. But it was also decided that any international control system should be flexible enough to constantly revise the list of regulated diseases. Diagnostic methods are available for the six diseases listed, and the commission accepted these techniques.

Possible methods to control diseases in transferred stocks have also been considered. Quarantining imported fish and inspecting individual consignments at the point of importation or exportation without quarantine were rejected as being perhaps a supplementary tool in certain cases, but not providing practical methods of inspection. It has been suggested that certification requirements be based on source inspections at the originating fish culture establishment. This would entail periodic inspections made at the exporting fish cultural facilities, and the maintenance of a disease case history

of the facility. Easily planned source inspections eliminate logistic and time constraints apparent in quarantines and frontier inspections of individual consignments. Source inspections would allocate manpower and facilities necessary for disease inspection more efficiently. From this type of inspection, it was also proposed that fish health could be classified with relation to diseases, and particularly to the fish rearing conditions. These classifications would be similar to those adopted for other cultivated animals such as swine and poultry. The classifications would include Specific Pathogen Free (SPF); Coded Pathogen Free (CPF); Specific Disease Free (SDF); and Non-Controlled. SPF status would require fish to be free of all species-specific pathogens, and therefore raised in an establishment which rigorously controls the water supply. At present, this classification is only theoretical. CPF animals would be free of all diseases that were listed by international agreement. The water in the facility could not be contaminated by restocking or from wild fish. Some salmon hatcheries exist with this classification.

Rearing establishments could be classified SDF, if supplied with water in which some pathogens could exist, but free from certain specified disease agents. For example, furunculosis could be present, but the hatchery could be classified as free of IPN.

The European Federation of Salmon Breeders has expressed trepidation over some regulations proposed and implemented for disease control. According to this organization it would be highly suitable for each country to study disease eradication programs using coded and specific disease-free fish. The organization also believes that an information service should be established to collect data concerning fish farms registered in such a manner. Such a program has been successful in Denmark. Since 1965, efforts to eradicate VHS and IPN have been ongoing. At the outset the program was voluntary and involved the emptying, disinfecting, and restocking of farms. In 1969 an act passed by parliament supported this voluntary effort for eradicating VHS and at this time there are now 380 fish rearing establishments registered VHS free.

In France, as well as Denmark, the attitude has been adopted that whole sectors of a watershed feeding a CPF or SPF establishment should be placed under sanitary protection. Restocking or contaminating these waterways with fish not classified the same or better is forbidden.

It has also been suggested that an important part of any disease control is a continual awareness of the total disease situation, nationally and internationally. It has been proposed that some reporting system be established to provide accurate information on morbidity and mortality rates, geographical distribution, seasonal incidence, and rate of dissemination of diseases. A central registry could review reports, and then distribute information on the location and nature of epizootics to appropriate recipients.

Even though a willingness to accept that long-range benefits to be gained from disease control exist, there is fear of immediate economic losses. These may range from loss of market, at least temporarily, to increased fish rearing costs, broodstock destruction, water supply modification, medical treatments, inspections and certifications, special transport precautions, and possible quarantines. All may be difficult and costly for government and private fish culturists. However, it has been pointed out that even a handful of diseased fish may be sufficient to destroy an entire hatchery's stock or infect the stocks of an entire river system. In defining losses from fish disease one must consider the cost of replacing stocks, costs of eradicating diseases or containment, loss of markets to competitors, and other aspects.

Obviously not all disseminated fish disease losses can be attributed to international trade. But international organizations studying this problem have basically agreed that a need for an equitable system of international control exists.

REVIEW OF NORTH AMERICAN FISH DISEASE CONTROL REGULATIONS

ROBERT E. OLSON, Assistant Professor, Department of Zoology, Marine Science Center, Oregon State University

A myriad of diseases accompanied the expansion of salmonid and other fish culture in North America. They have caused significant losses on numerous occasions. Attempts to control fish diseases have included research on the diseases and methods for their control, and, in recent years, both voluntary and government-imposed regulations.

An event that had a strong influence on the development of governmental controls at all levels was the introduction of whirling disease into this country from Europe. For this reason, I will briefly discuss the history of the dissemination of *Myxosoma cerebralis*, the myxosporidan parasite that causes whirling disease.

It is considered that this parasite has been present in Europe for a long period of time, and that it occurs in brown trout *(Salmo trutta)* there without causing apparent disease. However, when rainbow trout *(Salmo gairdneri)* were exported from the U.S. to Germany in about 1900, the rainbow trout became infected and whirling disease resulted.

The disease most severely affects small trout, which may die if infections are heavy. The parasite invades the cartilage, erodes it, and weakens the skeletal structure. When the cartilaginous auditory-equilibrium capsule weakens, the fish loses its orientation and goes into an erratic, tail-chasing whirl. Damage to the vertebral column causes a blackened tail, and pinches caudal nerves. Damage to the head skeleton causes misshapen heads.

From 1903 to 1952 whirling disease was known only in rainbow trout from Germany, France and Denmark. During World War II, live rainbow trout were transferred freely in Europe, and later a market for frozen table trout was established that reached other continents including North America. This allowed whirling disease to spread into

many areas worldwide.

Some countries that now control the import of salmonids have avoided whirling disease. These countries include Australia, Japan and England.

In the United States, the disease has been found in Pennsylvania, Connecticut, Virginia, West Virginia, New Jersey, Ohio, Michigan, Nevada, California and Massachusetts. It has caused major problems in several of these areas and thousands of fish have been destroyed in an attempt to prevent it from spreading.

Stimulated by the experience with whirling disease, a federal law, Title 50, was amended in 1958 to prevent importing salmonids and salmonid eggs unless they are determined free of whirling disease and viral hemorrhagic septicemia (VHS). VHS, a viral disease of salmonids in Europe, has so far not appeared in North America.

Individual states have also developed fish disease control regulations, and the results of a survey conducted in 1978 indicate that 30 of the 45 states responding have some form of regulation.

In 1970, Roger Herman conducted a similar survey and found that 14 of 45 states responding had regulations pertaining to fish disease control. So the trend is toward increasing governmental regulation at least at the state level.

The purpose of these regulations is to prevent contact between a disease agent and potential hosts to confine fish disease agents to their present ranges. Erradicating a disease once it is present in an area is much more difficult to achieve. Eradication usually succeeds only under optimal conditions and where the water supply can be completely controlled.

Other than the Title 50 amendment, attempts to pass federal laws that provide uniform fish disease control in the United States have not succeeded. The reasons are complex, but one aspect is the extreme heterogeneity of United States fish culture depending on the geographic area. Another is that those working in the fish health area cannot agree among themselves on how to best control fish diseases, and which diseases to control.

It has been somewhat easier to develop fish disease control policies on a regional basis. At least two regional policies

exist in the United States. They are the Colorado River Drainage Fish Disease Control Policy implemented in 1973 and the Great Lakes Fishery Commission Fish Disease Control Policy.

Also hindering the development of rational fish disease control regulations has been an absence of methods to detect certain fish pathogens, and agreement on the standard methods to use when a variety of methods become available.

Great progress has been made toward solving the standard methods problem, and two publications, now available, address themselves specifically to that topic. These are *Suggested Procedures for the Detection and Identification of Certain Infectious Diseases of Fishes* published in 1975 by the Fish Health Section of the American Fisheries Society, and the *Canadian Fish Health Protection Regulations Manual of Compliance* developed under the authority of the Fisheries Act of Canada. These two publications now comprise what many consider the standard methods to be used when determining the statistical absence of certain fish pathogens. Nevertheless, improved diagnostic techniques are still needed to gain precision and speed in diagnosing several fish diseases.

As mentioned previously, the seven states with waters in the Colorado River drainage agreed to a fish disease control policy in 1973. They are Arizona, California, Colorado, Nevada, New Mexico, Utah and Wyoming. The policy applies only to the waters within those states that drain into the Colorado River and provides that the responsible agency in each state will make every reasonable effort to prevent the introduction of the following fish diseases into the drainage system:

1. IHN (Infectious Hematopoietic Necrosis)
2. VHS (Viral Hemorrhagic Septicemia)
3. CCV (Channel Catfish Virus)
4. Whirling Disease (*Myxosoma cerebralis*)
5. Ceratomyxosis (*Ceratomyxa shasta*)
6. Bacterial Kidney Disease
7. European gill rot (*Branchiomyces* sp.)
8. Blood fluke of salmonids (*Sanguinicola* sp.)

To carry out this policy, a certifying team of fish disease specialists, that the states and the federal government identified are authorized to inspect fish culture facilities in the Colorado drainage, or facilities that plant fish into the drainage system.

The fish disease control policy provides

that:
  1.   Before any fish cultural station may
stock game fish or conduct fish cultural
activities in the drainage system, the sta-
tion must be certified free of the pathogens
listed in the policy.

  2.   When fish cultural stations experi-
ence significant fish losses and have fish
showing clinical symptoms of any disease,
it cannot plant fish into the drainage
system until the disease problems are
solved and the station reinspected.

  3.   All game fish that federal, state
and private fish cultural facilities plant
into the Colorado River drainage system
shall be free of the diseases or the patho-
gens inducing the diseases listed in the
policy.

  Unlike the Colorado River drainage fish
disease control policy, the Great Lakes
Fishery Commission fish disease control
efforts were not formally adopted by the
responsible agencies in the member states.
Rather, the Great Lakes policy is followed
voluntarily and attempts more to coordinate
than regulate.

  All states with waters draining into the
Great Lakes are members, but the interest
in fish disease control varies with the ex-
tent of fisheries involvement in a particu-
lar state.   For example, Illinois and
Indiana have limited Great Lakes shoreline
and so have less concern than some of the
other states.

  In general, voluntary agreements and peer
pressure instead of formal regulations ac-
complish fish disease control in the Great
Lakes.   Diseases considered certifiable by
this group include VHS, IHN, whirling
disease, ceratomyxosis and enteric
redmouth disease.   Reportable diseases are
infectious pancreatic necrosis, furunculo-
sis and bacterial kidney disease.

  A fish disease control program of even
larger scope is the Canadian Fish Disease
Control Policy which applies to all of
Canada.   On January 1, 1977, the Fish
Health Protection Regulations came into
force under the authority of the Fisheries
Act of Canada.   A manual of compliance was
published to explain the application of
this national policy.   The manual presents
guidelines for producers, defines the role
of fish health officials and local fish
health officers, and outlines the sampling,
handling, and diagnostic procedures that
constitute inspections leading to certifi-
cation.

  The design of the Fish Health Protection
Regulations prevents the spread of infectious
diseases by inspecting production sources of
fish stocks, and controlling the movement of
infected fish stocks.   The regulations apply
to members of the Salmonidae family and in-
clude live cultured fish, fish eggs and
dead products of cultured fish destined to
move into Canada or across provincial
boundaries within Canada.

  A certificate allowing transport of sal-
monids, salmonid eggs, and dead salmonid
products will be issued if the following
conditions are met:

  1.   Live cultured salmonids and eggs must
come from a source free of VHS, IHN, IPN,
*Myxosoma cerebralis, Ceratomyxa shasta,
Aeromonas salmonicida, Corynebacterium
salmoninus* and *Yersinia ruckeri.*

  2.   Eggs of wild fish must be taken from
fish free of those disease agents listed
above.

  3.   Dead cultured fish must come from a
source free of VHS and *Myxosoma cerebralis.*

  In addition, the following disease agents
must be reported if found: Myxobacteria,
motile *Aeromonas* spp., *Pseudomonas* spp.,
and *Vibrio* spp.   The presence of these
latter agents does not automatically prevent
certification, but it may, at the discretion
of the local fish health officer.

  All production sources must be certified
free of the agents in the list of certifi-
able diseases based on annual inspections
through January 1, 1980.   After that date,
consecutive satisfactory inspections must
be conducted at approximately six-month
intervals for a production source to retain
its certified status.

  Although a comparable program does not
exist at the national level in the United
States, the U.S. Fish and Wildlife Service
does have its own fish health policy that
applies to the national fish hatchery
system.

  This policy aims toward the development
of disease-free broodstock; the inclusion
of fish disease considerations in fish stock-
ing activities; fish disease monitoring
programs of all federal hatchery and research
facilities; and to provide leadership,
competence, training and research in the
development of improved fish health.

  As a result of this policy, all shipments

from and to national fish hatcheries must have a disease statement indicating the presence or absence of all certifiable diseases. The diseases in this category according to the U.S. Fish and Wildlife Service are caused by the viruses VHS, IHN and IPN; the myxosporidan, *Myxosoma cerebralis*; Bacterial Kidney Disease; and Enteric Redmouth Disease. Other disease agents that are considered certifiable in suspect areas, areas in which the agents are known to occur, include *Ceratomyxa shasta* and *Herpes virus, salmonis*.

Fish disease control regulations in the individual states vary from no regulation of any kind to well-defined regulations. The latter state precisely which disease agents will not be allowed into the state, and spell out the steps that must be taken to obtain an import permit. Between these extremes are many variations on the fish disease control theme that make regulations difficult to summarize on a national basis. Nevertheless, I have attempted to do so in the table that was handed out to you (Table I). The information in it is obviously an oversimplification, and each state's regulations should be examined individually in order to fully understand them. The summary is based on information we received after writing to the responsible agency in each of the 50 states. Five states did not respond (Table II). Of the 44 that did respond, 10 have no regulations pertaining to fish disease control (Table II).

Five states indicated that an import permit was required, but an inspection for fish diseases was not included in the regulations (Table III). In these states, the purpose of the import permit requirement was usually to prevent the introduction of exotic fish species into the state and not to prevent or control fish diseases.

Twelve states require, or have the authority to require, import permits, and have regulations that mention some form of fish disease inspection (Table III), but they do not list specific diseases for which the fish must be examined. This aspect is apparently left to the discretion of the responsible agency.

Eighteen states have import regulations and disease inspection requirements that mention specific diseases that must be absent before transport can be approved (Table IV). These 18 states include the seven members of the Colorado River Drainage Fish Disease Control group. Of these, Arizona and Nevada have regulations for non-

Colorado River drainage waters that are less stringent than the regional policy. Colorado and Wyoming have regulations that are more stringent than the regional fish disease control policy. The less stringent Arizona and Nevada regulations do not mention specific diseases, and in the case of Nevada, do not mention disease inspection. Both Colorado and Wyoming require inspection for more disease agents than the regional policy requires.

Thirteen diseases or disease agents are listed by the 18 states that mention specific agents in their regulations. The most listed by any individual is 11. Both Colorado and Wyoming mention this number.

The only diseases included in the lists of all 18 states are viral hemorrhagic septicemia (VHS) and whirling disease (Table V).

It is apparent that there is still a distance to go in the development of fish disease control regulations that are effective yet not so restrictive that they eliminate many fish culture operations. Research on the biology of fish disease agents, and on methods of diagnosis and control, is still a very important ingredient in developing the understanding necessary to both protect and foster fish culture in this country.

Table 1. Summary of Fish Disease Control Regulation
and Inspection in the United States

State	Importa-tion permit	Disease Inspec-tion	IPN	IHN	VHS	Myxosoma cerebralis	Cerato-myxa shasta	Corynebac-terium salmoninus	Yersinia ruckeri	Aeromonas salmonicida	Branch-iomyces	Sanguin-icola	Other
Ala.	NO	NO	-	-	-	-	-	-	-	-	-	-	-
Alaska	YES	YES	Specific Diseases Not Mentioned										
Ariz.*	YES	YES	Specific Diseases Not Mentioned										
Ark.	NO	NO	-	-	-	-	-	-	-	-	-	-	-
Cal.*	YES	YES	X	X	X	X	-	-	-	-	-	-	
Col.*	YES	YES	X	X	X	X	X	X	-	X	X	X	CCV Vibro spp.
Conn. (Effect. 1979)	YES	YES	X	X	X	X	X	X	X	-	-	-	UDN*
Del.	NO	NO											
Flor.	NO	NO											
Geor.	YES	YES	Specific Diseases Not Mentioned										
Hawaii	YES	?	(Commercial Suppliers of state hatcheries must supply virus-free certification)										
Idaho	NO	NO											
Ill.	YES	YES	Specific Diseases Not Mentioned										

Table 1 Continued

State	Importation permit	Disease Inspection	IPN	IHN	VHS	Myxosoma cerebralis	Ceratomyxa shasta	Corynebacterium salmoninus	Yersinia ruckeri	Aeromonas salmonicida	Branchiomyces	Sanguinicola	Other
Ind.	YES	YES	Specific Diseases Not Mentioned										
Iowa	NO	NO											
Kent.	No Information												
Louis.	YES	NO											
Maine	YES	YES	X	X	X		-	-	-	-	-	-	-
Maryl.	YES	YES	Specific Diseases Not Mentioned										
Mass.	YES	YES	Specific Diseases Not Mentioned										
Mich.	YES	YES	X	X	X	X	-	-	X	-	-	-	-
Minn.	NO	NO											
Miss.	No Information												
Misso.	YES	YES	X	-	X	X	-	-	-	-	-	-	
Mont.	YES	YES	X	X	X	X	X	X	-	X	-	-	
Neb.	YES	YES	X	-	X	X	-	-	-	-	-	-	CCV
Nev.*	YES	NO											
N.H.	YES	NO											
N.J.	YES	NO											
N.M.	YES	YES	-	X	X	X	X	X	-	-	X	X	CCV
N.Y.	YES	YES											
N.C.	YES	YES											

* Currently Applies Only To State Hatcheries

Regulations Currently under Review

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Table 1 Continued

State	Importation permit	Disease Inspection	IPN	IHN	VHS	Myxosoma cerebralis	Ceratomyxa shasta	Corynebacterium salmoninus	Yersinia ruckeri	Aeromonas salmonicida	Branchiomyces	Sanguinicola	Other
N.D.	YES	YES	Specific Diseases Not Mentioned										
Ohio	YES	NO											
Okla.	YES	NO											
Oreg.	YES	YES	X	X	X	Regulations Currently Under Review							
Penn.	YES	YES	Specific Diseases Not Mentioned										
R.I.	NO	NO											
S.C.	No Information												
S.D.	YES	YES	X	-	X	X	-	-	-	-	-	-	-
Tenn.	No Information												
Texas	NO	NO											
Utah*	YES	YES	X	X	X	X	X	X	-	-	X	X	CCV
Verm.	YES	YES	X	X	X	X	-	-	-	-	-	-	-
Virg.	No Information												
Wash.	YES	YES	Specific Diseases Not Mentioned										
W.V.	YES	YES	X	X	X	X	-	-	-	-	-	-	-
Wisc.	YES	YES	-	X	X	X	X	-	-	X	-	-	-
Wyom.*	YES	YES	X	X	X	X	X	X	X	X	X	X	CCV

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Table 1 Continued

State	Importa- taion permit	Disease Inspec- tion	IPN	IHN	VHS	*Myxosoma cerebralis*	*Cerato- myxa shasta*	*Corynebac- terium salmoninus*	*Yersinia ruckeri*	*Aeromonas salmonicida*	Branch- iomyces	*Sanguin- icola*	Other
Colo- rado River Dis. Con. Pol.	YES	YES	-	X	X	X	X	X	-	-	X	X	CCV

*These states adhere to the Colorado River Drainage Fish Disease Control Policy which applies to the Colorado River Drainage System, but not to other waters in the several states.

Abbreviations: IPN - Infectious Pancreatic Necrosis Virus

IHN - Infectious Hematopoietic Necrosis Virus

VHS - Viral Hemorrhagic Septicemia

CCV - Channel Catfish Virus

UDN - Ulcerative Dermal Necrosis

States not responding to request for
fish disease control information:

Kentucky, Mississippi, South Carolina,
Tennessee, Virginia

States that have no regulations per-
taining to fish disease control:

Alabama, Arkansas, Delaware, Florida,
Idaho*, Iowa, Kansas, Minnesota,
Rhode Island, Texas

*Suppliers of Idaho state hatcheries
must be certified virus free.

Table 2.

States that require importation
permits and documentation that
specific fish diseases or agents
are absent:

Arizona*, California*, Colorado*,
Connecticut, Maine, Michigan,
Missouri, Montana, Nebraska,
Nevada*, New Mexico*, Oregon,
South Dakota, Utah*, Vermont,
West Virginia, Wisconsin,
Wyoming*

*Members of Colorado River Drainage
Fish Disease Control group.
Regulations for non-Colorado
River drainage waters in these
states vary from the CRD Fish
Disease Control Policy.

Table 4.

States that require a fish importation
permit, but do not include inspection
for fish diseases in their regulations:

Louisiana, New Hampshire, New Jersey,
Ohio, Oklahoma

States that require or have the
authority to require importation
permits and have regulations that
mention some form of disease inspec-
tion, but do not indicate specific
disease agents:

Alaska, Georgia, Hawaii, Illinois,
Indiana, Maryland, Massachusetts,
New York, North Carolina, North
Dakota, Pennsylvania, Washington

Table 3.

Disease or Disease Agent	Number of states mention-ing disease agent in their regula-tions
VHS	18
Whirling Disease (Myxosoma cerebralis)	18
IPN	14
IHN	13
Vibrio spp.	1
Bacterial Kidney	8
Enteric Redmouth	4
Ceratomyxosis (Ceratomyxa shasta)	9
Furunculosis (Aeromonas salmonicide)	3
Salmonid Blood Fluke (Sanguinicola sp.)	6
European Gill Rot (Branchiomyces sp.)	5
Channel Catfish Virus (CCV)	6
Ulcerative Dermal Necrosis (UDN)	1

Table 5.

FISH DISEASE GUIDELINES FOR THE PRODUCERS OF
CULTIVATED FISH IN OREGON

EARL PULFORD, Fish and Wildlife Program
Manager for the Oregon Department of
Fish and Wildlife, announced the depart-
ment's tentative guidelines for fish
disease certification and transport.
The following is a copy of the guidelines
that the department provided.

PURPOSE

Cultivation and movement of fish is
rapidly increasing in Oregon. The dissemi-
nation of fish diseases in and to all parts
of the state is now a serious problem. To
meet this problem, the Oregon Department of
Fish and Wildlife (ODFW) has adopted admini-
strative rules aimed at disease control.
The purpose of the following is to provide
guidelines for all producers of fish, both
state and private, to follow for the control
of fish diseases.

GENERAL REQUIREMENTS (All Hatcheries and
Fish Rearing Facilities)

A. Disease examinations must be conducted by
a qualified pathologist acceptable to
ODFW. A list of acceptable pathologists
is available upon request.

B. Fish or eggs will not be moved from one
major watershed to another within the
state unless authorized by ODFW. This
authorization may require a disease exami-
nation for certifiable diseases and/or
the history of the facility and watershed.
involved.

C. In order to prevent the transmission of a
certifiable disease by water, any fish
authorized to be transported from a
station with a history of a certifiable
disease must be hauled in water from a
source approved by ODFW.

D. Water used to sanitize equipment, and/or
facilities, must be disposed of in a
manner that no harmful concentrations of
sanitizing agents will directly reach
other waters.

E. Any live fish or eggs originating outside of the United States must be cleared by the United States Fish and Wildlife Service (Title 50 requirement) in addition to approval from ODFW.

F. All live fish or eggs transported by private parties into or within Oregon must be accompanied by a fish transport permit, aquaria use excepted. (ORS 498.222)

1. Requests to import live fish or eggs will not be accepted unless accompanied by a disease inspection report certified by a pathologist acceptable to ODFW except when the fish are being transported to a quarantine facility authorized by ODFW (transport authorization required). Inspection for virus must be completed prior to removal from the quarantine facility.

2. The intended use of the fish (pond rearing, sale, ocean ranching, research, etc.) must be shown on the application for a transport permit along with information on species, size, number, brood, and identifying lot number.

3. The transport request becomes a transport and release permit when signed by an authorized ODFW employee and allows stocking in the water designated on the request permit (ORS 498.222). Release of salmon under an ocean ranching permit must have a separate release authorization (ORS 508.715).

4. An ODFW fish truck delivery slip will be issued for all fish hauled from state facilities. This truck slip will be used in lieu of the fish transport permit in such cases. Eggs must be accompanied by an egg shipment report.

G. No disease inspection certification may be accepted for transport of live fish or eggs unless it has been accomplished within a reasonable length of time, but not to exceed one calendar year or the next (ensuing) egg take, whichever comes first (except there must be a satisfactory IHN examination of parent fish). Such certification must include the lot number and brood year of each group of fish being certified by that particular examination. Separate lot numbers must be used to identify different spawning and age groups within a facility.

1. ODFW pathologists may examine fish for private growers for certification if requests for such services are submitted 30 days in advance. Costs (labor, travel, and expenses) will be paid by the requesting person or company (OAR 635-07-180).

2. Fish in private hatcheries may be examined at any reasonable time by state pathologists after due notification. No charge will be made by ODFW for examinations made at the discretion of the ODFW (OAR 635-07-185).

SPECIAL REQUIREMENTS (Additional to the General Requirements)

I. *Salmonids*

A. Disease Categories
ODFW recognizes the threat of certain infectious diseases and further categorizes such diseases as follows:

1. Emergency Diseases:
   a. Whirling disease caused by *Myxosoma cerebralis*.
   b. Viral Hemorrhagic Septicemia (VHS).

2. Certifiable Diseases: (Those for which examinations must be conducted.)
   a. Emergency diseases listed in 1 above.
   b. Infectious Hematopoietic Necrosis (IHN).
   c. Infectious Pancreatic Necrosis (IPN).
   d. Ceratomyxosis caused by *Ceratomyxa shasta*.
   e. Enteric Redmouth (ERM) *Yersinia ruckeri*.
   f. Bacterial Kidney Disease (BKD).
   g. Furunculosis *(Aeromonas salmonicida)*.

3. Reportable diseases: Must be reported to ODFW.
   a. All diseases listed in 1 or 2 above.
   b. *Ichthyopthirius*
   c. *Costia*
   d. Copepods: *Lernaea Salmincola*
   e. Drug-resistant strains of:
      Motile aeromonads
      Pseudomonads
      Flexibacter
      Cytophaga
   f. Piscine Erythrocytic Necrosis (PEN)

g. *Herpesvirus salmonis*
h. Other diseases as determined by ODFW.

B. Specific Requirements

1. Those diseases in 1 (Emergency Diseases) are so categorized because (a) they are catastrophic diseases; (b) there is no known treatment for them; and (c) they have never been diagnosed as occurring in Oregon. Consequently, if one of the emergency diseases is diagnosed it must be promptly and effectively eradicated by destruction of the stocks involved together with disposal and sanitation according to procedures approved by ODFW.

2. Salmonids intended for import into the state must be examined for all Certifiable Diseases listed above according to procedures acceptable to the Fish Health Section of the American Fisheries Society; or modifications of these procedures approved by ODFW.

3. Eggs imported into the state must be from parents certified to be free of viruses causing VHS, IHN, IPN, and the protozoan *Myxosoma cerebralis*. All eggs shall be disinfected with the chemical Wescodyne, or equivalent, upon receipt, using approved procedures.

II. *Nonsalmonid Game Fish*

A. General Requirements

1. The supplier must submit a statement of the complete disease history of the shipping station, and, in addition, other appropriate fish disease examinations may be required.

2. Based on the disease history of a hatchery, treatment prior to transfer may be required. Type of treatment and schedule will be determined by ODFW after consultation with the supplier.

3. Stocks of fish within Oregon should be used in preference to importation from other sources. Brown bullhead *(Ictalurus nebulosus)*, yellow bullhead *(I. natulus)*, and black bullhead *(I. melas)* will not be imported.

4. Warm water species may not be transferred to certain state waters. Information on specific restrictions will be made available on request.

5. Waters which have not had bass populations, or been exposed to the bass tapeworm *(Proteocephalus amphloplitis)*, may only be stocked with bass tapeworm-free stock.

B. Specific Requirements

1. Only channel catfish from California known to be free of channel catfish virus will be considered for import.
2. All channel catfish must be free of *Henneguya exilis*.
3. European carp *(Cyrpinis carpio)* must be free of *Rhabdovirus carpio*.
4. All species of fish included in this section must be free of *Branchiomyces sanguinis*.
5. Requests for other species will be reviewed on an individual basis.

III. *Nongame Species*(Except aquaria fish but including mollusks and crustaceans)

All introductions or proposals to import nongame species will be reviewed on a case-by-case and point-of-planting, or use, basis. Except:

A. No *Ctenopharyngodon idella*, grass carp may be imported into or planted into waters of the state.

B. No species of walking catfish, family Clariidae, or piranha subfamily Serrasalminae of the family Characinadae, shall be imported, held in possession, or released into the waters of the state except as provided by ORS 498.242.

Oregon Department of Fish and Wildlife, December 5, 1978
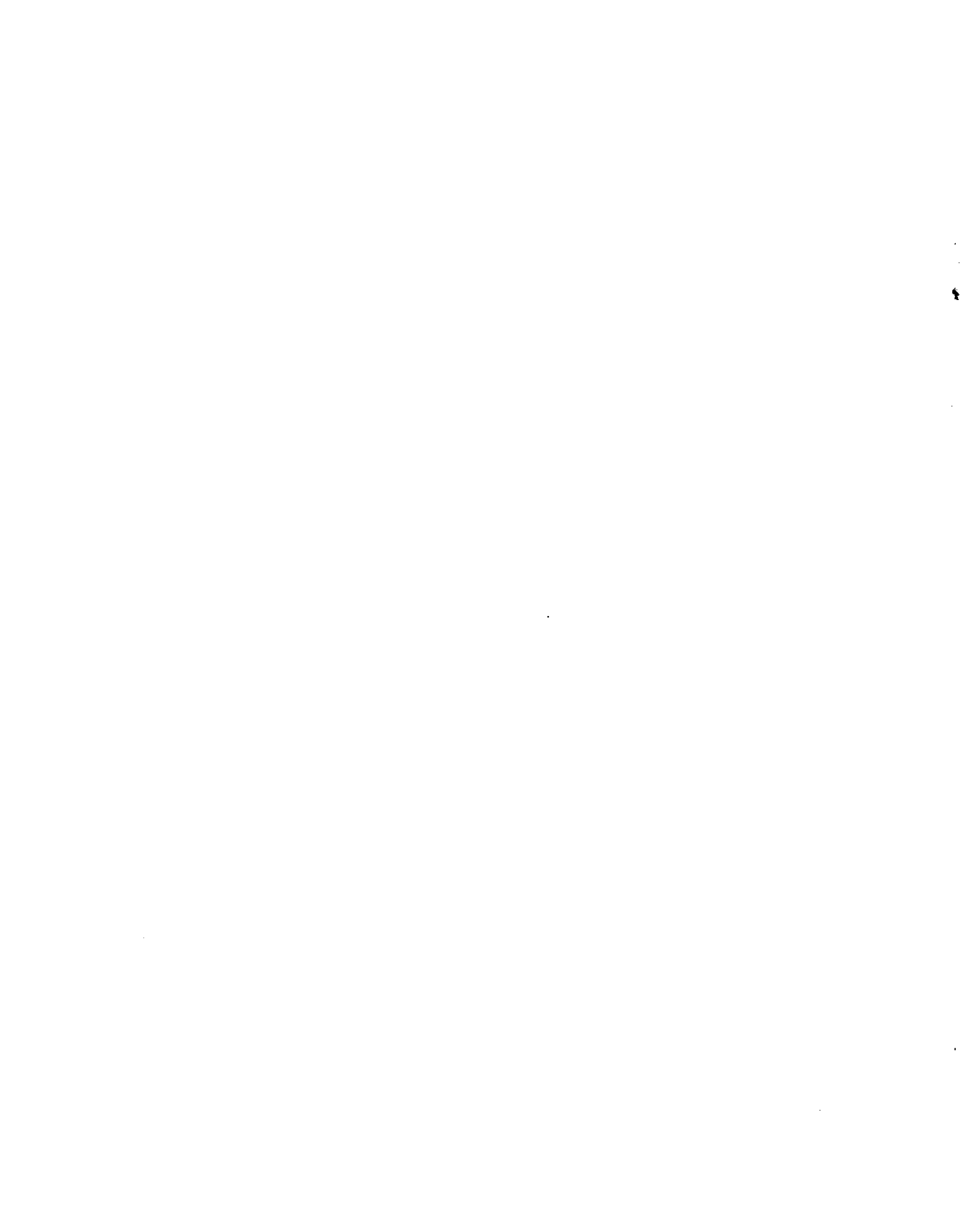
Part II Inspection techniques and problems

SAMPLING AND NECROPSY OF FISH FOR PARASITIC
AND BACTERIAL DISEASES

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   Department of Microbiology at Oregon State
   University, and presently Quality Control
   Manager for Oregon Aquafoods

A fish disease specialist diagnoses the cause of an infection, or certifies eggs and fish. When a fish culturist requires a disease specialist, the culturist often has a limited understanding of what the specialist requires regarding samples, and what he actually does with those samples. This presentation, and those that follow, will attempt to clarify some aspects of disease diagnosis and certification.

Culturists frequently ask how much time it takes this specialist to diagnose a disease or certify fish and eggs. The time necessary to complete an examination varies with the type of tests required, and also with the disease agents involved.

A disease diagnosis can be made much more rapidly than a certification. This is, in part, because sick fish contain disease-causing agents in relatively high numbers that the specialist can readily isolate and identify.

When certifying fish, the disease agents are usually present in very small numbers making their detection more difficult. Some protozoan diseases can be diagnosed very rapidly by microscopic observation of infected tissue, a process which takes just . a few minutes. However, it takes several days, sometimes weeks, and more sophisticated techniques before viruses can be detected and identified.

When a culturist submits fish for disease diagnosis, there is some information which is helpful to the specialist. This information includes:

1. A detailed description of disease symptoms.

2. A disease history of the facility and stock of fish.

3. Any possible stress conditions that

the animals may have encountered.

4. Any recent changes in the normal routine of the hatchery.

5. Other data including mortality rate, water temperature, diet and fish lot number.

Each of these helps the diagnostician identify the causative agent of the disease.

Fish culturists can also help disease specialists make a more rapid and accurate diagnosis by supplying appropriate samples for inspection. Samples of live, moribund and dead fish are required, as well as fish from adjacent ponds. All of these should be received at the laboratory fresh, cold and on ice, but not frozen.

Moribund fish are usually most useful for diagnostic purposes, because these nearly dead fish are infected with a maximum number of pathogens. Dead fish are also examined, but are sometimes not as useful because secondary infections and autolysis can occur. Examining live fish gives information on the severity of a disease outbreak. From these samples, it is possible to determine whether the infection is spreading from dead and moribund fish to ones that show no disease symptoms. Examining fish from other ponds within the facility indicates if other groups of fish have become infected.

To examine fish in the samples, the fish disease specialist performs a necropsy, or postmortem examination. After the fish has been sacrificed, blood smears are made for microscopic examination. These stained smears can reveal the presence of blood parasites, bacteria if they are present in very large numbers and piscine erythrocytic necrosis (PEN) inclusions.

The specialist next examines the fish for any gross external symptoms. These symptoms may include hemorrhages at the base of fins, on the body surface or around the operculum; inflammation of the vent; and frayed fins. The gills are also examined for any gross pathology. For microscopic observation, wet mounts of fin and gill pieces, and body scrapings are made. This is done by placing a drop of water on a microscope slide, adding the tissue to be studied and placing a cover slip over the preparation. Microscopic observation of these wet mounts are helpful in the diagnosis of external parasites including costia, trichodina, and ichthyopthirius; fungus infections; and myxobacteria, which cause gill disease or columnaris disease. A bacteriological

medium is inoculated with tissue from the gills so that any pathogenic bacteria can be detected.

The fish disease specialist begins the internal examination by aseptically opening the animal to expose the organs in the body cavity. The specialist notes pathological symptoms and uses a sterile loop to transfer a small amount of kidney tissue to bacteriological media for the detection of any systemic bacterial infections. The specialist also prepares kidney tissue wet mounts and stained smears for microscopic examination. The kidney, which filters the blood, is the primary organ used to detect systemic bacterial infections. In healthy fish this organ is sterile, but in a serious bacterial infection, bacteria are present in it.

A variety of myxosporidan parasites can exist in the gall bladder. To detect them the specialist makes and examines wet mounts of this organ. *Ceratomyxa shasta* spores can also be detected in samples of the rectum from fish infected with this parasite, and *Myxosoma cerebralis* can occur in developing cartilage of the head. A subsequent presentation will explain examination procedures for these two parasites in more detail.

Specialists can use histological techniques to detect pathological changes that indicate infectious disease conditions as well as the effects of toxicants and pollutants. Histopathological studies involve imbedding tissue into paraffin wax blocks, followed by the cutting of this tissue into very thin sections 5 to 7 micrometers thick. The sections are placed on glass slides, stained and examined microscopically. These examinations reveal tissue changes which occur as a result of pathological conditions.

This has been a brief description of the samples that a specialist requires for fish disease diagnosis, what is done with those samples, and what can be learned from them. Subsequent presentations will deal more specifically with the identification of selected fish pathogens.

THE DIAGNOSIS AND IDENTIFICATION OF
BACTERIAL FISH PATHOGENS

JIM WINTON, graduate student in the Depart-
    ment of Microbiology, Oregon State Uni-
    versity

A previous paper covered those procedures
referred to as a necropsy. These techniques
comprise the initial stages in making a
disease diagnosis and provide material for
further examination. This presentation will
be brief, and is not intended as a compre-
hensive review or short course in diagnostic
technique. It merely describes how we iso-
late and identify bacterial fish pathogens
at the Oregon State University Fish Disease
Laboratory.

The fish disease specialist relies on a
great deal of diverse information in making
a diagnosis including external signs and
symptoms, the history of the hatchery, geo-
graphic location, water temperature, species
of fish, and the time of year. Very impor-
tant, however, is the isolation of the
bacterial agent from the diseased fish and
the identification of that agent by various
techniques that will be described.

This presentation will review six of the
important bacterial diseases of salmonid
fish, discuss the sampling and diagnostic
procedures that follow the necropsy and lead
to the identification of a bacterial disease
agent, and mention some of the problem areas
in fish disease diagnosis and certification.

SIX REPRESENTATIVE DISEASES

In this section a brief review of six
diseases of salmonid fishes will be presented.
These are not the only bacterial diseases,
but simply examples to illustrate features
such as symptoms, distribution and significant
points in diagnosis for a few of the more
important diseases.

Bacterial Kidney Disease

This disease has been reported from North
America, Scotland, Japan, France and Italy,
and all salmonid fish are considered suscepti-
ble. The primary pathology involves grey-
white pustules in the kidney and exopthalmia,

or "popeye". The disease is often slow and chronic, but eventual mortality can be high. The causative agent is a small, nonmotile, gram-positive diplobacillus recently classified as *Corynebacterium salmoninus*. Diagnosis usually rests on microscopic observation of the organism in smears of kidney tissue, via gram stain, fluorescent antibody test, or in an immunodiffusion test described below.

Furunculosis

This disease is distributed essentially worldwide and all freshwater fish are considered susceptible. It is a generalized septicemia which may result in external lesions and hemorrhage. In other cases, the disease spreads rapidly with heavy mortality and an absence of symptoms. The causative agent is a nonmotile, gram-negative rod classified as *Aeromonas salmonicida*. Diagnosis depends on isolation of the bacterium from the kidney of the diseased fish and identification as *A. salmonicida* by biochemical or serological techniques.

Enteric Redmouth Disease

This disease is normally associated with rainbow trout in North America, although all salmonids are considered susceptible. It causes a general septicema, and one of the observed symptoms is a red hemorrhaged area on the head and mouth. However, symptoms may be more generalized.

A motile, gram-negative rod, which has recently been classified as *Yersinia ruckeri*, causes the disease. The organism is generally recovered from the kidney and biochemical and serological procedures are used to identify it.

Motile Aeromonas Septicemia

This disease is also a generalized septicemia of worldwide distribution found in all freshwater fish. The symptoms resemble other septicemias that gram-negative rods cause, and include hemorrhage and ulceration. In some cases, a rapid form of the disease with few symptoms is observed. The causative agent is a motile, gram-negative rod and most commonly, *Aeromonas hydrophila*. Specialists usually identify it by biochemical methods after isolating it from the kidney.

Vibriosis

This disease is primarily associated with any species of fish raised in marine situations, although a few outbreaks have been reported in freshwater. Like many of the other gram-negative rod speticemias, the primary pathology involves hemorrhage and ulceration in the subacute condition, Diagnosis rests on the isolation and identification of *Vibrio anguillarum* from the tissues of the diseased fish.

Columnaris

All freshwater fish are considered susceptible to columnaris disease, which has a worldwide distribution and is generally associated with warmer water. The disease may result in greyish-white external lesions in some cases, but quite often involves erosion of the gills. Diagnosis is by means of isolation on a special medium of a long, thin gram-negative bacterium showing a gliding motility. The organism is classified as *Flexibacter columnaris* and represents one of the myxobacteria which cause disease in fish.

BACTERIAL IDENTIFICATION

This stage of disease diagnosis begins where the necropsy leaves off. A careful necropsy makes identification both possible and informative. They key to successful identification is isolating the causative agent of disease and then using one or more techniques to correctly identify it.

During the later stages of the necropsy, material from lesions, gills and the kidney is placed on one of several bacteriological media to allow the growth of various bacteria. Any disease bacteria, as well as other bacteria present, will grow on one or more of these media. One of the first tasks, then, is to examine the plates for the presence of bacterial colonies and to select colonies for further testing. Not only must the original tissue sampled contain the disease organism, but they should be present in sufficient numbers to form a large number of colonies on the bacterial media. This allows the pathologist to select several of these predominant colony types. Once isolated, a strategy for the identification of bacterial fish pathogens is employed. This can be diagrammed in the form of a dichotomous key (Figure 1).

The key allows the pathologist, using a series of standard tests, to narrow the possible choices until only one is left. The key in Figure 1 begins with the gram stain which separates organisms on the basis of color into two groups. Gram-positive organisms stain blue while gram-negative organisms are red. Next, the size and shape of the bacterial cell is considered. A group of long, thin gram-negative rods, the myxo-

bacteria, can be separated from those short gram-negative rods. Among these short rods another color reaction, the oxidase test, is used to separate those organisms with a series of enzymes capable of turning a special reagent blue-black from those bacteria lacking the enzymes. This strategy is continued using additional tests indicated until the identity of the organism is revealed.

In addition to the biochemical tests, gram stain, colony morphology and cell shape, a number of other tests have been developed which make use of antibodies to identify bacteria. These tests are of several types, but all rely on the preparation of specifically reacting antiserum, usually made by injecting rabbits with a known organism. The serum of the rabbits is harvested, and when placed in contact with the bacterium against which it was made, will react specifically in a variety of ways. One common method of using the serum is by a rapid slide agglutination test. Here, cells from an unknown bacterial colony are placed together with known antiserum on a slide. If the bacteria are the same as those used to make the antiserum, a flocculating precipitate will be seen. This reaction is commonly used to confirm the identity of an organism following biochemical tests. Another method is to couple a fluorescent dye to the antibody and react it with a smear of bacteria on a slide. Under an ultraviolet microscope the bacteria are seen to glow if they react with the antiserum. A third technique, which has been used especially with bacterial kidney disease, is to place some of the kidney of the fish in a small well of an agar plate near another well containing antiserum to the kidney disease bacterium. If such bacteria are present in the kidney tissue, a sharp line of precipitation will be seen between the two wells. This immunodiffusion test provides strong presumptive evidence that the fish is infected.

Additional techniques have been developed recently to speed and make easier the isolation and diagnosis of bacterial fish pathogens. Packaged biochemical test kits used in human medicine are finding their way into the fish pathology laboratory to allow a variety of reactions to be run economically and quickly. Special growth media which incorporate various compounds have been developed to both aid in the growth of disease bacteria, and to help in their identification. Such developments make the diagnosis easier, faster and more certain.

PROBLEMS IN BACTERIAL DIAGNOSIS

Several problems exist in isolating and identifying bacteria from fish. The first of these rests on the necessity of obtaining an adequate sample. In many cases of high mortality, bacteria are easily isolated, but for chronic diseases like bacterial kidney disease or carriers of disease in which only a few fish are lightly infected, the detection of bacteria may be more difficult. This presents a special problem when the pathologist is asked to certify a population of fish free of disease in the absence of any obvious symptoms or history of the hatchery. As discussed in the section on necropsy, the selection of samples may be very important in allowing any disease bacteria present to be detected.

The isolation of unusual or mutant forms of disease organisms is also a problem. As with any biological entity, a degree of variation in the biochemical reactions, growth characteristics or reactions with antiserum may be encountered. This may confuse or slow the diagnosis until further testing can confirm the identity of the agent.

Sometimes mixed infections may result in diseased fish and the pathologist must make every effort to insure all possible disease organisms are accounted for. During heavy mortality, usually one organism will predominate but in certification of disease free status all possible disease agents must be considered.

In many cases, a compromise between speed and precision is necessary. The cost must be low as possible and the diagnosis made quickly. This is usually less of a problem in disease diagnosis than in disease free certification where larger numbers of fish need to be examined for more potential disease agents. Specialists need new techniques to do a better job. Research on them is currently in progress at several laboratories to help meet this need.

Infected tissue (aseptically obtained)

Gram reaction

Positive (see Bacterial Kidney Disease section)

Negative

Short rods (1-3 µm long)

Culture on TS Agar (20-25 C)

Cytochrome oxidase reaction

(+)

Motility

Non-motile

Brown diffusing pigment usually produced

Aeromonas salmonicida (see Furunculosis section)

Motile

O/F test (glucose)

Oxidative or no reaction

Pseudomonas spp[3] (see Pseudomonas Septicemia section)

Fermentative

Sensitive to novobiocin and compound 0/129; anaerogenic with glucose[5]

(−)

Aeromonas spp[3,6] (see Motile Aeromonas Septicemia section)

(+)

Vibrio spp[3] (see Vibriosis section)

(−)

Culture in tryptone broth (indole) and on TSI Agar

Indole negative A/A on TSI Agar

ERM bacterium[1] (see Enteric Redmouth section)

Indole positive K/AG, H₂S on TSI Agar

Edwardsiella tarda[2] (see Edwardsiella Septicemia section)

Long rods (35-120 µm long)

Require 15-20% seawater salts for growth on *Cytophaga* Agar

(+)

Yellow colonies, cells (5-8 µm x 075-10 µm) shows gliding motility; microcysts usually present

Sporocytophaga spp[4] (see Saltwater Myxobacteriosis section)

(−)

Yellow, dry, rhizoid colonies, cells (5-12 µm x 075 µm) show gliding motility, causes disease at water temperatures of 14 C and over

Chondrococcus columnaris[1] (see Columnaris Disease section)

Yellow, moist, colonies, cells (35-75 µm x 075 µm) show gliding motility, causes disease at water temperatures of 12 C and lower

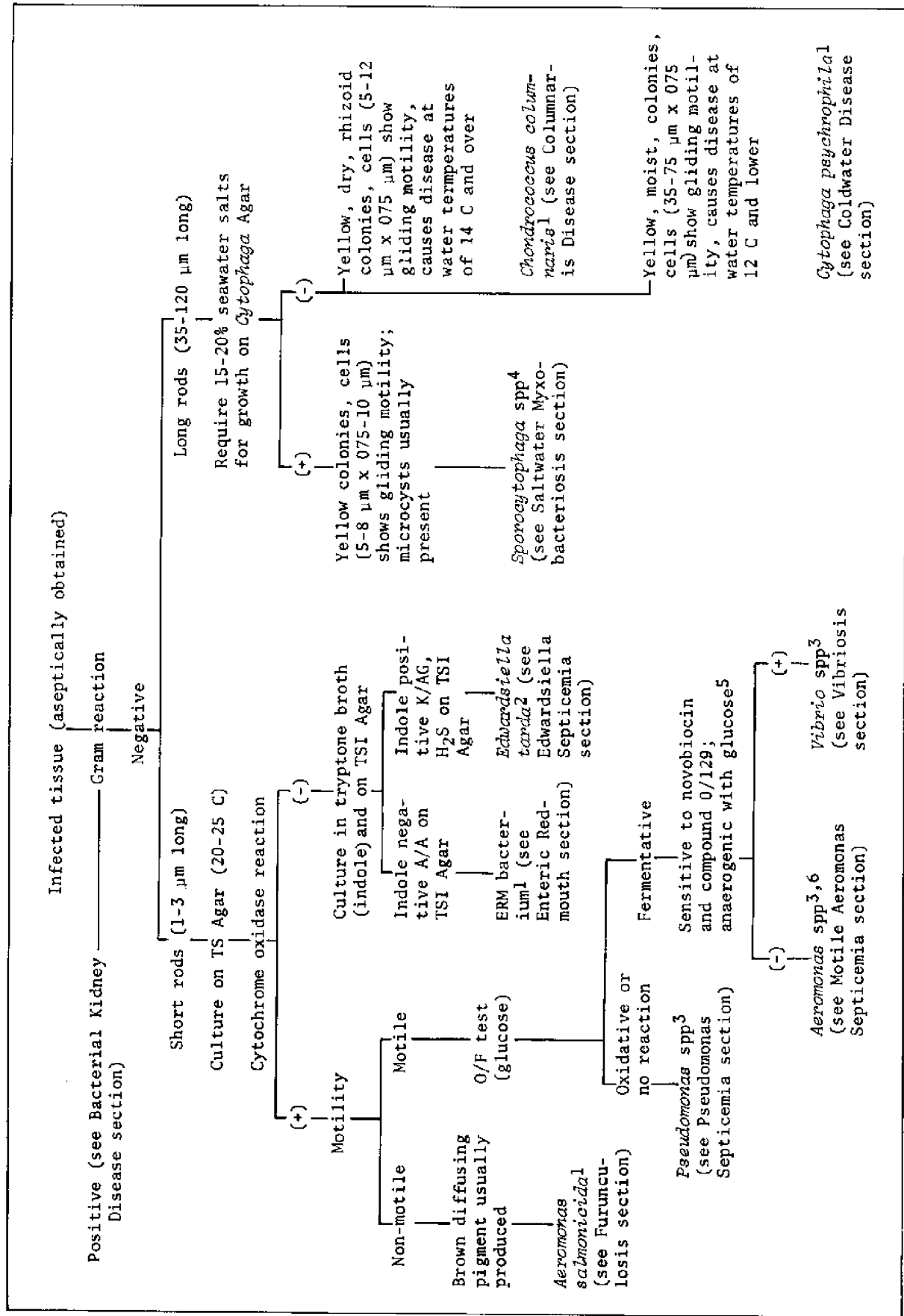*Cytophaga psychrophila*[1] (see Coldwater Disease section)

Fig. 1. Procedures for the differentiation of certain gram-negative bacteria associated with fish diseases (Reproduced from *Suggested Procedures for the Detection and Identification of Certain Infectious Diseases of Fish*)

DIAGNOSIS OF PARASITIC DISEASES — WHIRLING DISEASE AND CERATOMYXOSIS

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    Marine Science Center, Oregon State
    University

Fish play host to a multitude of parasites. In this country two of them, considered highly pathogenic and untreatable, are routinely required to be included in the pre-transfer fish health examination in an effort to confine the parasites to their present ranges. They are *Ceratomyxa shasta*, and *Myxosom cerebralis* or the whirling disease organism.

These two parasites, as with most parasites, cause little or no disease among fish stocks which have had a long association with them. Where the parasites are introduced into stocks that have had no previous contact with them, they can cause catastrophic losses.

The parasites are both freshwater myxosporidans, but their geographic range and the diseases they cause differ considerably.

Ceratomyxa shasta has been reported only within the Pacific Northwest from Washington to northern California (Table 6). This parasite can be isolated throughout the digestive system of infected fish, and can also invade many other tissues and organs. It has been reported in eyes, kidney, spleen, gills, gonads, muscle and connective tissue. The parasite infects fish at all stages of maturity.

The range of *Myxosoma cerebralis* is not nearly as limited. It has not been reported at all here in Washington and Oregon, but is a problem in other parts of this country and in many other countries of the world. It can only establish itself in developing cartilage of young fish.

Both of these parasites can produce a distinctive pathology in infected fish, but the only clear-cut way to determine that an individual carries the specific parasite is to isolate mature spores from the tissues.

Ceratomyxa shasta can distend the abdomen

and cause a bloody vent. It can also cause lesions or pustules, and if viscera are examined, they can show hemorrhages, thickening, perforations, or other damage.

The *Ceratomyxa shasta* spore, because of its small size, is invisible to the naked eye. Because the spore can be readily isolated from almost all parts of the digestive system of infected fish, the preparation for this test is a simple one. For this examination small pieces of rectum are collected and ground up on a tissue grinder. A drop of this material is placed on a slide. A minimum of twenty fields are examined under the microscope. If the whole fish is available in the lab, fluid from the gall bladder is also made into a wet mount and examined the same way.

The preparation for *Myxosoma cerebralis* is more involved. Spores of this organism are smaller than those of *Ceratomyxa shasta*, and oval in shape. Like *Ceratomyxa shasta*, they also display the two polar capsules that characterize this and all myxosporidians.

Fish infected with *Myxosoma cerebralis* can lose control of their pigmentation, and their tails turn black. Cartilage damage can leave the fish with misshapen heads, curved spines, and an impaired sense of balance which sends them into the whirling or frantic tail-chasing behavior that gives this disease its name.

The simplest standard method for isolating *Myxosoma cerebralis* is the plankton centrifuge method outlined in the American Fisheries Society Fish Health Section's *Suggested Procedures for the Detection and Identification of Certain Infectious Diseases of Fish*. This preparation disrupts the host tissue to free the spores. A filtration follows which separates the spores from the host tissue. Then the spores are concentrated to make finding them easier. Because it takes the spores a period of time to develop to a recognizable point, fish must be at least four-months old before examining them for this parasite.

Heads of fish to be examined for *Myxosoma cerebralis* are removed and combined into pools of five to 10 fish. Each pool has about 100g of tissue.

The heads are ground for five minutes at high speed in a Waring blender. Vacuum filtration to remove scales, bits of bone and other big pieces follows the grinding. The sample is poured through a coarse nylon

netting, then a finer one and ultimately passes through the stainless steel wire mesh millipore prefilter screen. Filtration is the limiting step as the screen plugs easily, and requires frequent rinsing.

The continuous flow plankton centrifuge works on much the same principle as an old-fashioned cream separator. The centrifuge has a funnel suspended above it. The filtrate is delivered through the funnel into a rapidly whirling cup inside the centrifuge.

The spores and other particulate matter remaining after filtration are pelleted on the walls of the centrifuge cup. The less dense materials travel up over the edge of the cup and out through rubber tubing, where a large flask in a sink below traps it.

After processing each pool of fish heads, the apparatus is dismantled and the pellets scraped from the wall of the cup.

This pelleted material is placed in a tube and resuspended in a small amount of water. A drop of this suspension is put on a slide, and a coverslip is added. Twenty fields are examined under the microscope.

This supernatent and all other materials from the preparation are autoclaved after completing this process. All equipment is soaked for 24 hours in a chlorine bath and then vigorously scrubbed with hot water and soap. Hard-to-clean parts, such as the separatory funnel and the filter screen, are further cleaned in an acid bath to prevent the spread of infection and the spore carryover from one preparation to the next.

With small fish this preparation goes quite smoothly, but for large, returning adult salmon examined prior to transferring eggs, the preparation is considerably more complicated. The volume of material is so great that heads have to be processed one at a time.

If the large fish heads are heated for five to 10 minutes in water at 50°C, all the soft tissues can be removed and bone and cartilage processed as small heads. This is extremely time consuming. It can take 35 to 40 hours for one person to process a 60 fish sample. To make this preparation go more quickly, heads are cut in half. The unused halves are kept in case the procedure must be repeated.

In another attempt to save time, the cranium and gill arches have been cut off and divided in half. If we remove the soft

tissues from these samples and decalcify them overnight, they can be combined into pools of five fish for processing. It is possible that this technique will be no less sensitive if even smaller amounts of tissue were used. It has been suggested that cartilage from the gill arches alone should be sufficient to detect the parasite in infected fish.

Efforts to reduce the amount of tissue used in the examination are complicated by the fact that it is still uncertain whether *Myxosoma cerebralis* can be isolated from the heads of returning adult salmon at all. So much of the cartilage of these mature fish has hardened into bone that spores may have been walled off and become inaccessible with these methods that were designed for smaller fish.

Examining adults may not really be helpful in curtailing the spread of whirling disease. Efforts to show transovarian transmission of the parasite have never been successful, but until it is decided that this is the case, or until we come up with a new method for detecting the parasite in returning adults, we'll continue to grind, to filter and to centrifuge.

Parasite	*Ceratomyxa shasta*	*Myxosoma cerebralis*
Disease	Ceratomyxosis	Whirling Disease
Distribution	Pacific Northwest	Europe, Asia, North America (probably other areas)
Sites of infection	Digestive system, other tissues and organs	Developing cartilage
Age of susceptible host	Juvenile to adult	Juvenile only
Symptoms of disease	Variable with host species (includes fluid-filled abdomen, hemorrhaged viscera, etc.)	Sunken heads Deformed spines Black tails Whirling

Table 6.

COLLECTION AND PREPARATION OF SAMPLES
FOR VIRAL EXAMINATION

WARREN GROBERG, Assistant Pathologist
Department of Fish and Wildlife

This discussion concerns field sampling
and the initial processing of salmonid fish
or their sexual products, ovarian fluid or
sperm for virological examination. It is
very important at the outset to realize that
the quality of the sample obtained in the
field will ultimately determine the time
required to analyze it and the reliability
of the results obtained.

Before discussing the specifics of samp-
ling, however, a brief description of viruses
as disease agents is in order. Viruses are
the smallest fish disease agents and are not
directly visible with the microscope. Elab-
orate techniques are required for their
detection and identification, and most im-
portantly, they can cause catastrophic losses
in fry and fingerlings. In addition, there
is no known treatment for fish viral diseases.

The viral agents we are primarily con-
cerned with in Pacific Northwest salmonids
are infectious pancreatic necrosis virus
(IPNV) and infectious hematopoietic necrosis
virus (IHNV). They are known to be present
and cause disease in this area. The names
are derived from the primary target tissue
that the viruses attack; however, in a
diseased fish the viruses may be recovered
from a variety of host tissues. In countries
and states where certification regulations
are in effect, these two viruses are usually
in the certifiable category as they are in
Oregon.

Also of concern is the agent of viral
hemorrhagic septicemia (VHS), as yet, con-
fined to Europe. Because of its limited
distribution and the potential for catastrophy
should it extend beyond its present range,
VHS is classed as an emergency disease.

Two recently discovered viruses in the
reportable category are *Herpesvirus salmonis*
and piscine erythrocytic necrosis virus (PEN).
The significance of both agents has yet to
be resolved.

Herpesvirus salmonis has been detected in the ovarian fluid of rainbow brood trout at the Winthrop National Fish Hatchery in Washington every year since 1971. A similar or identical virus has recently been isolated from adult and sockeye fry in Japan. In both countries, viral isolation has been associated with mortality; however, direct proof of viral etiology is still lacking.

Piscine erythrocytic necrosis virus has been identified in several salmonid species in the Pacific Northwest. It appears to cause extremely low blood hematocrits in infected hosts and secondary bacterial infections are often associated with the presence of this agent.

The sampling procedures to be described will provide tissue samples in which IPNV, IHNV, VHS virus and *Herpesvirus salmonis* can be detected from infected hosts. Detection of PEN virus requires fixing and staining of blood smears followed by microscopic examination.

The value of a vigorous sampling program is well demonstrated by the situation in Oregon. For many years the Oregon Department of Fish and Wildlife (ODFW) and Oregon State University, have cooperatively and extensively sampled fish populations in Oregon. The distribution of IPNV and IHNV is well documented from this work. Viral isolations have been made both during disease situations and during routine inspections of adults or young.

Eradication of IPNV appears to have been accomplished in trout at two central Oregon hatcheries following epizootics in 1973 and 1974 through foresighted management practices by ODFW. Infected and potentially infected stocks were destroyed, the ponds and equipment thoroughly sanitized and restocking with virus-free stocks undertaken. Extensive examination of adults and fry in the years since has failed to show any evidence of IPNV in fish at these locations. Although such measures are not always possible, these situations demonstrate that eradication is in some cases feasible. A thorough knowledge of the distribution of these agents can also allow managers to select noninfected brood stocks and limit the spread of viruses through controlled movement of fish or eggs.

During the course of conducting a fish viral examination the pathologist encounters certain problems. One involves obtaining the proper tissues and the other concerns

the time required to conduct an examination. In the discussion of viral examinations, we will try to highlight these areas to make them understood.

To determine the specific course for processing a set of samples, the diagnostician relies heavily upon what is known of the host specificity, tissue specificity and distribution of fish viruses. This is necessary to obtain the most reliable results in the most expedient manner.

Two situations arise which call for sampling fish for viral disease agents. The first, and most dreaded, is when a virus is suspected of causing disease. In this situation, 10 fish, pooled into two samples showing symptoms and morbidity, are a sufficient sample to allow for viral detection and identification if a virus is indeed the etiological agent of the disease. It is important to select fish near death since viruses replicate only in viable, living cells and will contain the highest numbers of virus particles. Dead fish are suitable for viral examination; however, dying fish are preferred.

Infectious hematopoietic necrosis and IPN most often occur in fry and fingerlings. Typical symptoms with either disease include exopthalmia or "popeye", darkening, hemorrhaging, abdominal swelling and aberrant motor behavior indicating nervous system involvement. Of course, any unusually high mortality with no diagnosed cause would provide the basis for a virological examination.

The second situation, and fortunately the more common, is when an inspection or certification is required for a fish population. This usually preceeds moving fish or eggs from this population. Sample size is an important consideration in this situation since viral isolation will most likely be from a few carriers harboring small numbers of viral particles. Using statistical methods, as applied to random samples, an agreed upon standard sampling scheme has been developed. To be 95% confident that a minimum incidence of 5% can be detected in a randomly sampled population of over 1,000, 60 fish must be sampled. These are pooled as five fish per sample so the result is 12 separate samples from 60 fish.

Another important consideration when sampling for viral examinations is to obtain tissue that will maximize the probability of virus detection. Viruses are very tissue-specific and unfortunately the tissues providing optimal recovery for IPNV and IHNV

differ. It has been demonstrated that IPNV is recovered at a higher frequency from the kidney and spleen of carriers than it is from sexual fluids. Therefore, when adults are not sacrificed at spawning, ovarian fluid is typically all that is sampled, and this is not optimal for detecting IPNV carriers. This is often the situation encountered with valuable trout brood stocks. The individual conducting the viral examination is responsible for explicitly documenting this when completing the examination report.

Ovarian fluid is the choice tissue for detecting IHNV from carriers. A lower incidence of recovery is made from kidney and spleen samples. Ovarian fluid is easily obtained, does not require sacrificing adults and is easily processed when compared to kidney-spleen tissue. Therefore, to properly inspect a large, spawning population of salmonids for IPNV and IHNV, 60 kidney-spleen samples and 60 ovarian fluid samples should be taken and processed. This is routinely done with spawning salmon, but kidney-spleen tissue is most often not taken when sampling brood trout.

It should be noted that sperm has not been mentioned as a possible tissue for viral detection. There are two reasons for this. First, sperm requires an additional step in sample processing, centrifugation, that ovarian fluid does not. Second, and more important, evidence indicates that in salmon, more females than males become IHNV carriers making females preferred for sampling. Virus can be recovered from sperm, however, and if circumstances warrant, sperm can be processed for virological examination.

Sampling sexually mature adults has been emphasized in this discussion. This is because IHNV is only detectable in infected fry or fingerlings, or, in sexually mature adult carriers. Between these life stages in an infected salmonid, IHNV is in an "eclipse phase" and current methods cannot detect it. The state of the virus during this period is unknown. Detection of IPNV, however, is possible at any life stage of a carrier.

It should be apparent, then, that although viral inspections or certifications are frequently done on salmonid fish at various life stages and with various fish tissues, only when certain conditions are met are these examinations completely valid for determining the presence or absence of both IPNV and IHNV. Ideally 60 whole fry or 60 kidney-spleen and ovarian fluid samples from sexually mature adults should be examined for certification.

A minimal amount of equipment is required for field sampling fish for viral agents. An ice chest, scalpel, forceps, sterile vials and 5 milliliter (ml) syringes are about all that is necessary. After the body cavity is opened 0.1 gram (g) each of kidney and spleen tissue are taken from a fish. These are pooled from five fish into a sterile vial or tube to give a total weight of about 1.0 g total tissue from five fish. This is important because in the laboratory premeasured aliquots of reagents and the equipment used are designed to handle standard weights of tissue. Too little tissue decreases the chance of virus detection, and too much leads to toxicity on the cell assay system and a prolonged examination period.

Ovarian fluid is easily obtained, after eggs are taken, by aspirating the fluid into a 5 ml syringe while swirling it through the egg mass. There are two important points to be observed in taking ovarian fluid. First, sperm should be added after the ovarian fluid sample is obtained because the sperm will be carried over to the cell assay system and interfere with the microscopic examination. Second, when possible, fish sacrificed at spawning should be prebled to limit the amount of blood in the ovarian fluid. When spawning live fish, ovarian fluid should be taken to minimize the blood in the sample. Blood in the ovarian fluid sample not only interferes with the microscopic examination, but also leads to toxicity on the cell assay system and a prolonged examination period.

After obtaining samples, they should immediately be placed on ice to limit autolysis or degradation, inhibit microbial growth, and to prolong survival of viral particles that may be present. Higher temperatures favor autolysis and microbial growth. Both can lead to toxicity on the cell assay system. If high levels of microbial growth are allowed, some of these may survive the antibiotic incubation treatment designed to eliminate them, and contamination of the cell assay system will result. Problems with both toxicity and contamination require that considerable additional processing of samples be made that significantly add to the time required to complete an examination.

Samples are shipped or transported on ice and processed as soon as possible for the same reasons indicated previously. Samples must not be frozen. Freeze-thaw cycles inactivate many viruses and IPNV is very sensitive to this process. The number of IPNV

particles in a suspension can be reduced by up to 90% by a single freeze-thaw cycle, and greatly reduce the possibility of detecting IPNV. It is worth noting at this point, that poorly obtained samples, or improperly stored and transported samples, will invariably show the problems resulting from toxicity or contamination. Delayed and less reliable results are the net result.

Once in the laboratory, the samples must be prepared for inoculation onto the cell assay system. In the case of whole fry, viscera or kidney-spleen samples, the tissue must be disrupted to release any viral particles present. A 1:20 dilution of the sample is made in a balanced salt solution and homogenized at high speed. This preparation is centrifuged, and 0.5 ml of the supernatant solution containing the virus, if present, is dispensed into 2.0 ml of antibiotic incubation mix to give a final tissue dilution of 1:100. Ovarian fluid is less toxic than tissue and is added to the antibiotic mix at a 1:5 dilution. The antibiotic solution is incubated at room temperature for two hours, or about 12 hours at 4°C. This kills bacteria or fungi in the preparation that will obscure observation of viral effects. The antibiotics have no effect on any virus present.

At this point we have obtained the sample and processed it for inoculation onto the cell culture assay system. Care should have been taken to insure that:

1. The proper tissue was selected in the proper amount from fish of an appropriate age.

2. The sample size met suggested procedures and requirements for the population size incidence level and confidence interval,

3. Samples were taken and transported to minimize toxicity and contamination,

4. The samples were processed as soon as possible.

The next discussion will deal with the actual detection and identification of fish viruses.

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VIRAL DIAGNOSIS: ISOLATION AND IDENTIFICATION

R.P. HEDRICK, graduate student in the Department of Microbiology, Oregon State University

Virus particles are too small for fish disease specialists to observe with the light microscope. Infectious pancreatic necrosis virus (IPNV) is 70 nanometers (nm) in diameter, or approximately 1/14th the size of *E. coli*, a common bacterium. Direct observation of viruses is dependent on the use of the electron microsope, which can determine their shapes and sizes.

The electron microscope shows that infectious hematopoetic necrosis virus (IHNV) is a bullet-shaped particle measuring 70 x 150 nm. IPNV, on the other hand, is hexagonal or icosahedral in shape and measures 70 nm in diameter. Unfortunately, electron microscopy is expensive and impractical for screening multiple samples for viral content and is therefore a poor diagnostic tool. A less expensive and very sensitive method for detecting viruses employs the cell culture system.

Viruses are obligate, intracellular parasites. Their replication is dependent on living animals, or cells derived from tissues of these animals. To facilitiate the isolation and study of viruses, tissues have been removed from fish and propagated in vessels with a nutrient-rich, liquid medium. Tissues grown in this fashion will divide and form a one-cell thick layer, a monolayer, on the bottom of the culture vessel. After the cells form a complete monolayer they can be dispersed by enzymes and chelating agents then removed from the vessel and subcultured into more vessels for continued growth and propagation. Cells that have undergone a suitable number of subcultures are considered to be autonomous cell lines. If maintained and subcultured regularly, the lines continue to grow and divide infinitely.

Two cell lines are routinely employed in this laboratory. The STE-137 cell line was derived from steelhead trout (*Salmo gairdneri*) embryonic tissue and the CHSE-214 from chinook salmon (*Oncorhynchus tshawytsha*)

embryonic tissue. The CHSE-214 cell line has undergone over 300 subcultures during its 14 years of existence.

The cell culture system just described allows indirect observation of viruses. Viruses cannot be resolved with the light microscope, but the changes they cause to cells can be seen. Changes result from intracellular replication of the virus and are often detrimental. They are termed a cytopathic effect, or CPE. The type of CPE can be very characteristic for the virus involved. For example, IHNV CPE takes five to ten days to appear and is characterized by grape-like clusters of dying cells throughout the monolayer. The CPE for IPNV takes two to four days to appear and is characterized by spindle-shaped cells. With the aid of the cell culture system the virologist can isolate and identify the common viral pathogens of fish.

An integral part of sample analysis for virus is a knowledge of the species of fish, watershed, history and clinical signs of the population involved. Evidence of this nature can be helpful in a presumptive diagnosis, but ultimate confirmation relies on the isolation and identification of the etiological agent. The procedure outlined next is suitable for the isolation and identification of IHNV, IPNV, viral hemorrhagic septicemia (VHS) and *Herpesvirus salmonus*.

Homogenized and centrifuged samples from kidney and spleen tissues as well as sex fluids are incubated in an antibiotic solution as described earlier. These samples are distributed in 0.1 ml aliquots to 96 well dishes with monolayers in each well. Care is taken to keep pools of five fish each separate. Mock-infected cells are included as controls on each 96-well dish. Fresh media is added and the dish is sealed with an adhesive mylar film. Replicate plates are incubated at 10°C and 16°C. The dishes are examined daily with an inverted microscope for any changes in the cell monolayers in each well. The types of changes that may occur include:

1. Toxicity is a generalized killing of cells destroying the entire monolayer as a result of the presence of cytotoxic substances. Toxicity increases when blood or crushed eggs are present in ovarian fluids, or when tissue samples are old or poorly stored. In the event of toxicity, the sample must be diluted 1:50 to 1:100 and subcultured to a new monolayer for continued observation. Toxicity increases the length of time required to process a sample. It can be minimized by properly taking and handling samples.

2. The monolayer may remain unchanged. This suggests that the sample is virus free, but to be certain that low-virus numbers are absent, a blind passage is performed. A blind passage may be defined as the subculturing of a sample 14 days after primary inoculation in the absence of CPE or toxicity. The recovery of low levels of IHNV or IPNV by blind passage has been reported. If no CPE occurs 14 days after the blind passage, the virologist reports that no evidence of virus was detected.

3. The cells may demonstrate CPE as a result of virus replication. The type and time for CPE to arise is recorded. The next step is to identify the isolated virus.

Fluorescent antibody and complement fixation tests have been described for identifying viruses, but the most reliable and widely used is the serum neutralization test. The neutralization test exploits the ability of specific antibodies to the virus to prevent replication and subsequent CPE in cell cultures. For example, antiserum specific to IPNV is incubated with a suspension of unknown virus obtained by the above isolation scheme. If the unknown virus is IPNV, the antibodies will attach to it and effectively neutralize its ability to cause CPE in cell cultures. A rigorous set of controls accompanies each neutralization test. The antiserum is checked with a positive control or unknown virus and antiserum alone is checked for its toxicity to cells. A cell control is also included. If the antiserum prevents CPE and all the controls are in order, the virologist can report the isolation and identification of the virus.

Viral diagnosis is dependent on the isolation and identification of the infectious agent in cell culture. This can be a tedious, time-consuming process. Figure 2 diagrams three possible pathways encountered by the virologist while processing a sample. Samples for viral analysis that are taken and handled properly can contribute to a more rapid diagnosis. Although the process is lengthy, it is currently the most practical and reliable technique for accurate viral analysis.
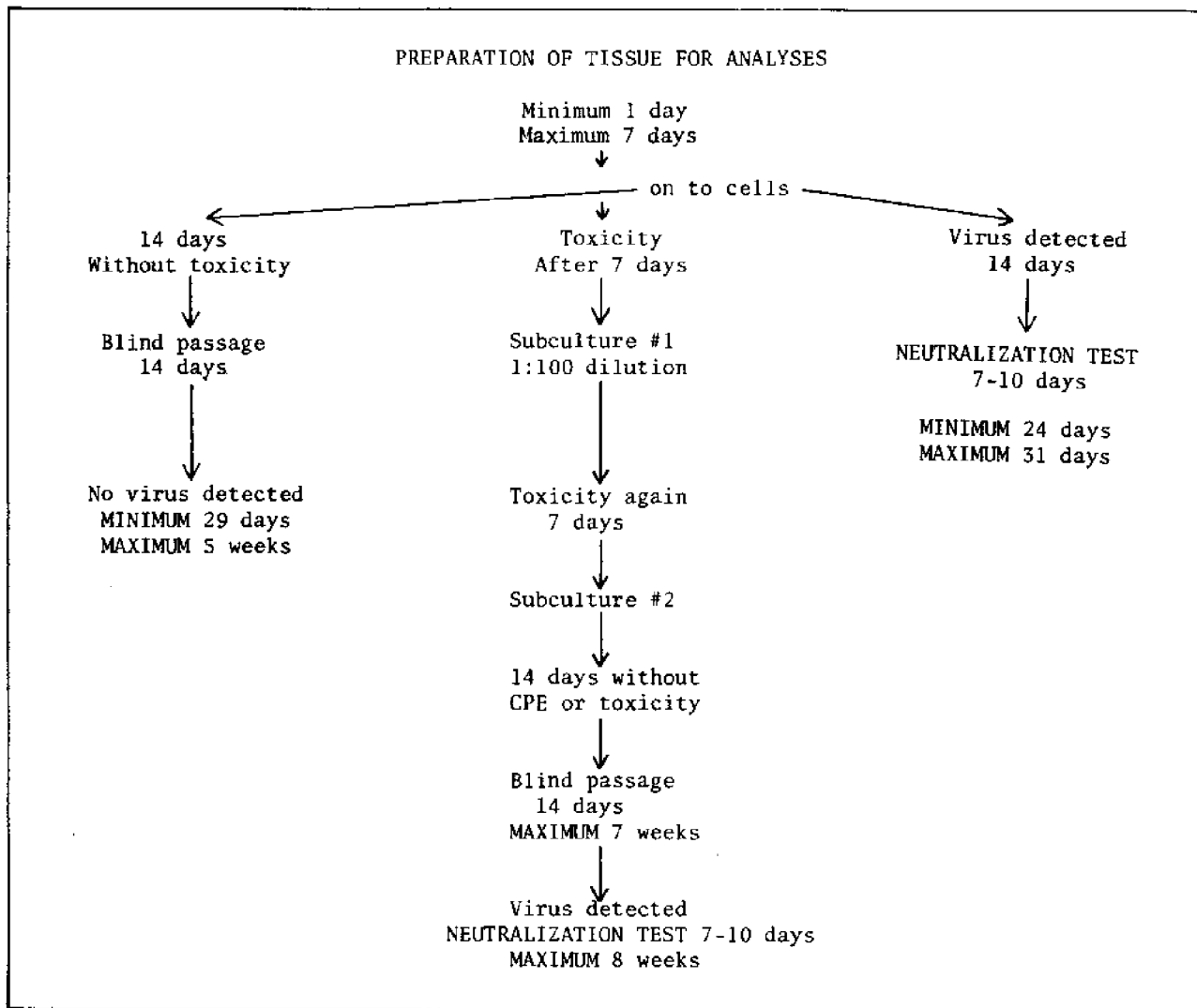
PREPARATION OF TISSUE FOR ANALYSES

Minimum 1 day
Maximum 7 days
↓
——— on to cells ———

| 14 days
Without toxicity | Toxicity
After 7 days | Virus detected
14 days |

14 days
Without toxicity
↓
Blind passage
14 days
|
↓
No virus detected
MINIMUM 29 days
MAXIMUM 5 weeks

Toxicity
After 7 days
↓
Subculture #1
1:100 dilution
|
↓
Toxicity again
7 days
↓
Subculture #2
↓
14 days without
CPE or toxicity
↓
Blind passage
14 days
MAXIMUM 7 weeks
↓
Virus detected
NEUTRALIZATION TEST 7-10 days
MAXIMUM 8 weeks

Virus detected
14 days
↓
NEUTRALIZATION TEST
7-10 days

MINIMUM 24 days
MAXIMUM 31 days

Fig. 2. Time schedule for viral analyses.

SUMMARY OF PANEL DISCUSSION

Moderated by JOHN FRYER, Department of Micro-
biology, Oregon State University

The general discussion on diagnostic
problems centered mainly on those involved
with viral certification. The major concern
of both aquaculturists and diagnosticians
was the extended length of time required to
determine the presence or absence of viral
agents. Delayed diagnosis becomes extremely
important when eggs are incubated at quaran-
tine facilities in warmer water allowing them
to hatch before viral certification is com-
pleted. This places logistic problems on
the aquaculturist because fish are much more
difficult to hold in quarantine than eggs,
and also pose greater problems in transporta-
tion. There was general consensus that some
synchrony of certification and development
of eggs is essential. One of the steps in
viral diagnosis, which prolongs the examina-
tion, is the blind passage, a procedure which
has been determined to increase the prob-
ability of finding any virus present by ap-
proximately 4%.

Because the numbers of fish from a popula-
tion to be checked is based on statistical
samplings, there was discussion on sample
sizes and how they might be increased so
that blind passages could be eliminated
without changing the confidence that virus
will be detected if present in the popula-
tion. It was pointed out that a large in-
crease in sample size would be necessary to
only slightly increase the confidence of
virus detection. Suggestions were made that
techniques used in veterinary medicine for
virus diagnosis be used in detecting fish
viruses, and although there are inherent
problems, several laboratories are involved
in research aimed at more rapid virus de-
tection.

With regard to sampling anadromous species,
a question was raised concerning the part of
the run to be sampled. Because a run may
continue over a period of several weeks
during which there are numerous spawnings,
it was asked what part of the run, or should
each spawning be certified? It was also
asked if one part was found to carry virus,

would that put the entire year-class in jeopardy.

A brief description of regulations for disease certification in the livestock industry was given by Dr. Dean Smith who is the assistant state veterinarian in Oregon. He also commented on the foresight being shown by those who are involved in the relatively new industry of aquaculture. After Dr. Smith's comments the discussion on health regulations governing transporting fish and fish eggs covered many topics. One that received a great deal of attention was quarantine facilities in which fish and/ or eggs are held while waiting to be certified. There was discussion concerning the situations that would require eggs or fish to be quarantined, whether or not one facility would be satisfactory for several users, and the physical form such facilities would take. Another topic which was discussed was the movement of adult fish between watersheds within the same state.

The rearing of salmonids is a unique industry in that the regulatory agency is also involved in the enterprise. This situation and the possibility of an autonomous regulatory agency, perhaps the USDA, was discussed. During the discussion it was pointed out that the poultry industry, which in many aspects is similar to aquaculture, is a self-regulating industry which has placed stringent health requirements on its members.

Some concern was expressed on the availability of diagnostic and certification services, and the activity offered by the Department of Microbiology and the Sea Grant College Program at Oregon State University was explained. Both diagnostic and certification services are being offered by this organization.

The conference closed with a suggestion that further meetings be held between private, state and federal organizations so that working groups could be organized that could seek solutions to problems involving health requirements of fish.