Diseases of Seawater Netpen-Reared Salmonid Fishes

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DISEASES OF SEAWATER NETPEN-REARED SALMONID FISHES

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FORWARD

In 1992 one of us (M.K.) published "Diseases of seawater netpen-reared salmonid fishes in the Pacific Northwest." Since this publication, there have been tremendous advances in fish health, particularly in the fields of diagnostics and vaccinology. Furthermore, with the continued growth of salmonid netpen aquaculture in the 1990's, several new (previously unrecognized) diseases have been reported. In this edition, we update the original manual and expand the text to encompass diseases of importance in salmonid seawater netpen culture in general. To accomplish this endeavour, Dr. T.T. Poppe has joined Dr. Kent as a co-author. In addition, we have elicited the expertise of other internationally recognized experts.

We thank the staff of the Fish Health, Parasitology and Genetics Section at the Pacific Biological Station for their assistance with the preparation of this manual. The staff of the Fish Pathology program (Dorothee Kieser, Carl Westby and Karen Mullen) provided a lot of the material that is included in this manual. We also thank Tom McDonald and David Whitaker for providing parasite material, Drs. Leo Margolis, Trevor Evelyn and David Speare (Atlantic Veterinary College, P.E.I., Canada) for review of the manuscript, John Bagshaw and Sheila Dawe for histological preparations, Jon Richard and Chris Whipps with assistance with field collections, and Pat Baglo for assistance with preparation of the manuscript. Sheila Dawe and David Whitaker also provided valuable assistance with the production of illustrations. Special thanks to Dr. Bob Devlin, at our West Vancouver Laboratory. Bob and his group were fundamental in the development of several PCR based diagnostic tests for parasites described in this text.

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INTRODUCTION

M.L. Kent

Culture of salmonid fishes in seawater netpens is rapidly expanding at several locations throughout the world. In the past, most research on diseases of salmonids has been directed towards those affecting fish during their freshwater phase of development. With the phenomenal increase in seawater netpen aquaculture in the past 10 years or so, several apparently new marine diseases of salmonid fishes have been recognized. Described here are important diseases and pathogens of salmonid fishes reared in seawater netpens. This text does not address diseases of specific importance in freshwater netpens, and henceforth "netpens" or "pen-reared" denotes seawater netpens. This manual is intended primarily for workers at the netpen sites and field laboratories. Therefore, diagnostic characteristics of each disease that can be obtained by macroscopic observation, simple stains or wet mount preparations are emphasized. Information on more involved laboratory diagnostic techniques that may be required for confirmatory diagnosis are also included. In addition, descriptions of histopathological changes are included to provide an understanding of the pathogenesis of specific diseases. It is expected that the manual will be used mostly by those with basic training in microbiology, parasitology, pathology, and fish health. However, we hope that aquaculturists in general will also make use of the manual, and a glossary is provided in the appendix to familiarize fish farmers with some of the technical terminology used in fish health. Preservative and media formulations, and scientific names of fishes referred to in the text are also listed in the appendix.

An abbreviated description of methods for conducting a disease investigation and a basic necropsy protocol are included. This manual is not intended to be a complete laboratory manual and more detailed descriptions of principles for diagnostics can be obtained in basic fish disease texts (e.g., Kabata 1985; Post 1987; Roberts 1989; Stoskopf 1993; Noga 1995; Bruno and Poppe 1996). Information obtained in the field may be adequate for only presumptive diagnosis, and more in-depth laboratory investigations are often required for positive diagnosis of certain diseases. Therefore, methods for preservation and transport of specimens to a laboratory for further microbiological, histopathological, and chemical examinations are included.

Control of diseases is very important to the economic viability of netpen fish farms, and for each specific disease we provide information on control and treatment. In addition, Drs. Brackett and Karreman (Chapter 4) present an overview of chemotherapy and vaccines for netpens. Dr. Stephen provides a review of outbreak investigations (Chapter 2). It is hoped that this manual will help fish health practitioners and fish farmers identify diseases in pen-reared salmon, and that in doing so it will provide a sound basis for implementing appropriate control and treatment procedures.

Fish Health in Netpens - General Considerations

Rearing fish in netpens may be economically expedient because of the relatively low construction costs involved. Also, there is no need for pumping water. However, this form of aquaculture may allow for the exacerbation of certain diseases and presents some unique fish health problems. With uncontrolled exchange of water in netpens, there is the potential for introduction of pathogens, pollution, and noxious algae. Certain pathogens, such as external parasites, are often only a minor problem in hatcheries or ponds because, in most cases, they are quickly eliminated with externally applied therapeutic agents. In contrast, these pathogens can be devastating in netpens because it is extremely difficult to apply and maintain appropriate levels of bath-administered drugs in this situation. For example, external parasitic copepods are one of the most serious problems for pen-reared Atlantic salmon.

Fish in netpens will often feed on naturally-occurring food organisms. These organisms may not normally be eaten by the fish and, when eaten, may result in unusual parasite infections (e.g., metacestodes of *Gilquinia squali* - see page 71). In addition, netpen liver disease (page 104) is apparently caused by feeding on naturally occurring netpen biota.

Although several causes of disease in pen-reared salmonids have been identified, there are few drugs available for their treatment. Therefore, most diseases are controlled by changing husbandry practices that avoid infections or improve the overall health status of the fish. In addition, there are now available vaccines that are very effective for controlling Gram negative bacterial infections. Many important diseases in netpens originate in fresh water (e.g., furunculosis and bacterial kidney disease). Often the best way to control these diseases is to identify fish in fresh water that have subclinical infections and to avoid their introduction into netpens.

Early detection of a disease may be more difficult in netpens

than in land-based system because water visibility is often reduced in the former. Often when a disease problem is recognized by the fish farmer, the disease has advanced to a stage where, even with immediate action, high mortality is unavoidable. In netpens, it may be difficult to detect subtle external lesions on fish or behavioral changes indicative of the onset of disease. Furthermore, moribund or dead fish that accumulate at the bottom of nets may not be detected until they are collected by divers or until the nets are raised. Added to these problems, a certain low-level of mortality is often considered acceptable by some fish farmers; these losses are classified as "natural mortality" and are often ignored. Fish in this category should, however, be examined because catastrophic disease outbreaks can usually only be avoided by early detection of a problem, and these fish sometimes represent the incipient stages of a serious disease. Frequent and consistent examination of "morts" and complete record keeping of mortalities in each stock is essential for preventing disease problems.

When an organism is cultured in a new geographic area it is often subject to diseases that do not affect the indigenous species. The Atlantic salmon is a very desirable species for netpen culture. Because this fish is not native to the Pacific Northwest, it may lack innate resistance to certain indigenous disease agents, to which Pacific salmon are resistant. There are already examples of this phenomenon; Kent et al. (1988b) described a toxic liver disease to which Atlantic salmon are more susceptible than Pacific salmon (see page 104), and a parasitic copepod, *Haemobaphes disphaerocephalus,* was found on pen-reared Atlantic salmon (Kent et al. 1997). This copepod normally infects eulachon in the Pacific Northwest, but has never been observed on Pacific salmon species.

Often previously unrecognized diseases are observed when an organism is reared under new environmental conditions. The rearing of salmonids in netpens is a relatively recent form of aquaculture. It is, therefore, not a surprise that in the past 10 years many "new" diseases have been documented in pen-reared salmon, and it is very likely that more diseases will be observed. Some of these diseases are caused by pathogens that were first recognized in netpens, then were later found to occur in wild or freshwater-reared fish (e.g., the rosette agent and the microsporean *Nucleospora salmonis*). However, the majority of diseases afflicting pen-reared salmon are caused by organisms that were first documented in wild fish or freshwater culture situations (e.g., *Renibacterium salmoninarum* and the IHN virus). A third category includes previously known pathogens, but which appear to be less pathogenic in fresh water or in wild salmon (e.g., *Loma salmonae*). The high likelihood of encountering an unreported disease in the netpen situation is a problem for the diagnostician because he/she cannot always rely on published references or past experience for making quick disease diagnosis.

OUTBREAK INVESTIGATIONS IN NETPENS

C. Stephen

The goal of a field investigation is to identify factors that can be manipulated to reduce the prevalence of disease. The occurrence of disease in a group of fish is rarely the result of a single factor and rarely has a simple solution. This is due to the multifactorial nature of disease in which host, agent and environmental factors must interact for a disease to occur. This is true even for infectious diseases where the presence of the infectious agent is often insufficient to result in disease. Experience with terrestrial species has shown that differences in the amount of disease between populations generally do not reflect microbiological difference between groups, but can instead be attributed to differences in host and management conditions (Hancock and Wilkes 1988). The objective of a field investigation is to identify and remove or control the factors that are responsible for the disease. To meet this objective, one must be prepared to conduct a comprehensive review of the pathological, environmental, management and host factors that give rise to the occurrence of the disease being investigated.

The first step of a field investigation is to describe the disease event. The pathological lesions, etiologic agents or environmental samples associated with a disease can often provide vital clues for disease control. It is wise to consult your diagnostic laboratory before embarking on a field investigation to ensure that diagnostic samples are appropriately collected, stored and shipped. Whereas laboratory analyses can be a valuable component of a field investigation, the costs of extensive testing can be high. Thus, the selection of specific laboratory tests should be directed to answering key questions, particularly those that can lead to disease control decisions. Details of sample collection for pathologic and microbiologic purposes are presented in Chapter 3. Similar care must be taken when selecting water, feed or other environmental samples for analysis.

It is important next to decide which tests and how many tests to use. From a clinical management perspective, a test should not be conducted unless its outcome is likely to affect health management decisions. In some cases, however, it is important to collect samples for research purposes to gain further insight into the causes of the particular disease being investigated. Regardless of the reason for testing, investigators must be aware of the performance characteristics of the tests being used. There are 4 main measures that are used to describe the performance of a test which dichotomize the fish or populations health status (Table 2-1). Each of these measures requires a "gold standard" which can reveal the true health status of the fish. A fifth measure, the kappa value, is a measure of how well two tests agree on the disease classification of a subject and can be used to compare tests when a gold standard does not exist (Martin et al. 1987). An understanding of how the prevalence of disease in the population, the number of tests performed and the number of individuals sampled per group affect a tests ability to accurately classify a fish or fish populations disease status, which is essential for appropriate interpretation of laboratory results (Martin et al. 1992). Unfortunately, very little information exists on the clinical performance of tests used for salmon.

As important as selecting the appropriate samples for diagnostic tests is the selection of fish to be sampled. Which fish are examined during the investigation will depend

Table 2-1. Measures of diagnostic test performance.

	DISEASE PRESENT	DISEASE ABSENT	TOTAL
TEST POSITIVE	True positive (a)	False positive (b)	Total test positive (a+b)
TEST NEGATIVE	False negative (c)	True negative (d)	Total test negative (c+d)
TOTAL	Total number with disease (a+c)	Total number without disease (b+d)	a+b+c+d

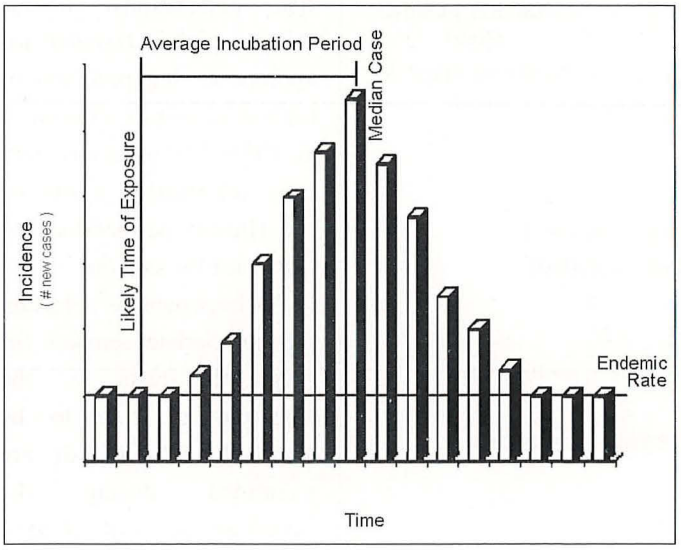
Sensitivity: Proportion of fish with the disease that will test positive = a/(a+c)
Specificity: Proportion of fish without disease that will test negative = d/(b+d)

Positive Predictive Value:*
Proportion of fish that test positive that truly have the disease = a/(a+b)
Negative Predictive Value:*
Proportion of fish that test negative that truly do not have the disease = d/(c+d)

*Influenced by prevalence of disease in the tested population

upon the questions that must be answered. For example, to establish the prevalence of disease in a group, random samples of the entire population are ideal, whereas investigations solely concerned with establishing the reason for deaths may restrict sampling to recently dead or dying fish. As it is rarely feasible to examine every individual in the affected population, field investigations usually rely upon examination of a sample of the group of interest. Rarely is it possible to obtain truly random samples of cultured fish populations. It is therefore important to understand the nature and magnitude of any biases that are created by relying upon a specific population sub-sample during an investigation. For example, research in chinook salmon seacages in British Columbia has shown that samples of surface accessible moribund fish can overestimate the prevalence of chronic disease in the entire cage (Stephen and Ribble 1995a). In such cases, examination of recently dead fish can provide more accurate estimates of the proportional mortality ratio (Stephen and Ribble 1997).

The second stage of the investigation involves describing the disease at a population level. For any disease to occur, there must be susceptible fish and opportunity for exposure to causal agents. The goal of this portion of the investigation is to identify the time and places where exposure or susceptibility was altered so that the key determinants that caused the disease can be identified. An essential first step of all field investigations is to generate an epidemic curve (Figure 2-1). The epidemic curve not only documents the magnitude of the problem, but also provides important clues as to possible times of exposure to causal agents, key times to check for environmental or management changes, and the nature of spread of the disease. Great care must be taken to verify data that is used to generate an epidemic curve. Particular attention should be placed on evaluating the accuracy and consistency with which cases were defined and counted. An accurate description of the spatial distribution can identify the potential locations where the susceptibility and/or exposure of the population were altered. Particular attention should be placed on determining where the first cases occurred and the pattern of the spread of disease through the affected population.



It is also important to describe the characteristics of the affected fish. Information to collect includes; (1) the age, sex, species, and strain of affected fish, (2) the performance characteristics of the affected population, such as food consumption and growth rates, and (3) the management history of the fish including their source, pattern of movement or mixing; types and sources of feed and water; and treatment and vaccination history. An attack rate table can be used to help identify potential key determinants or to identify environmental factors that should be further investigated (Table 2-2).

Often management practices or uncontrollable environmental conditions can impose restrictions on the number and nature of factors that can be manipulated to control or prevent disease. The descriptive phase of the investigation helps to assess which host, agent or environmental variables can practically and effectively be altered. In stage three of the field investigation, the information collected from the affected fish must be compared to unaffected fish. Because disease occurs as a result of the interaction of multiple risk factors rarely will a field investigation reveal a single problem. Consequently, we need a way to describe the relative contribution of various risk factors to the particular disease occurrence being investigated. This is done by comparing the characteristics of affected individuals and populations to those that are unaffected. While there exists several mathematical methods for comparing complex interactions of risk factors, they ultimately all try to estimate the relative risk of a particular factor. The relative risk provides a measure of the likelihood that disease will develop under a specific circumstance. The relative risk can be defined as the ratio of the incidence of disease in the affected population to the incidence of disease in the unaffected population. Ratios not significantly different than 1 indicate that the factor is not a causal risk, while those greater than 1 indicate the factor contributes to the risk of developing disease. Great care must be taken in selecting the comparison groups. Particular attention must be taken to correctly determine their disease status to insure they are true controls and not sub-clinical or recovered cases.

After completing the three phases above, hypotheses regarding the key determinants must be generated. These hypotheses can be tested either by mounting experimental or observational studies which can refine our understanding of the

Table 2-2. Example of an attack rate table. The attack rate is the proportion of fish with specific attributes or exposures that develop disease. Attack rates are used to identify potential risk factors in disease outbreaks that occur over a short time.

	Fish exposed to factor			Fish not exposed to factor			
Factor	**Cases**	**Total exposed**	**Attack Rate(%)**	**Cases**	**Total not exposed**	**Attack Rate (%)**	**Differences in Attack Rates**
Surface water	44	92	47.82	15	187	8.02	39.80
Tank 1	59	255	23.13	2	24	8.33	14.80
Feed X	59	265	22.26	2	14	14.28	7.98
Vaccine A	18	105	17.14	42	174	24.13	-6.99

causal relationships or they can be tested by implementing treatment or control programs targeted at hypothesized key determinants. Each approach has advantages and disadvantages. Whereas experimental studies provide the opportunity to more closely control confounding or extraneous variables, they are time consuming and can rarely replicate the interaction of factors present under field conditions. Observational studies (cohort studies, case-control studies and cross-sectional studies) vary in their costs and time needs. Although less able to control extraneous variables than in an experimental study, observational studies better replicate field conditions and can often provide risk reduction recommendations in the absence of precise knowledge of the pathophysiology or etiology of the disease of concern. However, both experimental and observational studies generally do not address the needs of the fish manager who wants to stop the problem now! Therefore, hypotheses generated by field investigations are often tested first by launching a control program. There are, in general, a limited number of ways by which a disease can be controlled or prevented at a population level. They can broadly be divided into actions to reduce susceptibility or actions to reduce exposure (Table 2-3). Key determinants that have been identified as the target for control should be assessed for their economic feasibility and acceptability to fish managers before being selected as key control targets.

Whereas much of this manual details the microbiological and pathological characteristics of diseases of cultured salmon and suggests chemical means for their control, it is essential to remember that disease in populations rarely result from the action of pathogens or parasites alone. Effective disease control or prevention programs must also pay significant attention to the host and environmental factors which affect the susceptibility of fish and probability of exposure to pathogenic agents. And thus to identify methods that can be used to reduce disease incidence in a field setting.

Table 2-3: General Disease Control Options

Reduce Population Susceptibility to Pathogenic Agents
- Improved husbandry and/or nutrition
- Mass vaccination
- Selective breeding
- Environmental management and hygiene

Reduce Population Exposure to Causal Factors
- Depopulation or selective slaughter
- Quarantine or isolation
- Mass treatment
- Environmental management
- Site and water source selection
- Biological controls

Education
- Methods of prevention
- Methods of early detection
- Methods of early intervention

NECROPSY PROTOCOLS

M.L. Kent

The mere presence of a pathogen in a sick fish does not necessarily mean that it is the cause of the disease. It is important, therefore, to conduct a thorough investigation, beyond just detecting the presence or absence of known pathogens, to determine the cause of the disease. Histological examinations, in addition to gross necropsies and in vitro culture of microorganisms, are often required to determine the association of the observed pathogens in the disease being investigated. Furthermore, because fish in netpens often have mixed infections, it is often necessary to examine several fish from the affected population to differentiate primary pathogens, opportunistic pathogens, and secondary causes of the disease.

Diagnosis of a disease begins at the farm, and some of the most crucial information can be collected by the farmer by thorough record keeping and careful observations of affected fish (see previous chapter). The following information is important for disease diagnosis and implementing control strategies.

Background Information

To assist with obtaining an accurate diagnosis, the following information should be obtained: time of seawater entry, origin (stock), smolt producer, vaccine status, previous disease problems in the affected stock in fresh water and in the netpen, diet, medication history, and mortality rate (See Chapter 2 on Outbreak Investigations).

Suboptimal water conditions often exacerbate diseases and, because of lack of control of water movement in netpens, water quality problems may be unavoidable. The following are water quality parameters of particular concern for netpens and should be considered in a disease investigation: dissolved oxygen, temperature, salinity and phytoplankton density.

Fish Examination

Behavior. Fish behavior may be useful for indication of an emerging disease and some behavioral changes are useful for presumptive diagnoses. For example, if fish are flashing (rubbing on nets), this may indicate that they are infected with external parasites. Other indications of disease include: cessation of feeding, lethargy, and abnormal position in the netpen (e.g., at surface or at the bottom). Abnormal respiratory pattern may indicate gill damage, and whirling or spiralling swimming often indicates neurological damage.

Selecting fish for Necropsy. Examinations should be conducted on diseased fish that are collected while still alive (moribund). It is important to determine the primary cause of mortality and to differentiate this from secondary or opportunistic pathogens that may have taken advantage of already diseased fish. To accomplish this, several affected fish should be examined whenever possible. In addition, for unusual diseases, the sample should include apparently normal, asymptomatic fish so that early pathological changes and the underlying cause of morbidity can be determined. Dead fish may be suitable for some parasitological examinations and for observing obvious macroscopic pathological changes. However, bacteriological examinations conducted on dead fish can yield misleading results, and many histological changes are obliterated by post-mortem autolysis.

Moribund fish are usually collected from the surface. However, the pattern of disease in these "slow swimmers" does not always accurately reflect the disease status in the overall population (Stephen and Ribble 1995a). Therefore, very fresh dead or moribund fish collected from the bottom of pens (e.g., by divers) should also be included during a disease examination.

External Examination. Note surface abnormalities (e.g., frayed fins, cloudy eyes, ulcers, skin discolorations, parasites, and tumors). Prepare wet mounts of the skin mucus and a few scales by scraping the surface of the fish with a coverslip and placing the coverslip on a glass slide. Some sea water may be added to the preparation so that the area between the slide and the coverslip is completely filled with liquid. Examine the wet mount with a compound microscope, starting with low power. Reducing the light and lowering the condenser will produce higher contrast, which will make microscopic parasites and other pathogens more visible.

Gills. Remove the operculum. Note color of gills (pale gills usually indicate anemia). Check for parasites, cysts, excessive mucus, and hemorrhages. A dissecting microscope is useful for detecting larger parasites. Prepare a wet mount of a few filaments and examine for small parasites, fungi, and bacteria

using a compound microscope. Wet mounts of gills are prepared by removing a few filaments with scissors, placing the filaments in a large drop of sea water on a glass slide, and overlaying with a coverslip.

Internal Examination. Open the visceral cavity. Note if ascites, hemorrhages or other abnormalities are present. Expose the kidney by removing the swimbladder and note any kidney abnormalities. Many diseases cause enlargement or discoloration of the kidney. Examine the heart for any abnormalities. Remove and open the intestinal tract by cutting length-wise and examine for parasites with a dissecting microscope or magnifying glass. Dissection of the gastrointestinal tract in 0.9% saline is helpful for finding helminth parasites. Examine squash preparations of organs with a compound microscope to detect encysted parasites, fungi or granulomas. Squash preparations are made by removing a small piece of tissue, and gently squashing it between a slide and coverslip so that a thin preparation suitable for examination with a compound microscope or dissecting microscope is made.

Imprints. Leishman's Giemsa (see Appendix III) or Diff-Quik (Harleco) stained imprints of kidney, spleen or other affected organs are useful for detection of protozoa and bacteria. Remove a piece of tissue (approximately 0.5 X 0.5 cm^2), blot on clean paper towel to remove most of the blood, and lightly touch the cut surface of the tissue on a clean glass slide. Several imprints from the same piece of tissue can be made on one slide. Air dry the preparation for approximately 1/2 h. Fix the slide for 5-10 min in absolute methanol for Giemsa stains. The slide can then be stained with Giemsa or Diff-Quik or shipped to a diagnostic laboratory.

Histology. It is critical to fix tissues for histology as soon as possible after fish are killed to avoid post-mortem changes. If possible, do not use dead fish from the netpens because significant autolytic changes may occur in 15-20 min after death. Put tissues in formaldehyde based fixative (see Appendix III) for histology. Place small pieces of each organ in the fixative at approximately 1:20 (v/v) tissue to fixative. We have found Davidson's solution to be the best all around fixative for fish tissues for histological examination.

Electron Microscopy. All the above principles for histology applies for collecting specimens for electron microscopy, but freshness of tissues at fixation and proper infiltration of tissues is even more critical. Therefore, small pieces of tissue should be minced in cold glutaraldehyde based fixative into cubes about 3 mm X 3 mm. The fixed tissue is stored overnight in this solution, then transferred to the appropriate buffer solution. Samples in EM fixatives and buffers should be refrigerated. There are many EM fixatives and buffers, and Appendix III provides recipes for those we use in our laboratory. Transmission electron microscopy can be performed with limited success on tissues fixed in neutral buffered formalin, but is very poor with acidic fixatives, such as Davidson's or Bouin's solutions.

Bacteriology. Some bacterial diseases, such as bacterial kidney disease, can be identified by simple Gram stains. However, the diagnosis of many bacterial diseases requires isolation of the bacteria in culture. In this case, live fish should be delivered to the laboratory, but this may not be practical in some situations. For this reason, methods for obtaining initial cultures in the field are outlined below. The bacterial cultures can then be sent to a laboratory for complete identification.

Use only freshly sacrificed fish. Dead fish from the pens are essentially worthless. Disinfect the surface of the fish with 70% ethanol, flame-sterilize a scalpel, open the visceral cavity making sure not to cut into the gastrointestinal tract. Push aside the swimbladder with flame-sterilized forceps and insert a sterile swab or loop into the kidney. Streak the specimen on Tryptic Soy Agar and Marine Agar (Difco) bacteriological plates, seal the plates, keep at 15-25 ^{o}C, and send the plates to the microbiology laboratory for further diagnosis.

For gliding bacteria (*Cytophaga* and *Flexibacter* spp.), we recommend Marine Agar (Difco). Although the lesions may exhibit massive numbers of gliding bacteria, other bacteria (e.g., *Vibrio* spp.) will usually outgrow the former. Therefore, the best way to obtain pure cultures of gliding bacteria is to homogenize the tissue in sterile sea water, and inoculate plates in serial log dilutions.

Gram-stained preparations may reveal bacteria when they are numerous in infected tissue. Smear or imprint suspect tissues thinly on a glass slide, air dry and fix the slide by gently heating the slide over an open flame for 3-5 seconds. Gram stain kits are available from scientific supply houses and include instructions for their use.

Virology. As with bacterial diseases, isolation of viruses in culture may be required to diagnose a viral disease, and culture is best conducted on specimens collected from freshly killed fish. If this is not practical, the fish should be refrigerated for no longer than 24 hr before examination. As a last resort, fish for virus examination can be frozen. The specimens are then transported to a qualified fish virology laboratory.

Molecular Biology. DNA-based diagnostic tests utilizing polymerase chain reaction (PCR) are increasingly becoming common in fish health diagnostics because they are very sensitive and specific. Because of their extreme sensitivity, great care should be taken when collecting samples in the field to avoid cross contamination between samples. Samples are either frozen immediately or preserved in ethanol; it is usually much more difficult to perform these tests on formalin fixed material. Bleach and wash instruments between samples to avoid cross contamination.

Transport of Specimens

It is not always possible to obtain live or fresh specimens. If only dead fish can be provided for laboratory examination, they should be refrigerated and examined within 24 hr. If fish cannot be delivered to a laboratory within 24 hr, then fish should be preserved by freezing and/or fixation in tissue preservatives (see below). Each method of preservation has certain advantages and disadvantages, as indicated in Table 3-1.

Transport of Tissues in Fixative. For shipment of fixed tissues, replace fixative with 70% alcohol and soak overnight. Drain off excessive fluids, wrap tissues in alcohol-soaked paper towels, and seal in a leak-proof container.

Transport of Frozen Fish. Freeze fish in a plastic bag and ship in an insulated container with ice packs.

Transport of Refrigerated Fish. Place fish in a plastic bag and surround the bag with ice or ice packs. Ship in a leak-proof, insulated container.

Table 3-1. Preservation methods of fish tissues and their uses in fish disease diagnostic examinations.

+++ = optimal; ++ = satisfactory in most cases; + = suboptimal, can be used if no other tissue available; 0 = useless.

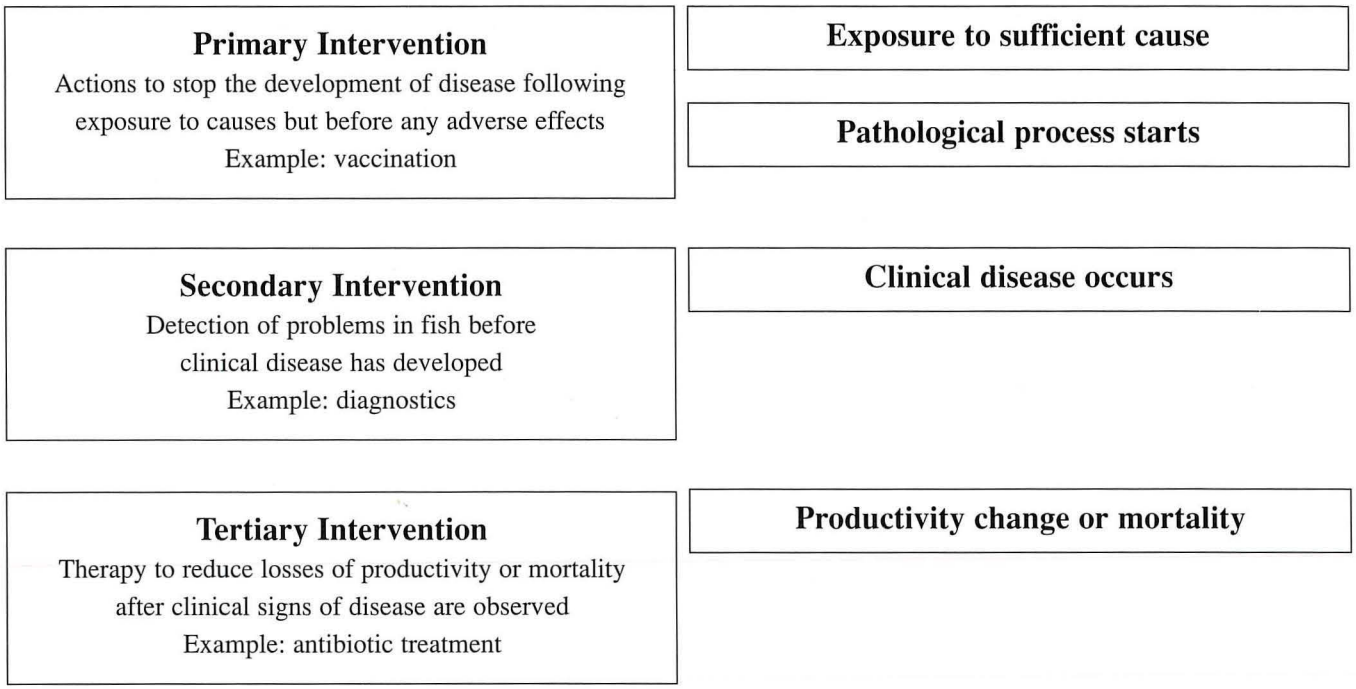
	Fresh	Refrigerated	Frozen	Preserved*
Parasitology	+++	++	++	+
Bacteriology culture	+++	+	+	0
Virology culture	+++	++	+	0
Toxicology (chemical analysis)	+++	++	+++	0 to +++
Histology	+++	+	+	+++
Electron Microscopy	+++	+	0	+++
Molecular Biology	+++	+	+++	+++

*Preserved in formalin-based fixative (e.g., Davidson's solution) for histology, glutaraldehyde-based fixative for electron microscopy, 95% ethanol for molecular biology (e.g., PCR testing). Methanol preservation is suitable for some chemical analyses.

DISEASE TREATMENT IN NETPEN AQUACULTURE

Jim Brackett and Grace Karreman

The goal of disease control, including treatment, in netpen aquaculture is to optimize production. Design of a disease control program is based on assessments of the risks of disease, the available tools to minimize risks or control diseases, and the cost-efficacy of the potential actions. Disease control can involve a broad spectrum of activities, including intervention in the disease process at different levels (Martin et al. 1987):



Intervention at all levels of the disease process, including treatment, will be required in a comprehensive disease control program.

Detection of problems

As discussed in the previous chapter, accurate and early detection of a disease problem is important to minimize impacts of the disease. Detection of changes in productivity (e.g., reduced feeding or lowered feed conversion ratios), or survivorship is critical in effective initiation of intervention and disease control. Detection of these changes necessitates complete and accurate records. Fish in netpens are easy to observe compared with wild fish. Netpen fish can be sampled and enumerated regularly, enabling early observation of developing problems. Decisions based on efforts to minimize risk and maximize production require frequent weight sampling and counting to measure performance.

Frequent collection of dead fish from netpens is an important task in disease control. Furthermore, pathogen loading in the netpen environment is reduced with frequent removal of fish dying from infectious diseases. Collection of dead fish can be accomplished by diving inside each pen and taking the dead fish to the surface for counting, examination for cause of death and proper disposal. Devices to trap and collect the dead fish can be installed at the bottom of each pen, permitting removal without diving in the pen. Dead fish should be removed at least weekly, and more frequently if numbers of mortalities increase.

The number of dead fish from each individual pen should be recorded and compared with the number of fish in the pen to calculate a daily or weekly rate of mortality. Any change from "baseline" mortality rates should be identified quickly and the appropriate response initiated. The pattern of changes in mortality rate over the seasons or over the production cycle can

direct modifications in production management and disease control activities.

Risk factors

Many risk factors may be involved in production of a disease outbreak in pen-reared fish. Identification of these risk factors and determination of their relative importance in the outbreak will help in directing effective treatment and control efforts. Risk factors in production of disease are both intrinsic and extrinsic. Intrinsic factors include the general health of fish, their specific and non-specific immune status, species and age of the fish, etc. Extrinsic risk factors include the water temperature, reservoirs of disease organisms, etc.

The density of the fish in a netpen can be a significant risk factor for infectious diseases. Under some circumstances, density of the fish can affect general health through effects on feeding behaviour, physical trauma of the fish, territorial and social behaviour. For highly infectious diseases, the density of the fish in the netpen may affect the probability of effective contact and, therefore, the likelihood of infection and disease development.

Identification of a reservoir of the causative pathogen is an important component of managing risk factors. Continued exposure to the pathogen source will affect the selection and success of treatment and control measures. Management changes to reduce the exposure to the reservoir of infection will likely be more successful in disease control than repeated treatments. Treatment of a specific disease may have decreased success if other disease agents present significant risks at the same time. In these situations, control of all of the factors may be necessary to achieve satisfactory disease control. Predators can represent important risk factors for many infectious diseases. If exposure to these predators is not restricted, repeated outbreaks of infectious diseases may occur.

Farm Records and Decision-Making

Farm production records are a primary component of a successful and effective decision-making process for disease treatment. All of the factors discussed previously must be considered in light of the records that should include, at the minimum, descriptions of the growth of the fish and mortality rates. Mortality rate is associated with rate of disease spread but must be interpreted carefully. Most farms have an implicit threshold at which the disease losses are high enough above normal to contact a veterinarian for further assistance (e.g., 1%/month). But more important than the threshold for concern is the pattern of the mortalities and the age of fish in which the losses are occurring.

Figure 4-1 illustrates four patterns of mortality in which the cumulative mortality is identical (17%) but the patterns are very different. The rate at which mortality accumulates is illustrated in the second curve of each graph. These particular examples are based on an actual site at which harvesting commenced at 15 months. Examples A and B suffered most of their losses prior to harvest whereas example C, and particularly D, had ongoing mortalities during the harvesting period.

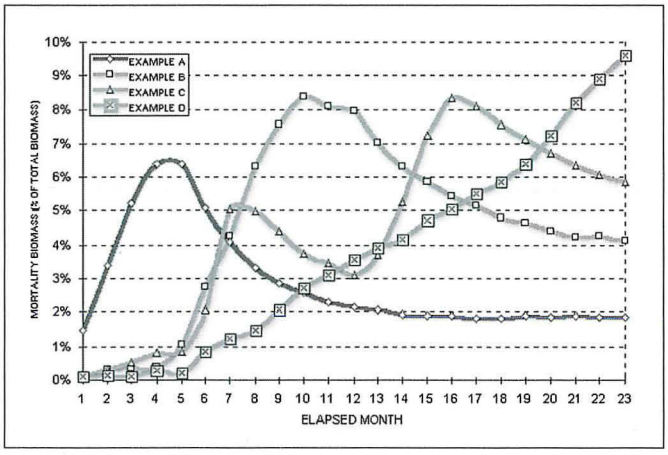


Figure 4-1. Different mortality patterns at farm sites over a 23 months growing cycle. A. Most of the mortalities occur in the first 8 months. B. Mortalities start later but most have occurred before 13 months. C. A bimodal pattern that starts at approximately 4 months and ends after 18 months. D. A steady rate of loss occurring throughout the grow-out cycle.

The percentage of the biomass lost to mortality is heavily influenced by the timing and the pattern of the mortalities. Biomass is the product of fish numbers and weights and is the preferred index for measuring inventory in the water. For this example the growth curve is unchanged though the mortality rate and pattern differs. Figure 4-2 shows the ratio of mortality biomass to total potential biomass (i.e., if there had been no losses due to mortality). The end ratio for example A is slightly less than 2% while that for example D is almost 10% because losses occurred so late in the cycle.

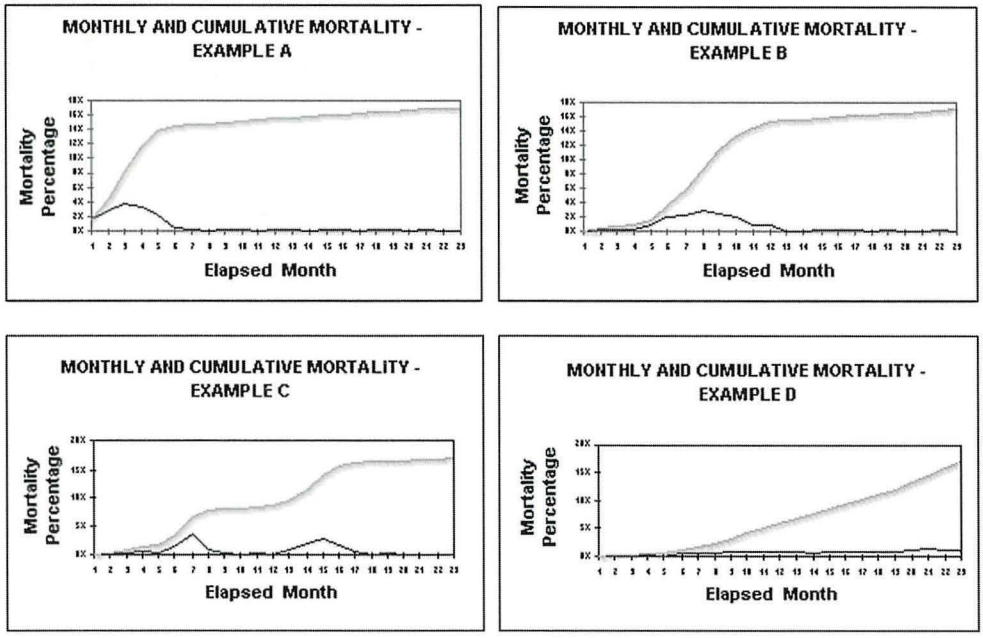


Figure 4-2. Mortality by biomass percentage compiled from Figure 4-1.

Providing a treatment for the disease exists, clearly it is beneficial to treat. Primary husbandry should always be addressed—there may be benefit in decreasing the density, to stop feeding for a certain period or decreasing existing stress if it can be properly identified. In cases of diseases that are not zoonoses and have no effect on product quality, early harvest of the pen of fish may represent the most economically reasonable option for disease management. Sometimes the use of chemotherapeutants is indicated, and these are most often administered in feed. These have associated costs: 1) the cost of the medication including milling and transport; 2) medication may decrease the feeding rate and thereby the growth; and 3) the withdrawal period ("drug free" period after treatment) may fall into the period when treated fish would normally be harvested. The cost benefit analysis will also help to direct the choice of treatment when more than one option is available. The lower cost treatment may not be the best choice if a higher cost product can result in more rapid results or greater efficacy.

Estimating the costs vs. benefits of a therapeutant treatment implies knowledge of several economic realities about the site. Figure 4-3 shows the relative shapes of the curves for biomass (the product of the growth and survival curves), cost of production and feed conversion ratio. Each site must know its predicted growth rate and feed conversion ratio's, as well as the "normal" mortality rate that might continue after treatment is finished. For example, while therapeutants may put fish off their feed during administration, afterwards there may be a rebound, or "compensatory" growth period. More difficult to determine but certainly true in terrestrial livestock, is that treatment of the entire pen might also help those fish with subclinical disease (i.e., those that have a slightly depressed growth rate but no other outward clinical signs). Finally, and most important, the site must know the desired harvesting schedule, including the predicted market weight and selling price of those fish under that schedule.

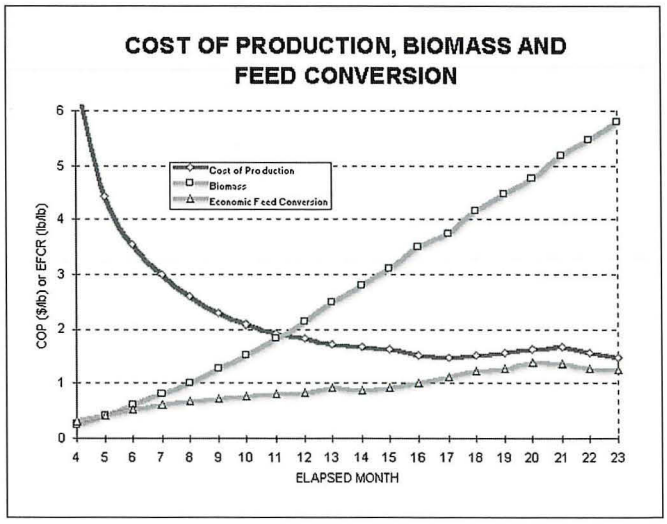


Figure 4-3: Cost of Production, Biomass and Feed Conversion Ratio Relationships

Table 1 illustrates some of the economic consequences of losses during the production cycle. There are many ways to calculate these numbers. For simplicity in our hypothetical examples, we fixed growth rate, the harvesting pattern and selling prices. In each example we allowed the input costs (feed, smolt cost, labour and others) to vary proportionally to

the number of fish left in the pen after the mortalities were taken out each month. Proceeding again from example A to D, there is a rise in cost of sales and corresponding decrease in margins. While our assumptions exaggerate this difference, this indicates the disproportional effect of having to feed large fish toward the end of the cycle which will not make it to harvest. For a 200 metric tonne (450,000 lb.) site, a $.10/lb difference in margins is worth $45,000. Whereas most therapeutant treatments are expensive, on this site there would be a favourable cost/benefit ratio for treatment.

Table 1. Economic Consequences of Losses

	Example A	Example B	Example C	Example D
Cost of Sales	$2.29	$2.34	$2.37	$2.45
Gross Margin	$0.94	$0.89	$0.86	$0.79

Selection of appropriate treatments

All therapeutants used to treat diseases of fish intended to be harvested for food must be "approved" and used according to federal and provincial legislation. In Canada, drugs regulated federally by the Bureau of Veterinary Drugs and are defined in the Food and Drug Act as "any substance or mixture of substances used in (a) the diagnosis, treatment, mitigation or prevention of a disease, disorder or abnormal physical state, or symptoms thereof, in man or animal." Pesticides are products applied externally to control parasites of fish and are regulated in Canada by the Pest Management Regulatory Agency. Vaccines regulations are administered in Canada by the Canadian Food Inspection Agency. These regulations ensure that the products used in disease control in food fish are safe and effective and do not leave harmful residues in food.

In addition to compliance with all regulations in the country where the fish are grown, the regulatory requirements of the countries to which fish and fish products are exported must be met. While harmonization of drug use legislation and requirements is sought by Canada, the United States and many other countries, there remain different standards that must be considered when selecting treatment choices in fish that might be exported after harvest. Drug use and potential residues are an important component of food inspection procedures in Canada and in the new Hazard Assessment at Critical Control Point (HACCP) program instituted in the United States.

Antibiotics

As in all animal production, the use of antibiotics to control bacterial infections are an important component of disease control. All animals are frequently confronted with bacterial infections and disease, and in netpen fish culture, treatment with antibiotics might be required to reduce morbidity and mortality to acceptable levels. Usually, antibiotic treatment is a component of a disease control program that also includes preventative actions such as vaccination, disinfection, screening of broodstock and other management practices.

Antibiotic treatments occur when the disease is observed clinically, that is, late in the development of the disease. The goal of producers is to prevent the development of the disease to this stage; however, when disease does occur, treatments are initiated as soon as the disease manifestations can be detected. Early identification and treatment of problems requires close observation, accurate records of mortalities and production rates, along with examination of dead or dying fish.

As netpen culture of salmon progresses, there is a strong trend to lower antibiotic use. This reduction arises from improved husbandry practices, lower mortality rate, faster detection, earlier treatment, prevention of infections by screening, disinfection and protection by vaccines for the most important diseases. Mortality rates in netpen salmon in British Columbia, Canada have dropped by 40 to 60% from 1990 to 1994 (Figure 4-4).

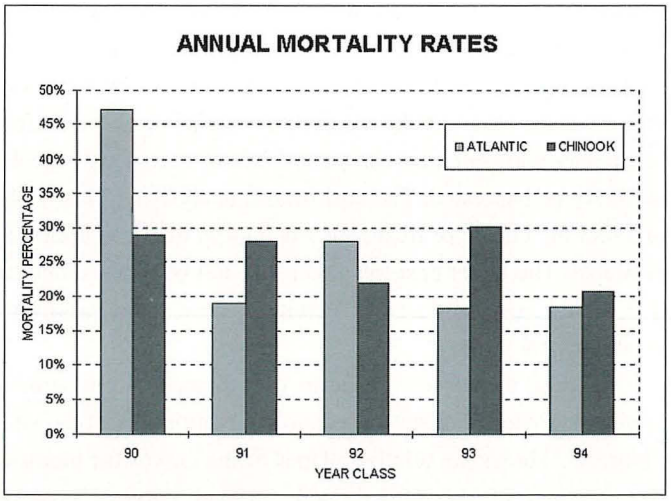


Figure 4-4. 1990-1994 mortality rates in chinook and Atlantic salmon in British Columbia

In Norway, losses due to disease have declined from 25% of the 1988 generation of Atlantic salmon to approximately 7% of the 1995 generation, while antibiotic use has declined from 592 grams per tonne of growth in the 1987 generation to 3 grams

per tonne of growth in the 1996 generation. In British Columbia, antibiotic use in feed in 1995 was reduced to 156 grams per tonne of production, a decline of 23% from the previous year (British Columbia Ministry of Agriculture Food and Fisheries 1997)

Selection of antibiotics. Antibiotics commonly used in netpen fish are also used in other animal production industries. There is a long history of safety of these compounds for use in food-producing animals and the efficacy against common pathogens is well-known. The label of an approved antibiotic will indicate the diseases that the antibiotic has been demonstrated to control. Clinical experience of the fish health staff and the prescribing veterinarian will also be helpful.
When pathogenic bacteria are cultured and identified as the cause of a disease problem that requires antibiotic treatment, an antibiotic sensitivity test should be done on the isolate. The sensitivity of the isolate to the antibiotics will guide in the selection of the antibiotic to treat the fish. Laboratory testing for antibiotic sensitivity should be used only as a guide, as clinical results will not always reflect the laboratory findings. Antibiotic sensitivity testing results are affected by many aspects of the testing (Smith et al. 1994). The methodology of the testing, including bacterial incubation, plate preparation, media used, antibiotic discs used and the measurement and interpretation of the zones of inhibition are not standardized among or within diagnostic laboratories and can have an influence on the test results, repeatability and comparisons with results from elsewhere.

Residues. The prevention of residues of an antibiotic in fish intended for human food is the primary consideration for use of a product in food fish. The antibiotics used in netpen fish have been tested to determine an appropriate time between the last use of the compound and harvest of the treated fish ("withdrawal time"). This time will depend on the dose of the drug, the metabolism, distribution and elimination in the fish, and the temperature of the environment during and after treatment. Data is generated for government agencies demonstrating the safety of the compound in humans. The expected amount of time between treatment and planned harvest of the fish will also be a consideration in selection of antibiotics. Antibiotics with a shorter withdrawal time would be a better choice in larger fish that are closer to the time of harvest.

Rotation of drugs. When more than one antibiotic is available and likely to be successful in treating a disease, rotation of the products for subsequent outbreaks in a pen or on a farm is recommended. This is common practice in human and animal medicine, and is intended to help reduce problems of resistant populations of bacteria. Repeated use of the same antibiotic places selective pressure on the bacterial population and encourages expression of antibiotic resistance in bacteria. This resistance is not permanent and will disappear when the selective pressure is removed, but may restrict the success of disease control efforts, especially if the number of antibiotics available for use is limited.

Medicated feed. Delivery of drugs in medicated feeds is the most common method of treating fish in netpens. It is the least labour intensive and least stressful for fish. Antibiotics are most commonly added to the feed during milling at the end of the pellet manufacturing or can be hand mixed at the farm site. Care must be taken when mixing medicated ingredients to ensure proper safe handling and calculation of proper dosages. Mixing instructions are provided on product labels and designated in the requirements for licensed production of medicated feeds. In Canada, guidelines and instructions are contained in the Medicating Ingredients Brochure for feed mills. Commercially prepared medicated feeds are available from a variety of manufacturers and require a veterinary prescription (for prescription drugs).

There are, however, disadvantages associated with administration of antibiotics in feed that can impact on the effectiveness of antibiotic treatment. Unhealthy fish are often reluctant to accept feed resulting in variation in the total amount of feed consumed and the dose administered for treatment. Palatability problems have been encountered with certain antibiotics and can exacerbate this problem, further reducing the effectiveness of treatment. Inaccurate accounting of the number of fish per pen and inaccurate estimation of the average weight of the fish at the time of treatment can also affect the effectiveness of the therapeutic regime. When calculating the total amount of therapeutant for a population of fish, several factors should be considered. In most cases the antibiotic is provided as a pre-mix and not in its pure form. Calculating the correct dose requires a knowledge of the total biomass of the population and rate of administration of feed per body weight. Some antibiotic formulations interact with components of the feed or elements in the marine environment which decrease the bioavailability and efficacy of treatment. When choosing an antibiotic for treatment of a bacterial infection, consideration should be given to these factors to maximize the effectiveness of treatment.

DRUGS USED IN NETPEN AQUACULTURE

Antibiotics

Oxytetracycline. This is the most commonly used antibiotic in netpen culture in Canada. This compound has a long history of use in animal production and is effective in the control of common bacterial infections of fish. It is approved for use in food fish in Canada (TM Aqua, Pfizer). Common uses of oxytetracycline in netpen salmon culture include the treatment of furunculosis (*Aeromonas salmonicida*) (Groman et al.1992; Mitchell 1992) and vibriosis caused by various *Vibrio* spp. Another use for oxytetracycline is the treatment of bacterial kidney disease (*Renibacterium salmoninarum*). These treatments are delivered orally by incorporating the drug premix in the feed. Injection of oxytetracycline into the dorsal sinus or into the peritoneal cavity of netpen fish has been used in some circumstances, but is limited by the difficulties and risks associated with the handling of an entire pen of fish for injection procedures at a time when a disease is causing problems. Oxytetracycline is also used to help control bacterial kidney disease and furunculosis in broodstock fish not intended for food. These treatments are by injection of a long-acting oxytetracycline product into the dorsal sinus.

Bacteria commonly develop resistance to oxytetracycline following repeated use in salmon. This phenomenon is also seen with disease treatment with oxytetracycline in other species as well. The resistance is temporary and rotation of other drugs into a treatment regime, and ensuring proper dosage and consumption of all of the medicated feed by the fish will minimize problems associated with resistance.

A potential problem with the use of oxytetracycline in fish treatments is the ability of calcium and other cations in the feed and water to bind with the oxytetracycline molecule. This binding will reduce the bioavailability of the drug and could affect clinical results. In spite of this low bioavailability, positive clinical results with oxytetracycline are observed.

Potentiated Sulfonamides. These consist of a mixture of two active ingredients: sulfadimethoxine and ormetoprim (Romet 30, Roche) or sulfadiazine and trimethoprim(Tribrissen, Schering). The two active ingredients attack bacteria at sequential metabolic processes, increasing efficacy and decreasing the potential for development of resistance. Both Romet 30 and Tribrissen have been accepted for use in food fish in Canada. They are commonly used for treatment of furunculosis and vibriosis by delivery in feed (Mitchell 1992; Maestrone, 1984). Both of these products can be used in rotation with oxytetracycline for these applications, although the similarity of the two potentiated sulfonamides means that bacterial resistance to one of them will also preclude the use of the other. Under some conditions, potentiated sulfonamide products may inhibit feed consumption in treated fish. This reduced feeding can make it difficult to deliver the entire course of treatment to affected fish and can reduce treatment efficacy.

Florfenicol. A newly approved antibiotic in netpen aquaculture in Canada is florfenicol (Aqua Flor, Schering). Aqua Flor has shown good efficacy for treatment of furunculosis and other diseases (Nordmo et al. 1994, Sheppard et al. 1994). It is administered in the feed. Florfenicol is a valuable product to use in rotation with oxytetracycline and the potentiated sulfonamides because of low levels of resistance observed in pathogens and because cross-resistance induced by other antibiotics is unlikely.

Erythromycin. This antibiotic is commonly used for the control of bacterial kidney disease. In netpen culture, the most common method of use is by dorsal sinus injection in broodstock (Evelyn et al. 1986a; Brown et al. 1990; Lee and Evelyn 1994). The compound is concentrated in the developing eggs during a time window prior to spawning, resulting in levels that persist through egg development to help reduce infection in the eggs. Oral treatment with erythromycin is not commonly carried out in netpen fish. Problems with palatability with erythromycin salts and damage to the compound during feed manufacturing have been limiting factors. Furthermore, erythromycin treatment can be relatively expensive, making cost-efficacy a significant issue.

Parasiticides

The most common parasites causing problems in netpen salmon aquaculture are sea lice (*Lepeophtheirus* sp. and *Caligus* sp.). Parasiticides are used to control the parasites when their numbers reach levels that result in problems. Control and treatment considerations are discussed indepth in the section on sea lice.

In Canada, sea lice have been treated with Azamethiphos (Salmosan, Ciba Geigy) under a limited registration. The compound is administered by bath treatment. Ivermectin products can be used for oral treatment under veterinary prescription. Insect growth regulators which inhibit chitin synthesis are currently under investigation for oral treatment. Risk factors for sea lice problems and non-therapeutic control measures are the focus of investigations in the industry and an Integrated Sea Lice Management program is under

development in Canada in collaboration among producers, support industries, academic institutions and government agencies.

Formalin (Parasite S, Western Chemicals, Syndel) is used for the control of fungal egg infections and external parasites in fresh water, but has had only limited use in sea water applications. Attempts to control costiosis with formalin after transfer to salt water have not been successful. Furthermore, management of bath treatments with formalin in netpens is problematic.

There are no compounds commercially available for treatment of systemic protozoal infections. However, fumagillin has been used experimentally in the laboratory and in limited field trials. Fumagillin treatments are discussed further in pages 57–58.

Anesthetics

Anesthetics are an important tool in effective management of netpen fish production. In netpens, they are most commonly used for weight sampling and for handling of broodstock for sorting and for spawning. As indicated earlier, accurate information on size and growth is essential in optimizing netpen production. In some circumstances, for example, the identification of a new disease risk, or availability of a new vaccine, netpen fish are anesthetized for vaccination in saltwater. Obviously, the disease risk and expected vaccine efficacy must outweigh the expense and risks of handling fish in the netpens.

Fish in a netpen can be introduced to an anesthetic bath by crowding them into a smaller volume and then pumping or netting the fish to deliver them to the anesthetic. In all cases, gentle and careful handling is required to avoid damage and reduce stress. Because it is not easy to observe fish during recovery in netpens, a recovery tarp suspended just below the surface is recommended to assist in monitoring recovery.

Tricaine Methanesulfonate. Tricaine methanesulfonate (TMS Powder, Syndel) is the most commonly used anesthetic for netpen fish in Canada. The dose can be adjusted to achieve the desired time for induction and for recovery of the fish. Because treated fish can be harvested 5 days after treatment, the product can be used even with large fish and can be used to assist in developing harvest plans.

Metomidate. This compound marketed as Marinil (Wildlife, Syndel) is used for sedation and anesthesia of fish. It is approved in Canada for several non-food fish species. In netpen aquaculture, it is used for sedation of smolts during delivery to saltwater sites. At low doses, it reduces activity and stress in the transported fish. Higher doses produce general anesthesia. Marinil is used for weight sampling, similarly to tricaine methanesulfonate. Fish are crowded and placed in anesthetic bath tanks. Following handling, fish are returned to recovery tanks or to shallow netpens for observation until recovery is complete. The dose of Marinil, and its sedative or anesthetic effect on the fish, can be easily and accurately adjusted to achieve the desired results.

Benzocaine. This is similar in activity to tricaine methanesulfonate but is not soluble in water. Organic solvents are required to dissolve benzocaine before mixing in the anesthetic bath. Benzocaine effects on fish are almost identical to tricaine methanesulfonate. Benzocaine is not approved for use on fish in Canada.

Vaccines

Injection vaccination of salmonids has dramatically reduced losses caused by several diseases in netpen culture. An excellent review of the status of vaccines for fish as of 1996 is found in Gudding et al. (1997). Commercial vaccines at this time are derived from killed, whole cell antigens. However, there have been recent advancements in the development of vaccines using recombinant DNA technologies (Leong et al. 1997). Furunculosis and vibriosis, including cold water vibrosis, are well-controlled throughout the entire sea water production phase following vaccination of smolts in fresh water prior to moving to netpens. In almost all cases, a single injection of oil-based emulsion vaccine containing a mixture of bacterins for the common diseases is adequate for protection for two years or more.

Additional vaccines under development or in limited use during development include products for the control of infectious hematopoietic necrosis, infectious pancreatic necrosis, bacterial mouthrot, bacterial kidney disease, and rickettsia infections. Efforts are underway to improve efficacy and reduce potential adverse effects of existing products. Enhanced production of antigens and changes in adjuvant systems are among the areas of development.

Vaccines used in Canada are licensed for use by the Canadian Food Inspection Agency, similar to government agencies in other countries. Products must meet stringent standards for potency and safety to satisfy licensing requirements. There are ongoing efforts to harmonize regulatory standards for fish vaccines in several countries to permit more rapid and cost-effective commercialization of vaccines.

In some cases, vaccination of fish in netpens is

recommended. This situation usually arises when a new vaccine becomes available after fish have already been moved to sea water. Fish are crowded, placed into anesthetic baths and vaccinated by crews on barges or boats beside the netpens. Obviously, the risks of handling the fish and the costs of administration of the anesthetic and vaccine must be weighed against the potential reduction in losses due to the disease in question.

Chinook salmon in netpens can be vaccinated by immersion to enhance protection to vibriosis. Fish are crowded in the netpen and placed into a tank containing the vaccine, then returned to the netpen. This vaccination is a booster for previous immersion vaccinations performed while the fish were in fresh water. The repeat vaccination is timed so that the fish have had time to adjust to the move to sea water, but before the expected challenge by *Vibrio* spp. a few weeks post-transfer.

The decision to vaccinate is based on assessment of the risks of encountering the diseases and the expected protection provided by the vaccine. The costs of the vaccine and the vaccination process, generally less than $Can 0.15 to 0.20 per fish, must be compared with the potential costs of mortality, reduced growth and disease treatment.

Vaccines and vaccination may induce adverse effects in vaccinated fish. Adverse reactions can include reduced growth due to post-vaccination anorexia, peritoneal adhesions or melanization from vaccine-induced inflammation (see page 102). Other adverse reactions include post-handling problems such as fungal dermatitis or clinical outbreaks of other diseases in carrier or sub-clinically diseased fish. These adverse effects and the associated costs of reduced production must be considered in the cost-benefit analysis performed to assist in design of vaccination programs and selection of vaccines.

The protection provided by vaccines can be overwhelmed if fish are exposed to large challenges or are immunologically compromised by factors such as poor nutrition or sub-optimal rearing conditions. The important risk factors for diseases must be reduced and farm management practices must be optimized in disease control programs that include vaccination.

BACTERIAL DISEASES

T. P. T. Evelyn, M. L. Kent, T.T. Poppe, and P. Bustos

A number of bacterial diseases cause serious and recurring losses in pen-reared salmon; others are emerging diseases of some concern. One of the important diseases, bacterial kidney disease (BKD), is caused by *Renibacterium salmoninarum*, a Gram-positive bacterium. The other important or emerging bacterial diseases of pen-reared salmon are caused by Gram-negative bacteria: typical vibriosis, caused by *Vibrio anguillarum* and *V. ordalii*; cold-water vibriosis or Hitra disease caused by *V. salmonicida*; winter ulcers caused by *Vibrio* spp.; furunculosis, caused by *Aeromonas salmonicida*; yersiniosis, caused by *Yersinia ruckeri;* myxobacteriosis, caused by *Cytophaga-Flexibacter* spp.; and systemic diseases such as salmonid rickettsial septicemia or piscirickettsiosis, caused by rickettsias, in particular *Piscirickettsia salmonis*. All salmon species reared in netpens are susceptible to these bacterial diseases, but some diseases are more problematic in certain species than in others. For example, chinook, coho, and sockeye salmon appear to be more susceptible to BKD than Atlantic salmon, whereas furunculosis and myxobacteriosis are more of a problem in Atlantic salmon than in Pacific salmon species.

Bacterial diseases probably cause more mortality in pen-reared salmon than diseases due to other infectious agents. However, unlike most other diseases of fish, there are commercially-available drugs that may be effective for treating bacterial diseases. In addition, BKD and furunculosis can be managed to a certain extent by screening fish and avoiding infections, and efficacious vaccines are available for vibriosis, furunculosis, and yersiniosis.

Bacterial Kidney Disease

Bacterial kidney disease is probably the most significant cause of mortality in pen-reared chinook and coho salmon, but Atlantic salmon are also susceptible to the disease, although to a lesser degree (Brackett et al. 1991). In addition, sockeye, pink, and chum salmon are extremely susceptible to BKD (Brett et al. 1978; Evelyn 1988a), but these species have thus far been reared in netpens mostly on an experimental basis. The causative agent, *Renibacterium salmoninarum*, is a non-motile Gram-positive bacterium that infects inflammatory cells, primarily macrophages, and produces a systemic infection. It induces severe, chronic inflammation in the kidney, other visceral organs, the eye, brain, and to a lesser extent the muscle.

All ages of salmon are susceptible to BKD. The bacterium is transmitted vertically through the egg (Bullock et al. 1978; Evelyn et al. 1988a). Clinical disease may occur in fresh water. In addition, fish can carry the infection with them to sea water, and may serve as a source of infection for their cohorts. In netpens, these fish may show clinical disease shortly after introduction to netpens. However, most epizootics start in the first winter following the introduction of the fish to netpens, and peak in the following spring. These epizootics are probably due to horizontal transmission of the pathogen from heavily infected older fish that are already on the farm when multiple year classes are reared at the same site. The seasonality of BKD appears to be diminishing, and outbreaks have been observed throughout the year.

CLINICAL SIGNS AND GROSS PATHOLOGY. Fish with BKD can exhibit a variety of macroscopic changes, often depending on whether the disease is present in acute or chronic form, or on the primary location of the infection. Affected fish may be dark, lethargic, and exhibit a swollen abdomen due to ascites. Some fish may exhibit exophthalmus and other eye lesions, and multiple blood-filled blisters may be present on the skin. The latter are particularly common on fish undergoing sexual maturation, and is often referred to as "spawning rash" (Fig. 5-5). The gills are usually pale due to anemia (Fig. 5-1b). Internal examination usually reveals multifocal, greyish-white nodules in the kidney; these lesions may also occur in the spleen and liver (Fig. 5-1a). Some fish may exhibit only a few, very large granulomas, whereas others show miliary lesions throughout the viscera. Formation of a white, diffuse membranous layer (pseudocapsule) over the spleen and heart is also a frequent finding (Fig. 5-1a). In acute cases, where the lesions are very diffuse, the granulomas may not be visible to the naked eye. The kidney and spleen are usually enlarged, and serosanguineous or cloudy fluid often accumulates in the visceral cavity. Hemorrhages are often observed in the liver, intestine, pyloric fat, and muscle. Large, focal, cystic cavities may also occur in the skeletal muscle. In some cases, the meninges are the only tissues in the fish that are severely affected (Speare et al. 1993; Speare 1997). These fish usually exhibit spiralling or whirling swimming behavior, and usually show no macroscopic changes in the viscera.

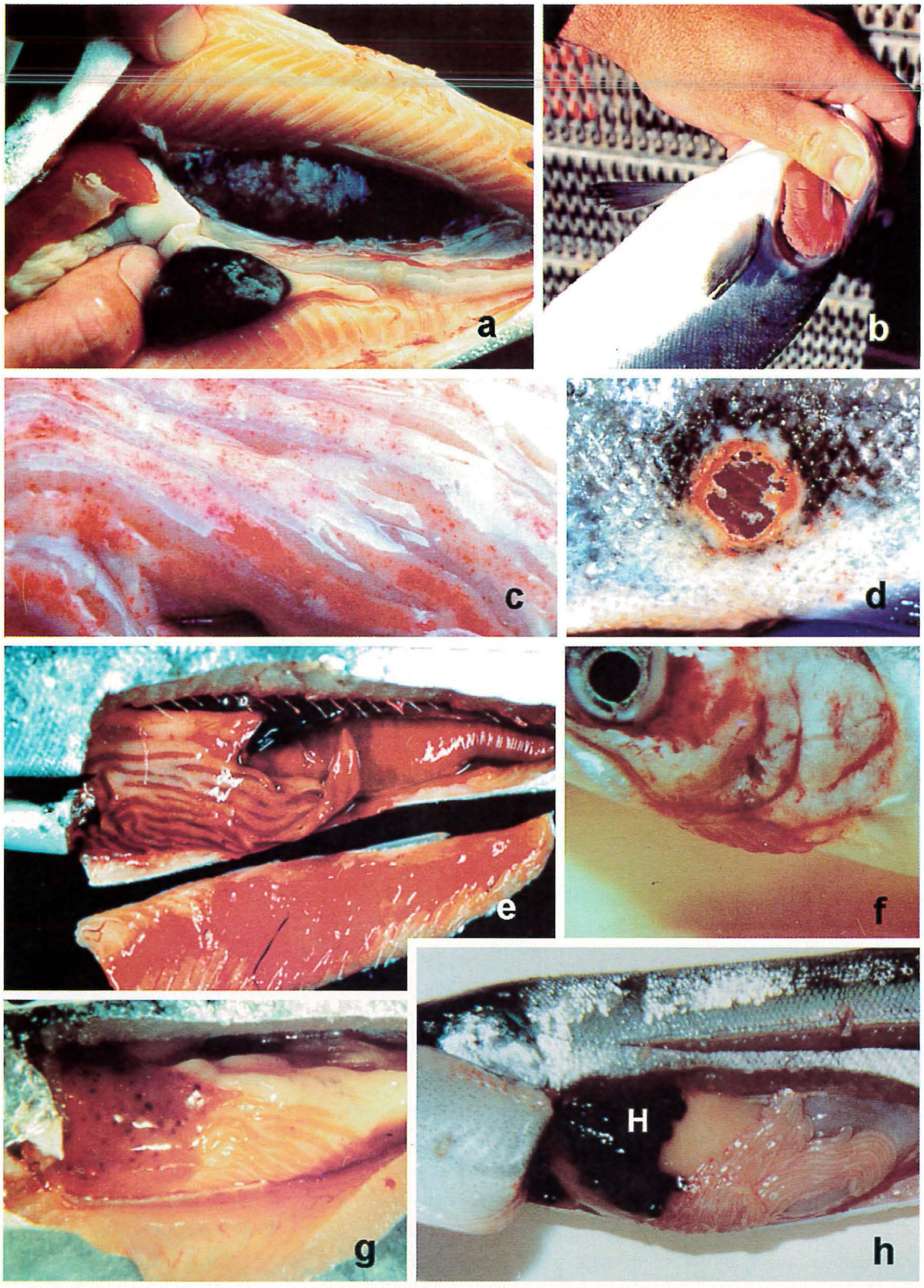


Figure 5-1. a and b, Coho salmon with BKD. a. The kidney is enlarged and has focal, white, granulomatous lesions, and a pseudocapsule covers the enlarged spleen b. Pale gills due to anemia. **c-e. Bacterial diseases in Atlantic salmon.** c. *Vibrio salmonicida* infection. Note petechial hemorrhage in the perivisceral fat between the pyloric caeca. d. Winter ulcer in Atlantic salmon skin. e. Furunculosis. The viscera exhibits extensive hemorrhage and the lower intestine is enlarged. **f-h. Chinook salmon post-smolts with vibriosis.** f. Hemorrhages in the skin on the head and operculum. g. multifocal hemorrhages in the liver. h. large, coalescing hematomas (H) in the liver.

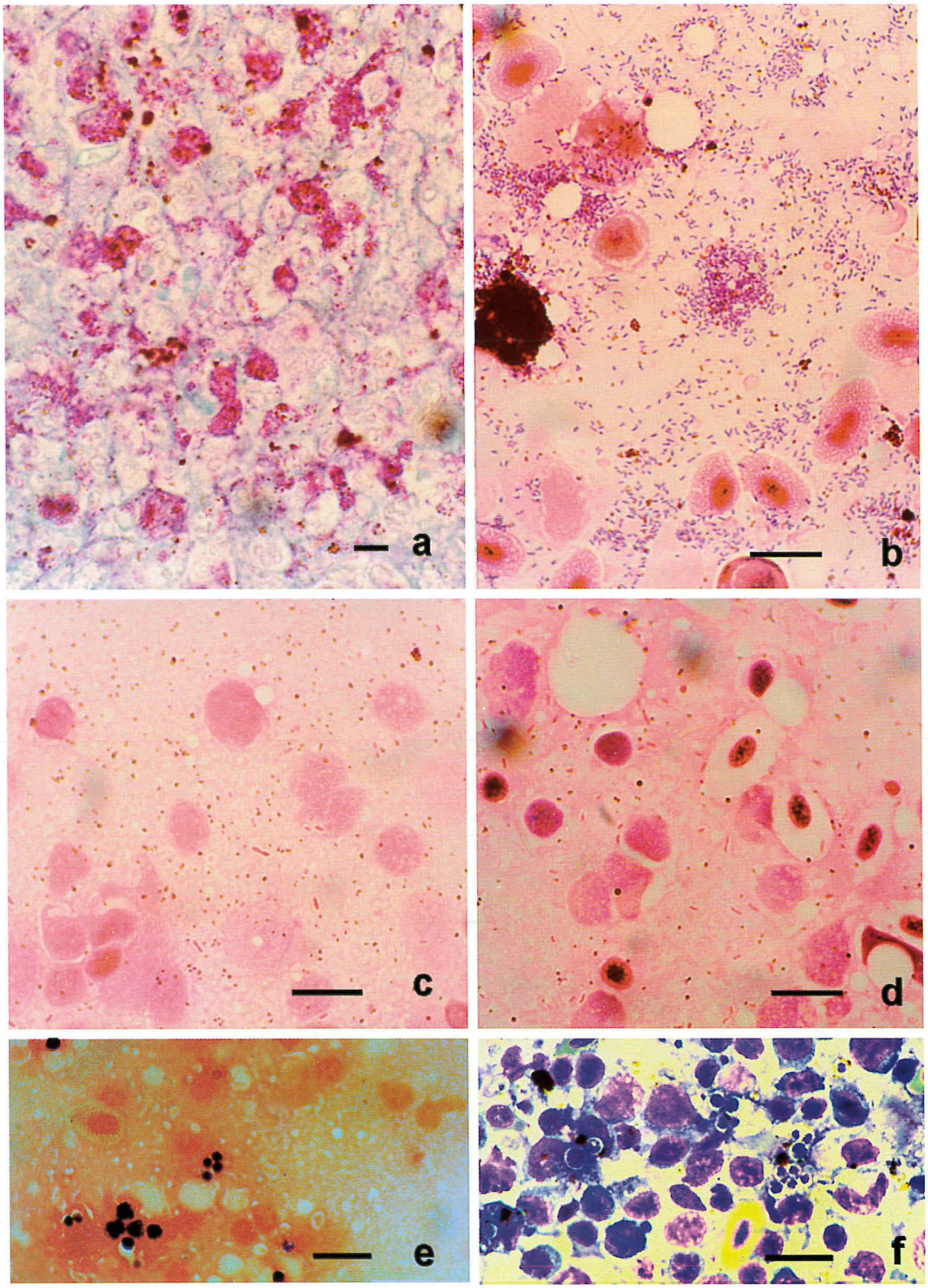


Figure 5-2. Microbial pathogens of pen-reared salmon in tissue smears, imprints stained or histological sections from kidneys. Bar = 10 µm a. kidney section of chinook salmon with BKD stained with Periodic acid-Schiff (PAS). Note the PAS-positive (red) bacteria in phagocytes in the kidney interstitium. b. *Renibacterium salmoninarum*, Gram. c. *Vibrio anguillarum*, Gram. d. *Aeromonas salmonicida*, Gram. e. rosette agent, Gram. f. rosette agent, Giemsa.

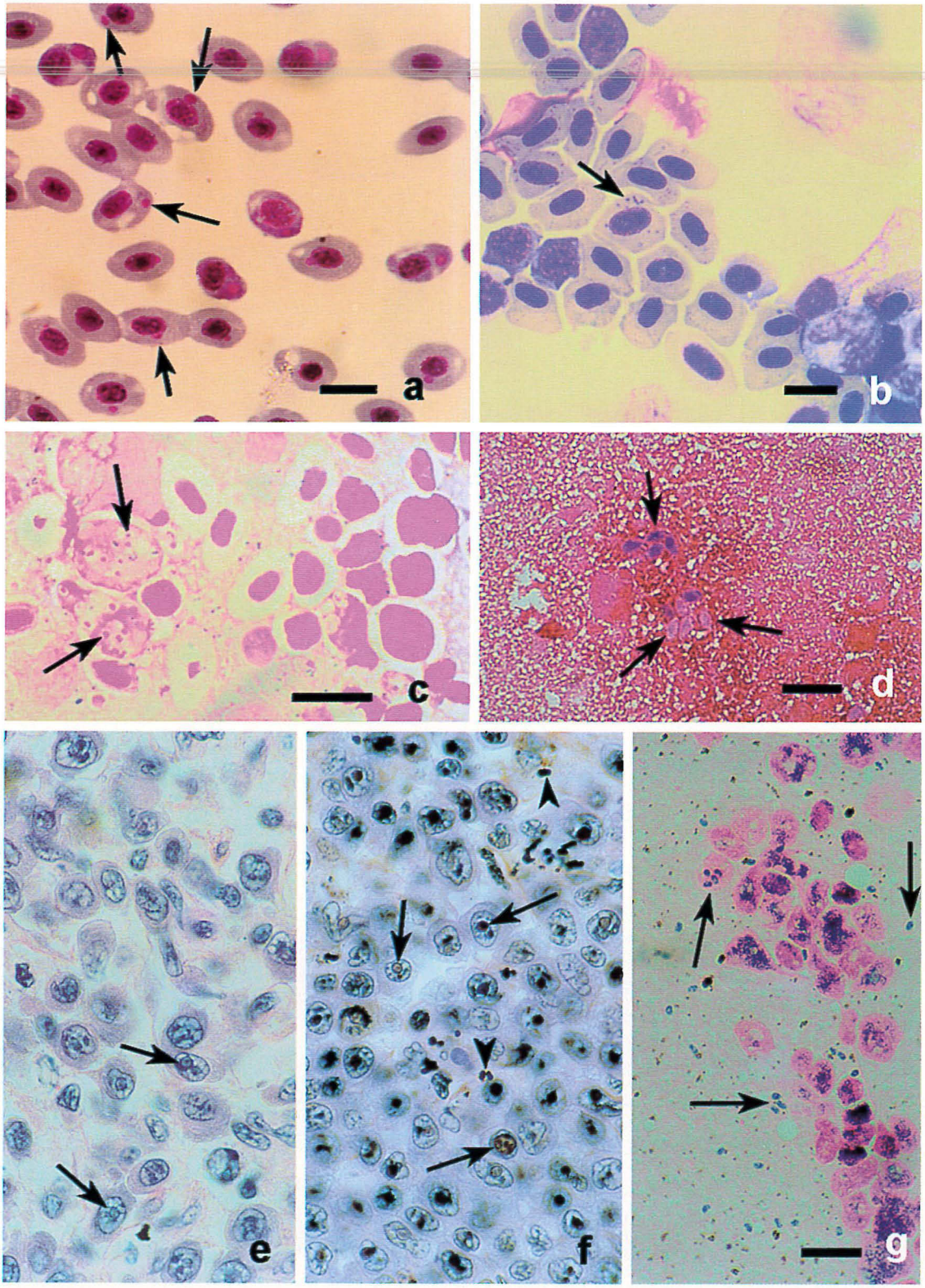


Figure 5-3. Microbial pathogens (arrowheads) of pen-reared salmon in blood smears, imprints stained or histological sections. Bar = 10 µm. a. VEN inclusions in red blood cells, Giemsa. b. EIBS inclusions in red blood cells, Giemsa. c. *Piscirickettsia salmonis*, Gram stain of kidney imprints. d. Spores of *Loma salmonae*, Gram stain of kidney imprints. e, f. *Nucleospora salmonis* in histological sections (arrows) e. H&E. f. Warthin-Starry/H&E. Arrowheads = spores. g. *Nucleospora salmonis*, Gram stain of kidney imprint.

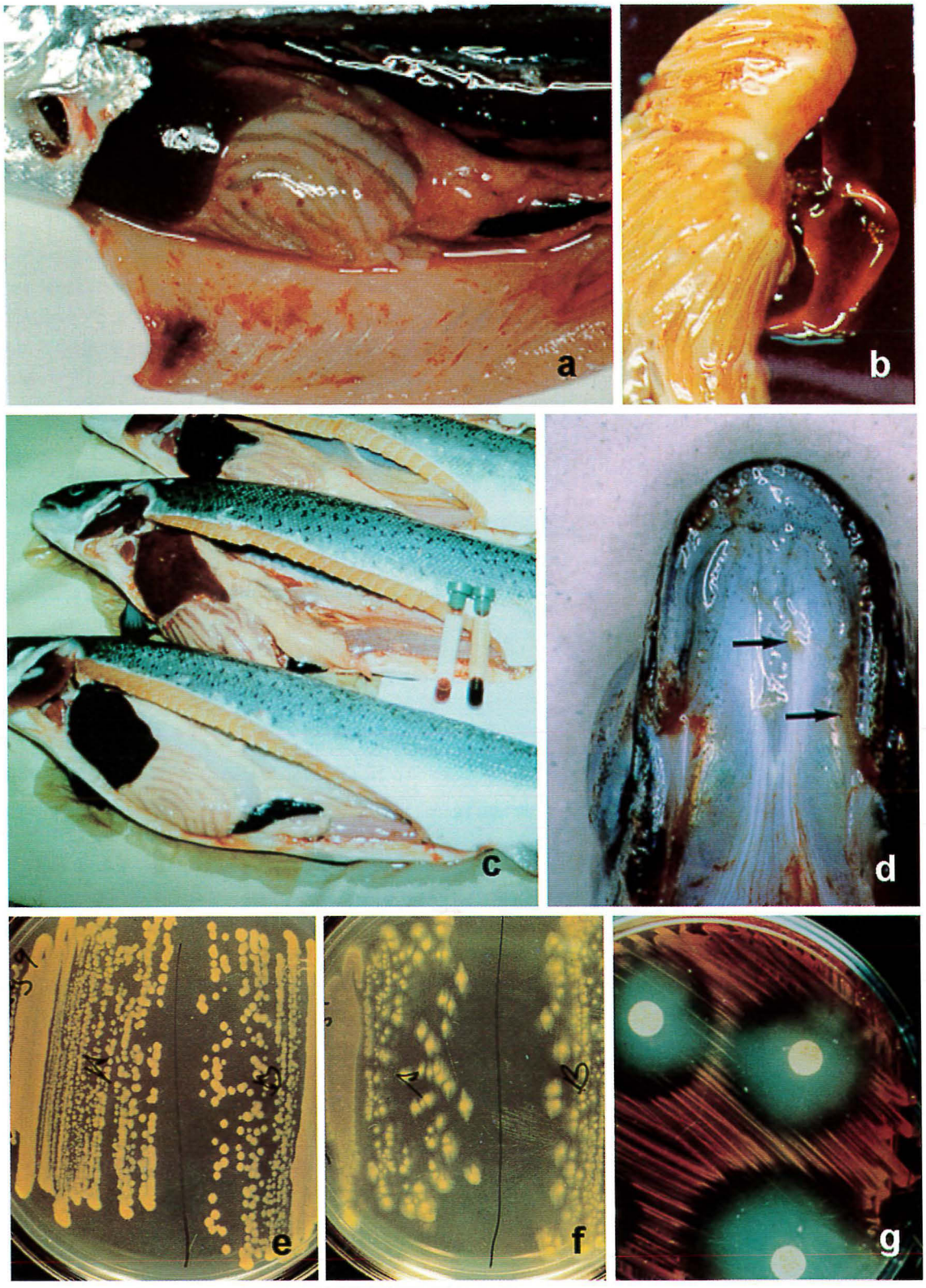


Figure 5-4. Microbial infections and bacteria culture plates. a. IHN infection in Atlantic salmon. Note visceral hemorrhages. b. Petechial hemorrhage in peripancreatic fat around pyloric caeca in Atlantic salmon with pancreas disease. c. ISA in Atlantic salmon. Note dark livers of affected fish (upper and lower) compared to normal fish (middle). d. Myxobacterial "mouth rot." Note focal yellow nodules (arrows). e f. Myxobacteria (*Cytophaga* sp.) from Atlantic salmon skin lesions. e. Bacteria grown on Marine Agar (Difco). f. Bacteria grown on Seawater *Cytophaga* medium. g. *Aeromonas salmonicida* grown on trypic soy agar. Note the diffusible, brown pigment.

MICROSCOPY. Gram-stained smears of infected organs from fish with clinical BKD exhibit numerous, small (0.5 X 1 µm) Gram-positive bacilli, many of which occur in aggregates within phagocytic cells (Fig. 5-2b). Histologically, BKD is best described as a bacteremia, characterized by systemic, diffuse chronic inflammation (Wolke 1975; Bruno 1986). Multifocal, coalescing granulomas are found in all affected tissues (Fig. 5-6). In Pacific salmon, the granulomas are diffuse with poorly defined borders, whereas the granulomas in Atlantic salmon are more encapsulated and contain numerous epithelioid cells. Caseation may occur in the centers of older granulomas, giving rise to cystic lesions. Tissue sections stained with Periodic acid-Schiff (PAS) will readily reveal the organism within phagocytic cells in the granulomas (Fig. 5-2a). With brain infections, the bacterium localizes in the meninges where it occurs in ependymal cells (Speare et al. 1993).

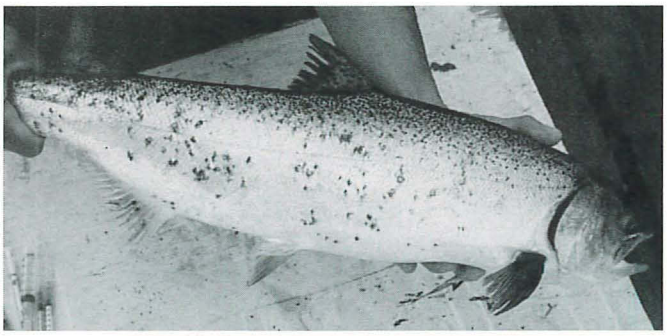


Figure 5-5. Sexually maturing chinook salmon with "spawning rash" caused by *Renibacterium salmoninarum* (Courtesy of D. Elliott).

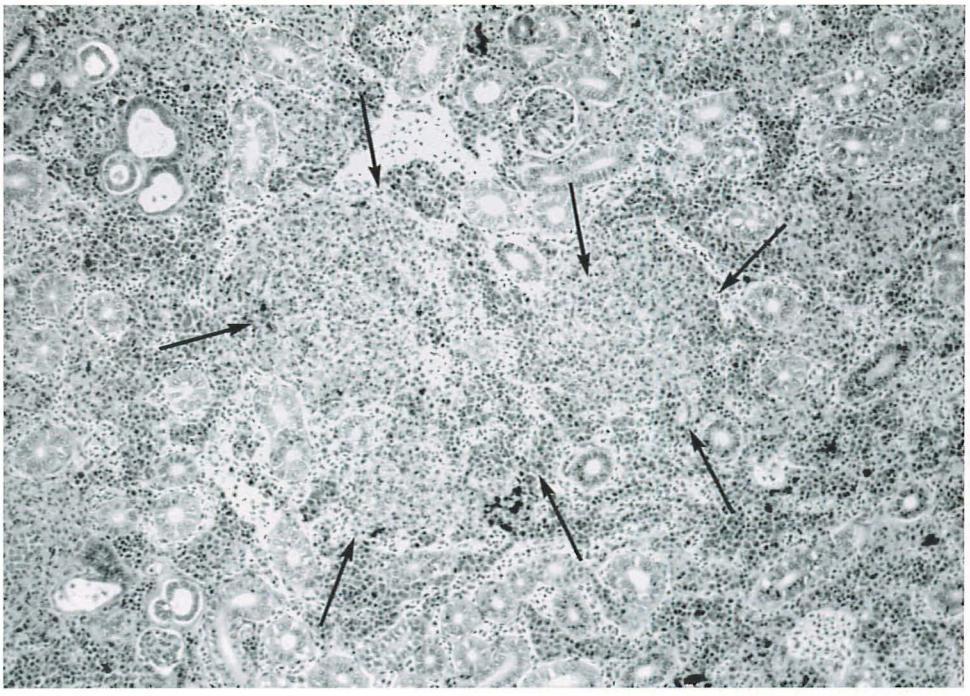


Figure 5-6. Tissue section of kidney from a chinook salmon with BKD. Note the granulomas (demarked by arrows) in the kidney interstitium demarked by arrows. H & E.

DIAGNOSIS. Presumptive diagnosis is achieved by observing the characteristic granulomatous lesions in affected fish. However, other pathogens may cause granulomatous lesions (e.g., *Ichthyophonus hoferi*, *Ceratomyxa shasta*, encysted metazoan parasites). In addition, not all fish with BKD exhibit macroscopically visible granulomas. Therefore, confirmatory diagnosis of the disease is based on the detection of the bacterium in either Gram-stained smears or PAS-stained tissue sections along with the characteristic histological changes.

A number of diagnostic tests have been developed to detect the bacterium in subclinical infections. Serological methods such as the indirect and direct fluorescent antibody tests (IFAT and DFAT) (Bullock and Stuckey 1975a; Bullock et al 1980) have been used to screen smolts for the infection before their transfer to sea water, and the ovarian fluid or kidney tissue of ripe females has been examined using these methods to avoid the taking of infected eggs. Elliott and Barila (1987) described a membrane filtration technique to enhance the detection of the bacterium from coelomic fluid. This test is extremely sensitive, and is about 100 times less likely to overlook the presence of the pathogen in the coelomic fluid than the serological tests mentioned above (Lee and Evelyn, unpubl. data). Cvitanich (1994) developed a quantitative DFAT test (QFAT) which has been used by several fish farms in British Columbia to assess the status of *R. salmoninarum* infections in selected populations.

An ELISA test to detect *R. salmoninarum* antigens in fish tissues has also been developed by Pascho and Mulcahy (1987). This test and its many variants are very sensitive and are particularly well adapted for screening large numbers of fish for the presence of the pathogen. The test has proved useful for selecting brood stock or smolts that are free of *R. salmoninarum*, and provides the basis for avoiding infections with the bacterium (Pascho et al. 1991).

More recently, tests based on the polymerase chain reaction (PCR), have been developed for detecting the bacterium in fish tissues. The PCR tests, although not yet used

routinely for this purpose, appear to be highly sensitive and specific (see for example, Brown et al. 1994; Brown et al 1995; Pascho et al. 1998). Interestingly, our recent studies using kidney samples from moribun chinook salmon to compare the sensitivity of ELISA and PCR have shown them to yield comparable results. The occasional fish negative by PCR but positive by ELISA turned out to have only brain infections (Kent et al. unpubl. data). In addition, some low or borderline positives by ELISA are proving negative by PCR. The ELISA test detects a soluble antigen produced by the bacterium (the p57 protein), and Pascho et al. (1997) demonstrated that this antigen can persist in salmon for many months in the absence of the bacterium. This observation may also account for descrepancies seen between the ELISA and PCR. Until this discrepancy is resolved, it is probably best to be conservative and to regard such samples as positive.

Renibacterium salmoninarum can be cultured on enriched media, such as KDM-2 (Evelyn 1977) or charcoal agar (Daly and Stevenson 1985). Evelyn et al. (1989; 1990) developed special culture techniques for enhancing the growth of this fastidious and slow-growing bacterium, thus greatly increasing the speed and sensitivity with which the bacterium can be cultured. Notwithstanding this, culture cannot be recommended as a routine method for detecting the infection. It is a lengthy procedure and overgrowth of the culture plates with fast-growing contaminating bacteria and fungi often occurs even when antibiotics for suppressing these contaminants are included in the culture medium (Austin et al. 1983).

CONTROL AND TREATMENT: Elliott et al. (1989) and Evelyn (1993) recently reviewed control strategies for BKD. Vaccines affective against BKD are lacking and the disease is difficult to treat with antibiotics. Oral treatment with erythromycin phosphate is efficacious for BKD (Groman and Klontz 1983; Austin 1985), but does not eliminate the infection from all fish (Wolf and Dunbar 1959; Austin 1985). In addition, fish may reject feed medicated with erythromycin, which may reduce the effectiveness of the drug (Schreck and Moffitt 1987). Furthermore, this drug is too expensive to use for production fish in netpens and it is not licensed for use in food fishes. Because of cost-considerations, oral treatment with oxytetracycline has routinely been used to treat BKD in netpens throughout the Pacific Northwest. However, reports on well-controlled studies to evaluate the efficacy of oxytetracycline for controlling BKD in netpens appear to be lacking.

Injection of erythromycin is useful for controlling BKD in brood stock and for preventing egg-mediated parent-to-progeny (vertical) transmission of the bacterium (Lee and Evelyn 1994). Fish are injected medially between the epaxial muscles, just anterior to the dorsal fin, with 80 mg erythromycin/kg fish 9 - 57 d before spawning. Eggs from female treated in this manner contain levels of antibiotic sufficient to kill the bacterium in eggs. As an additional precaution, eggs from treated broodstock should be surface-disinfected with an iodophore (100 ppm iodine for 15 min) to eliminate the pathogen on the egg surface.

To summarize, the best way to control BKD is to avoid the infection. Ideally, eggs from infected females should not be used. In addition, because no diagnostic test will detect all infected brood stock, the females serving as sources of eggs should be injected with erythromycin as described shortly before spawning. Resulting fry should be reared in freshwater that is not contaminated by infected fish, and, if practical, only smolts that are apparently free of the pathogen should be transferred to netpens.

Avoiding horizontal transmission in the netpens is also very important. Bacterial kidney disease is transmissible in sea water (Evelyn 1988a; Murray et al. 1992), and many smolts probably contract the infection from heavily infected fish of the previous year class that are maintained on the same site. Therefore, it is highly advisable to maintain single year class sites.

Vibriosis (caused by *Vibrio anguillarum and V. ordalii*)

Vibriosis is a systemic disease that affects many marine fishes and invertebrates (Anderson and Conroy 1970; Colwell and Grimes 1984; Egidius 1987). Frerichs and Roberts (1989) considered vibriosis to be the most significant disease of wild and cultured marine and brackish water fishes. In salmonids, typical vibriosis is caused by *Vibrio anguillarum* or *V. ordalii*. Diseases caused by other vibrios (e.g., cold-water vibriosis or Hitra disease, caused by *V. salmonicida,* and winter ulcers disease caused by *Vibrio* spp.) are dealt with separately under their own headings.

Vibrio anguillarum accounts for almost all of the outbreaks of vibriosis in farmed salmon worldwide, but in the Pacific Northwest and New Zealand where *V. ordalii* also occurs, infrequent and sporadic outbreaks of vibriosis due to this bacterium have been reported (Evelyn 1971; Harrell et al. 1976; Novotny 1978; Schiewe et al. 1981; Wards et al. 1991). *Vibrio ordalii* has also been reported from diseased fish in Japan (Muroga et al. 1986), but to date it has not been reported there as a problem in pen-reared salmon.

Vibrio anguillarum has been intensively studied for many

years because it is a major cause of vibriosis in a wide range of fish species worldwide. Unlike *V. ordalii*, strains which form a homogenous group, *V. anguillarum* strains show much heterogeneity. This heterogeneity is both phenotypic (Tajima et al. 1985) and serotypic (Kitao et al. 1984; Tajima et al. 1985; Sorenson and Larsen 1986). Fortunately, the strains of *V. anguillarum* that cause vibriosis in pen-reared salmon worldwide represent only one or two serotypes (based on the "O" antigens present). This greatly facilitates serological identification of the organism in disease outbreaks and it also simplifies the formulation of anti-vibriosis vaccines for controlling vibriosis.

Vibriosis can cause high, acute mortality in unvaccinated smolts, and mortalities as high as 90% have been reported (Cisar and Fryer 1969). Although all *Vibrio* species, except *V. cholera*, are considered to be marine bacteria, outbreaks of vibriosis occasionally occur in freshwater-reared salmon . The source of the bacterium in freshwater situations is not always clear, but in some instances it was obviously a diet that was formulated with marine products (reviewed by Evelyn 1988b). Vibriosis outbreaks are favored by temperatures between about 15 and 21 ^{o}C, and most outbreaks occur in smolts during their first summer in sea water. Vibriosis in the Pacific Northwest is mainly a problem of Pacific salmon (e.g., chinook and coho), but Atlantic salmon are also susceptible. Interestingly, however, all cases of vibriosis due to *V. ordalii* in pen-reared salmon reported to date have involved Pacific salmon.

CLINICAL SIGNS AND GROSS PATHOLOGY. Mortality caused by vibriosis may be very severe and rapid, and moribund fish in such cases may exhibit no gross pathological changes other than darkening and lethargy. Hemorrhagic abscesses are often seen in Atlantic salmon with vibriosis in Europe. As typical of septicemias caused by Gram-negative bacteria, fish with vibriosis may exhibit erythema at the base of the fins, petechiae in the skin, and frank hemorrhages on the body surface (Fig. 5-1f). Fish may also exhibit bilateral exophthalmia and frayed fins. Internally, congestion and petechiae are usually evident in the visceral organs, particularly in the gut and liver (Fig. 5-1g). Large, multiple coalescing hematomas in the liver (piliosis hepitis) are often seen in vibriosis (Fig. 5-1h). These lesions are very characteristic of vibriosis, but may also occur with a few other diseases (e.g., ISA). Affected fish also exhibit pallor of the gills (due to anemia) and enlargement of the spleen and kidney.

MICROSCOPY. Histological changes are consistent with septicemia caused by Gram-negative bacteria. With *V. anguillarum*, the bacterium occurs as single cells that are distributed throughout the vasculature. Necrosis and edema are associated with the infection in well-vascularized organs such as the liver, kidney, and spleen. Increased deposition of hemosiderin may be observed in the kidney interstitium and spleen. Fish may also exhibit severe cardiomyopathy. *Vibrio ordalii* infections, on the other hand, are characterized by focal lesions, and large colonies of the bacterium may be observed in the affected tissues.

DIAGNOSIS. Presumptive diagnosis is possible by macroscopic examination if the characteristic hematomas in the liver are present (Fig. 5-1h), and the causative Gram-negative bacilli are usually easy to detect in Gram-stained kidney smears (Fig. 5-2c). The other gross and clinical changes are not specific to vibriosis and are associated with a number of bacterial or viral systemic diseases. Confirmatory diagnosis is based on culture and identification of the causative organism from the kidney of suspect fish. Both *V. anguillarum* and *V. ordalii* are easily cultured on Tryptic Soy Agar with 1.5 % NaCl or on Marine Agar (Difco) at room temperature. Bacterial colonies are round, raised, and off-white in color. *Vibrio ordalii* grows more slowly than *V. anguillarum* and forms smaller colonies. Pure cultures of the suspect bacteria can be distinguished from one another using biochemical tests (Schiewe et al. 1981; Tajima et al. 1985; Scalati and Kusuda 1986). API-20E test strips (Analytab Co., Analytab Products, Plainview, NY, USA) can be used for rapid and easy identification of marine vibrios from fish (Kent 1982; Grisez et al. 1991). Using the API-20E test, *Vibrio anguillarum* is distinguished from other Gram-negative bacteria causing disease in fish by the following criteria: the bacterium yields positive oxidase, Voges-Proskauer, and gelatinase reactions; ferments sucrose, sorbitol, and arabinose; does not grow at 40 ^{o}C; and does not produce gas. *Vibrio ordalii*, in contrast, is Voges-Proskauer negative and does not ferment sorbitol or arabinose. Both bacteria are sensitive to the vibriostatic compound O/129 vibriostat (2:4 diamino 6:7 diisopropyl pteridine). These bacteria can also be identified serologically using slide agglutination tests. Rabbit antisera required for the tests are available commercially (Microtek-Bayer, Sidney, British Columbia). Interestingly, immunofluorescence tests applied directly to vibrio-infected tissues cannot be used for rapid diagnosis of the disease (Evelyn, T. P.T., unpubl. data). Apparently salmonid tissues (including tissues from naive fish) contain substances that block receptor sites on the vibrios that would normally react with the vibrio-specific antibodies in the diagnostic antisera.

CONTROL AND TREATMENT. Vibriosis in pen-reared salmon is best controlled by prevention, and vaccines for this purpose are commercially available. Vaccination is best carried out on fish that have attained immunocompetent size (at least 5 -10 g) and before they are introduced to netpens. The vaccines are conveniently administered by immersion methods, and if applied properly, they afford excellent protection (Evelyn 1984; 1988b). Failures of protection in vaccinated Pacific salmon, particularly with chinook, have occurred at a number of fish farms in British Columbia. The precise cause of these failures has not been determined, but the most likely explanation is that the fish may have been vaccinated before they were immunocompetent. Some farms have gone to revaccinating fish shortly after introduction to netpens, and results with revaccination in sea water have been promising. Revaccination should, however, be conducted with caution because the handling of fish shortly after their introduction to sea water may be very stressful. Although vaccination by immersion is feasible, the most recent trend is to administer the vaccine by intraperitoneal injection, usually in combination with other vaccines, e.g., with furunculosis vaccines which are most effective when injected. Injection of Atlantic and coho salmon smolts prior to seawater introduction has never been a problem because the smolts are large enough to be readily handled. However, until the technology for producing chinook salmon "super smolts" was widely available, the injection of chinook smolts was problematic. Multivalent vaccines administered by injection are being increasingly used because they can be formulated to protect against the particular diseases of concern and to contain adjuvants and immunomodulators which help to ensure that the vaccines produce a strong and durable protection.

Antibacterial drugs incorporated in feed are available for treating vibriosis, e.g., oxytetracycline, potentiated sulphonamides, quinolones, and florfenicol. Treatment is usually efficacious if the disease is recognized early, if the fish are still actively feeding, and if care is taken to select a drug to which the pathogen is still sensitive. However, in some countries not all of the drugs have been approved for use in fish intended for human consumption. Thus control of vibriosis should be effected primarily via an anti-vibriosis vaccination program.

Coldwater vibriosis (Hitra Disease)

Coldwater vibriosis is a bacterial septicemia caused by the psychrophilic *Vibrio salmonicida*. After its first occurrence in farmed Atlantic salmon in northern Norway in 1977 (Egidius et al. 1981), it has since been diagnosed in most fish-farming areas in Norway as well as in salmon-producing countries surrounding the North Atlantic (Bruno et al. 1986) and eastern Canada and the United States (O'Halloran and Henry 1993). The disease is also known as "Hitra disease" after severe outbreaks occurred in the Hitra region in Mid-Norway in the early eighties. The most severe outbreaks typically occur at low temperatures during the winter months, but may occur throughout the year. Although no toxins have been identified, the role of *V. salmonicida* in the etiology of the disease is unquestionable, but the role of environmental stress and nutrition should not be neglected. Although the bacterium may cause disease in other fish, such as Atlantic cod (Jørgensen et al. 1989), serious losses were first noted in farmed Atlantic salmon.

CLINICAL SIGNS AND GROSS PATHOLOGY. Clinical signs may be unspecific, but usually include lethargy and cessation of feeding. Affected fish turn darker, exhibit exophthalmia, a swollen vent, and pin-point hemorrhages along the belly and at the base of the pectoral, pelvic, and anal fins. The gills are usually pale. Internally, ascites and petechial hemorrhage in perivisceral fat, pyloric caeca, peritoneal surfaces, liver, and swimbladder are typical findings (Fig. 5-1c). The swimbladder may be filled with a blood-tinged fluid and the liver typically has a yellowish discoloration, sometimes with hemorrhage. In chronic cases, skin ulceration, fin rot, and a pseudomembranous peritonitis and epicarditis may also be found. The spleen is usually slightly lighter than normal in color. Hemorrhagic miscoloration of the posterior gut occurs frequently.

MICROSCOPY. Direct microscopy using phase contrast from ascitic fluid, swim-bladder contents, and blood reveals motile rod-shaped bacteria. The bacteria are also commonly seen in histological sections (Fig. 5-7). The bacterium may be found in high numbers in muscle and heart where it typically occurs between the outer compact and the inner spongious myocardium, but also in organs like kidney, spleen, and liver. There is usually extensive myocardial and muscle degeneration where the bacteria are found as loose aggregates or individual cells with the Giemsa stain (Fjølstad and Heyeraas 1985). There is also hemorrhage and necrosis with sloughing of the gut mucosa.

DIAGNOSIS. The diagnosis is based on isolation of *V. salmonicida* on 1.5 or 2% NaCl supplemented blood agar or TSA at 15 ^{o}C. Standard biochemical tests, including sensitivity

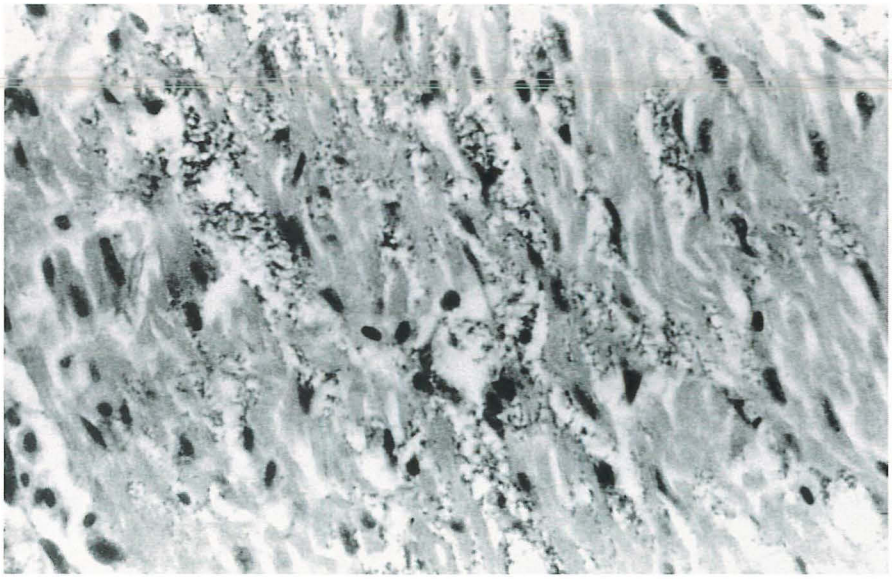


Figure 5-7. *Vibrio salmonicida* in Atlantic salmon. Histological section showing bacteria in necrotic muscle. Giemsa.

to the vibriostatic agent 0/129 and novobiocin usually identify the bacterium (Holm et al. 1985). The diagnosis is confirmed serologically using fluorescent antibody tests or agglutination. Microscopical demonstration of the bacterium in Giemsa-stained smears and muscle sections and immunohistochemistry (Evensen et al. 1991b) are useful for locating the organism.

TREATMENT AND CONTROL. *Vibrio salmonicida* is not considered as a very pathogenic bacterium and a massive exposure is required to infect the fish. As with other diseases, optimization of the environment and reduction of stressors, in particular during the winter months, are important measures to avoid outbreaks. Multivalent vaccines protecting against furunculosis, vibriosis, and cold-water vibriosis give excellent protection provided the vaccination programs are carried out in a proper way. Nevertheless, outbreaks do occur in properly vaccinated fish, particularly in northern Norway. Although the bacterium occurs commonly in the water and sediments close to cages, its numbers escalate during outbreaks and it is therefore important to isolate diseased fish from healthy fish (Enger et al. 1989).

The bacterium will normally respond to several antimicrobials, but it is important to start medication early, particularly during the winter months when the appetite is low and the fish go off the feed very easily. The sensitivity profile of the bacteria in the farm should be monitored regularly in order to start feeding with the best medicated feed as soon as possible.

Winter Ulcers

Winter ulcers is a serious problem in farmed Atlantic salmon, particularly in smolts put to sea in the autumn. This condition may lead to severe mortality during the first winter in seawater, but also causes considerable losses due to downgrading or rejection during processing. The sores typically occur during the cold winter months, but may also be observed at other times of the year. Although two vibrios have been associated with the disease (*Vibrio vulnificus* and *V. wodanis*), reduced osmoregulatory abilities at low temperatures obviously plays an important role in the development of the disease. Thrombosis of capillaries at low temperatures with subsequent vesicle formation and later disruption have also been shown to be involved in the pathogenesis (Salte et al. 1994).

CLINICAL SIGNS AND GROSS PATHOLOGY. Mortality may be slightly elevated in affected stocks. In advanced stages, the fish may become lethargic and congregate near the corners of the cages. The size of the ulcers may vary from pin-head to hand-sized and are typically located on the flanks of the fish (Fig. 5-1d). The transition zone towards normal skin may be hyperemic or hemorrhagic. When the ulcers heal with increasing temperatures, a white granulation tissue may grow in from the margins.

MICROSCOPY. Histology of exposed subcutaneous tissue or muscle shows with moderate to pronounced infiltration of inflammatory cells in loose connective tissue. There is often extensive muscle degeneration of the exposed muscle and numerous bacteria may be seen in the connective tissue and degenerated muscle close to the ulcers.

DIAGNOSIS. Diagnosis is based upon the characteristic ulcers that should be differentiated from physically inflicted wounds and ulcers caused by other bacteria.

TREATMENT AND CONTROL. Treatment with medicated feeds alleviates the problem temporarily, but mortalities tend to increase again some time after cessation of the medication. Recent results show that the addition of urea to the feed reduces the osmotic stress on the salmon in winter indicating that osmoregulatory dysfunction may be an important component of the disease.

Furunculosis

Furunculosis is a septicemic disease caused by the Gram-negative bacterium *Aeromonas salmonicida*. Excellent reviews (McCarthy and Roberts 1980; Paterson 1982; Austin and Austin 1987; Hastings 1988) and a new book (edited by Bernoth et al. 1997) on the disease and the causative agent are available. The disease has long been recognized as a serious problem in freshwater-reared salmonids. With the increase in netpen farming, it has also become a serious disease of salmonids reared in sea water, particularly in Atlantic salmon. Like BKD, infected fish can carry the infection into sea water, and horizontal transmission can occur in netpens (Smith et al. 1982). The disease is favored by high or rising temperatures, but it has occurred in netpens in the Pacific Northwest at temperatures as low as 6 °C. *Aeromonas salmonicida* has been reported from a large number of fishes, including marine species, and it is generally held that infected fish are the chief reservoirs of infection. This view is supported by the fact that fish may be cultured without the disease if water supplies to the culture facility are free of fishes that carry the pathogen. Notwithstanding this, survival of the pathogen outside of the host in fresh water, freshwater sediments, and sea water can be quite extensive (Rose et al. 1989, 1990). These properties of the bacterium probably explain why horizontal infections in fresh and sea water occur, even when the sites involved are separated (Munro et al. 1990). The overwhelming body of evidence indicates that the bacterium is not transmitted from parent to progeny via the eggs (vertically transmitted).

Three subspecies of the bacterium are currently recognized in Bergey's Manual of Systematic Bacteriology, all of them having been reported as causing infections in salmonids. In pen-reared salmon in the Pacific Northwest, however, only one subspecies has thus far been encountered as a problem: *A. salmonicida* subsp. *salmonicida*. This subspecies is the one most frequently involved in furunculosis outbreaks in pen-reared salmon world-wide. It produces a diffusing brown pigment when cultured on Tryptic Soy Agar (Fig. 5-4g), a property that helps with the diagnosis of furunculosis.

CLINICAL SIGNS AND GROSS PATHOLOGY. Fish with furunculosis may exhibit a wide spectrum of clinical and gross pathological changes, largely depending on whether the infection occurs in acute or chronic form. Early in more acute forms of the disease, fish may exhibit anorexia, darkening of the skin and lethargy. Later, fish exhibit hemorrhages and reddening of the skin and fins. In more chronic cases, fish exhibit "furuncles" (i.e., vesicles containing serosanguineous fluid that underlie the skin) or large, bloody ulcers.

Internally, fish exhibit diffuse reddening, hemorrhages in the visceral organs, and enlargement of the spleen (Fig. 5-1e).

MICROSCOPY. The most striking histological change in furunculosis is the lack of an inflammatory response to the infection. Colonies of the causative bacterium, associated with focal necrosis, are readily detected in the spleen, liver, kidney interstitium, myocardium, and gills (Fig. 5-8).

DIAGNOSIS. The presence of large, focal colonies of short bacilli in tissue sections can be used as a strong presumptive diagnosis of furunculosis. However, diagnosis of furunculosis is most often achieved by isolation of *A. salmonicida* from the kidney or other suspect fish tissues followed by serologic or phenotypic identification of the isolated bacterium. The

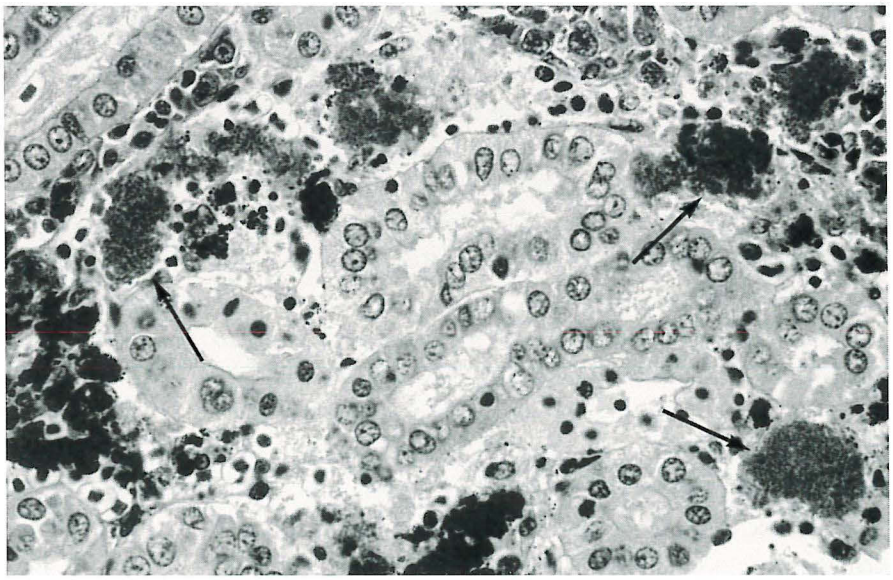


Figure 5-8. Colonies of *Aeromonas salmonicida* (arrows) in the kidney of chinook salmon. H & E.

widespread use of this rather slow diagnostic approach is probably due to the fact that the bacterium is easily cultured and one normally has to await the results of antibiotic sensitivity testing (which requires culturing the bacterium) if decisions are to be made on the antibiotic of choice for treating infections with the bacterium. Alternatively, the diagnosis may be more rapidly accomplished by a variety of sensitive and specific tests, applied directly to suspect fish tissues. For example, immunofluorescence techniques, ELISAs (see, for example, Yoshimizu et al. 1993; Hiney et al. 1994), and PCR assays (see, for example, Miyata et al. 1996; Hoie et al. 1997) have been developed by a number of laboratories for detecting the pathogen, but the procedures have usually been employed in connection with specialized environmental or epizootiological studies involving the pathogen rather than for the routine diagnosis of *A. salmonicida* infections.

Aeromonas salmonicida subsp. *salmonicida* grows readily on Tryptic Soy Agar, producing visible growth within 2 -3 days at room temperature along with a characteristic brown pigment that diffuses into the growth medium (Fig. 5-4g). Serological identification of the isolate can be achieved using a latex bead agglutination kit (Microtek R & D, Ltd, Victoria, B.C.). Also, the standard slide agglutination technique has been frequently employed for this purpose but its use is contraindicated because the bacterium autoagglutinates (i.e., is self agglutinating even in the absence of *A. salmonicida* antiserum). Phenotypic features for identifying the isolate are as follows: it is a short (approximately 1 X 2 µm), Gram-negative, non-motile rod; ferments glucose with the production of acid and gas; is oxidase positive; and does not grow at 37 °C.

CONTROL AND TREATMENT. Furunculosis is a difficult disease to control. Significant protection against the disease can, however, be achieved using vaccination, and this is the method of choice for controlling the disease. The vaccines that have proved most effective are ones that are intraperitoneally injected. These vaccines are commercially available, are usually formulated to contain oil-based adjuvants and/or immunomodulators, and are often administered along with other vaccines, e.g., anti-vibriosis and/or anti-coldwater vibriosis vaccines, as appropriate.

Despite vaccination, farmers sometimes have to resort to chemotherapy to control furunculosis. However, the pathogen often becomes resistant to antibiotics routinely used for treating the disease (Romet-30, oxytetracycline, and oxolinic acid) (Hastings and McKay 1987), and outbreaks often recur shortly after treatment has terminated. In addition, mixed infections can occur, in which some fish are infected with antibiotic sensitive strains of *A. salmonicida*, while others from the same population are infected with resistant bacteria. Therefore, the search for antibacterial agents effective against the bacterium is a continuing one (Inglis and Richards 1992) and it is important to culture several fish in an affected population when determining the antibiotic of choice for treating a particular outbreak.

Probably the best way to control furunculosis is to avoid using smolts with a history of the infection, and to place these smolts on farms where they represent the only year class present. Smolts free of the pathogen can be produced by using surface-disinfected eggs and by raising the fry in water from a source that does not contain wild or cultured fishes. (Note: the pathogen is not transmitted within salmonid eggs; surface disinfection of the eggs is used to avoid the possibility that the eggs may be surface-contaminated with the pathogen). As an additional precaution, apparently healthy smolts may be tested for absence of the bacterium prior to seawater introduction with a stress test (Bullock and Stuckey 1975b; McCarthy and Roberts 1980). The test facilitates detection of the pathogen in healthy "carrier" fish and involves injecting the fish with an immunosuppressive compound (e.g., prednisolone acetate) and holding the fish for 3 wk at 18°C before attempting to isolate the bacterium.

Yersiniosis

Yersiniosis or enteric redmouth disease (ERM) caused by the Gram-negative bacterium *Yersinia ruckeri* has been a well-known problem to the trout-farming industry in the USA since 1950 (Bullock et al. 1971), and has since been described from at least 20 countries. The bacterium is described from several different fish species under different environmental conditions indicating a widespread occurrence. Although initially regarded as a problem in freshwater aquaculture, yersiniosis has also caused considerable problems in seawater netpens.

CLINICAL SIGNS AND GROSS PATHOLOGY. In seawater, the characteristic hemorrhages in the mouth and jaw areas are usually lacking and the disease manifests itself as a rather unspecific septicemic condition. Affected fish may be darker than normal and show reduced activity. Skin hemorrhage and exophthalmos may also be evident. Additional gross signs may include congestion, ascites, splenomegaly, and pin point hemorrhages in visceral fat, muscle and on serosal surfaces. The gut contents may be watery or blood-tinged.

MICROSCOPY. Necrosis of hematopoietic tissue in the kidney and spleen occurs frequently as in other septicemic diseases. Focal necroses, venous and capillary congestion and hemorrhage may be seen in most affected organs, and bacterial colonies may be found subepithelially in the gills, in the myocardium (particularly between the outer compact and the inner spongious myocardium). Purulent epicarditis and meningitis have been found in isolated cases.

DIAGNOSIS. Isolation and identification of *Yersinia ruckeri*, a Gram-negative facultative anaerobic motile rod, is usually easily performed using standard bacteriological techniques (Austin and Austin 1993; Ewing et al. 1978; Waltman and Shotts 1984) or using a selective medium (Rodgers 1992). The diagnosis can also be based on immunohistochemistry techniques on formalin-fixed tissues from diseased fish.

CONTROL AND TREATMENT. Medicated feed with standard antibacterials commonly used in fish farming are usually effective. Sensitivity tests should be performed before treatment is started. The main source of infection is latent carriers, both among feral and farmed fish.

Effective vaccines are available, but their use has been abandoned in Norway as yersiniosis no longer represents a major problem. As the bacterium is very widespread in the aquatic environment, it is difficult to avoid contact. However, outbreaks are usually related to unfavourable environmental conditions, and clinical disease is not likely to occur as long as these are kept under control.

Myxobacteriosis

Cytophaga and *Flexibacter* spp. are important bacterial pathogens of cultured fishes and usually cause external lesions in freshwater and marine species (Anderson and Conroy 1969; Pacha and Ordal 1970). *Flexibacter columnaris* and *C. psychrophila* (now *Flavobacterium columnare* and *Fl. psychrophilum*) (Bernardet et al. 1996) are well recognized pathogens of fishes reared in fresh water (Pacha and Ordal 1970; Snieszko and Bullock 1976). In marine aquaculture, infections by *Flexibacter maritimus* have been observed in Japanese flounder and sea breams (family Sparidae) in Japan (Baxa et al. 1986, 1987; Hikida et al. 1979; Masumura and Wakabayashi 1977; Wakabayashi et al. 1984, 1986) and Europe (Bernardet et al. 1990). In Tasmania, Handlinger et al. (1997) identified *F. maritimus* associated with skin and gill lesions in pen-reared Atlantic salmon and rainbow trout.

Proper taxonomic identifications have not been conducted on many *Cytophaga* and *Flexibacter* spp. that have been associated with disease in marine fishes. However, the study by Bernardet et al. (1996) which included a number of marine species, including *Flexibacter maritimus*, concluded that none of the marine forms belonged to the genus *Flavobacterium*. These bacteria are usually referred to as 'myxobacteria' by fish health workers and aquaculturists, but this is technically incorrect because these bacteria belong to the order Cytophagales, not the order Myxobacteria. It would, therefore, be more appropriate to refer to the marine forms using collective terms such as "cytophaga-flexibacter-like bacteria" or "gliding bacteria". However, to remain consistent with the common terminology and to avoid confusion, we continue to refer to these bacteria as myxobacteria in this text.

Myxobacteria have been associated with skin lesions in seawater-reared salmonids for many years (Borg 1960; Rucker 1963; Anderson and Conroy 1969; Wood 1974; Sawyer 1976). These bacteria have at times been identified as *Sporocytophaga* sp. However, the presence of microcysts (an important diagnostic feature of this genus) has not been clearly demonstrated in these isolates.

Two types of myxobacterial infections have been associated with high mortality in pen-reared Atlantic salmon in the Pacific Northwest; one type causes large skin ulcers, and the other causes lesions primarily in the mouth. Myxobacteria infections are also seen in pen-reared Pacific salmon, but are not usually associated with severe epizootics. In Pacific salmon, myxobacteria are usually associated with frayed fins and erosion of the tail.

Myxobacterial skin lesions in Atlantic salmon

A *Cytophaga* sp. causes large skin lesions in Atlantic salmon smolts shortly after seawater entry (Kent et al. 1988a). Very similar skin lesions associated with *F. maritimus* infections were observed in pen-reared Atlantic salmon and rainbow trout in Tasmania (Handlinger et al. 1997). In the Pacific Northwest, lesions and associated mortalities usually peak at about 1-3 wk after introduction, and based on our observations, the disease subsides after about 3-4 wk. There appears to be a seasonality to the disease, and fish introduced later in the spring and summer usually exhibit fewer skin lesions. Infections are restricted to the skin and muscle. Fish with large lesions exhibit elevated plasma sodium levels (Kent et al. 1988a), which suggests that affected fish may ultimately die from an osmotic imbalance.

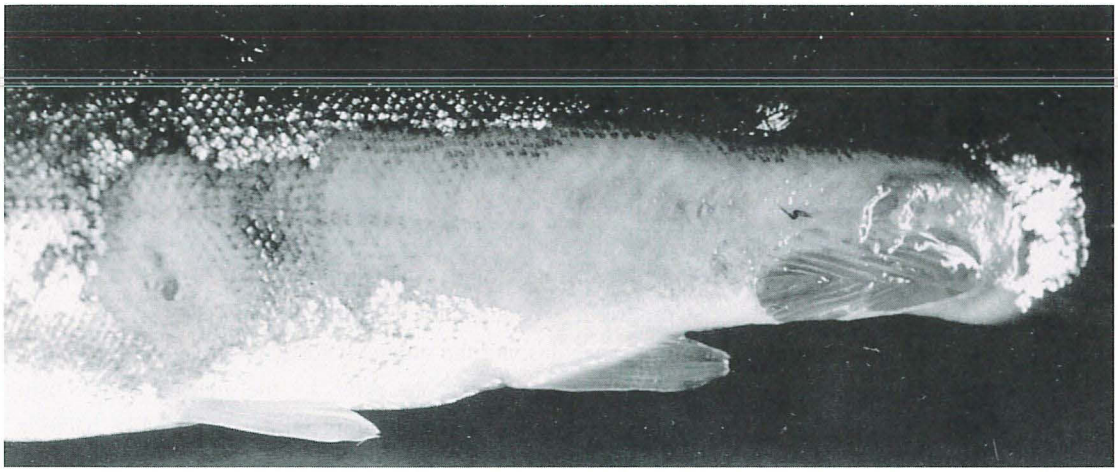


Figure 5-9. Myxobacterial skin infection; the dermis is exposed throughout on the flanks and the underlying muscle is exposed at the caudal peduncle.

CLINICAL SIGNS AND GROSS PATHOLOGY. Moribund fish exhibit large, white patches on the caudal peduncle and posterior region of the flanks when the dermis is intact. Infected fish may also exhibit more severe lesions in which areas of the skin are completely destroyed and the underlying muscle is exposed (Fig. 5-9).

MICROSCOPY. Wet mounts of the lesions reveal masses of filamentous bacteria (0.5-0.7 X 4-20 µm) (Fig. 5-10). Tissue sections of lesions where the skin is present reveals accumulations of filamentous bacteria on the surface of the skin, and throughout the epidermis and dermis. In more severely involved areas, large mats of filamentous bacteria replace the skin, and the bacteria invade deep into the underlying musculature. Affected muscle is hemorrhagic and necrotic, but little inflammation is observed. The bacterium is observed only in the muscle and skin, and tissue sections of the gills and visceral organs have revealed no bacteria or significant pathological changes (Kent et al. 1988a).

DIAGNOSIS. Diagnosis of myxobacterial infections can usually be accomplished by observing large numbers of filamentous bacteria in wet mount preparations. Further identification of bacteria can be accomplished by culture on either Cytophaga Medium (see Appendix III) made with 50% sterile sea water or Marine Agar (Difco), and incubation at approximately 15 °C. Isolation of myxobacteria in pure culture may be difficult from skin lesions due to contamination with other faster growing bacteria (e.g., vibrios). However, the lesions usually contain very large numbers of the myxobacteria and serial dilutions of affected tissue in sterile 50% sea water facilitates the isolation of the culprit bacteria in pure culture. The myxobacteria form small yellow-green, diffuse, rhizoid colonies on Cytophaga Medium (Fig. 5-4f), and yellow-orange, smooth colonies with entire margins on Marine Agar (Fig. 5-4e). More information on the characteristics of the myxobacteria isolated from Atlantic salmon skin lesions is found in Kent et al. (1988a). Reichenbach (1988) describes the characteristics of *Cytophaga* and *Flexibacter* spp. in general.

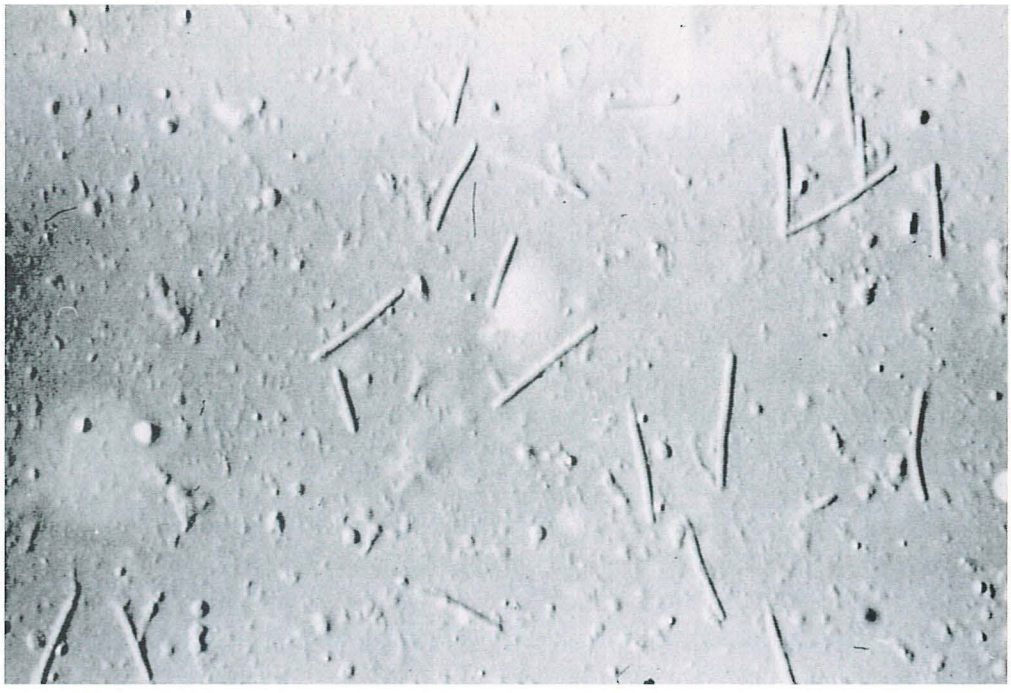


Figure 5-10. Myxobacteria (*Cytophaga* sp.) in a wet mount from a skin lesion of an Atlantic salmon.

TREATMENT AND CONTROL. External treatments with antibiotics are often used to control myxobacterial infections in fresh water, but such treatments are not usually practical in seawater netpens. These bacterial infections are often initiated in the skin where there are abrasions. Physical trauma during transport of smolts may allow the bacteria to establish an infection. Observations from fish farmers indicate that improved transport techniques and careful handling of fish greatly reduces the prevalence of the disease.

Myxobacterial Stomatitis in Atlantic salmon

Infections of the mouth and snout by myxobacteria have been observed in Atlantic salmon smolts during their first summer in sea water. The condition has occurred at many netpen sites in the Pacific Northwest, and is often associated with high mortalities (Hicks 1989; Frelier et al. 1994; Anonymous 1996). Pen-reared Arctic char have also been afflicted with the infection. The infection appears to begin around the teeth. It has been suggested that the infection is initiated in periodontal tissue that has been abraded by feeding on spiny crustaceans such as crab larvae and *Caprella* amphipods. Other potential predisposing factors suggested by farmers that may lead to the infection are 1) feeding on hard pellets, 2) fish biting net surfaces, and 3) stress-induced lesions in the mouth. Fish farmers in British Columbia report the condition is particularly troublesome at farms with high salinity water.

Based on culture characteristics, the myxobacterium from mouth lesions appears to be a different organism than the myxobacterium causing skin lesions. A similar myxobacterial stomatitis has been observed in wild Atlantic cod in the North Sea (Hilger et al. 1991).

CLINICAL SIGNS AND GROSS PATHOLOGY. Infected fish are lethargic, emaciated, and anorexic, and some affected fish may exhibit flashing or head shaking. Early in the infection, examination of the mouth reveals focal, yellow bacterial mats around the palate and teeth, including the vomer (Fig. 5-4d). The lesions may be single, but the opposing surface is often affected (Frelier et al. 1994). As the disease progresses, affected fish show multiple ulcers in the mouth with large bacterial mats overlying the lesions. The lesions may extend to the branchial arches and proximal oesophagus, and the lower and upper jaw may be completely eroded in very severe cases. Severely affected fish do not feed and their stomach is devoid of food.

MICROSCOPY. Wet mounts of the lesions reveal numerous filamentous myxobacterial cells. Histological examination of affected tissue reveals masses of the causative bacterium associated with focal ulcers, necrosis of the underlying bone, and a mixed inflammatory infiltrate (Frelier et al. 1994).

DIAGNOSIS. The disease can usually be diagnosed by observation of the distinctive yellow patches in the mouth of the affected fish. Positive diagnosis is achieved by observing filamentous bacteria in the mouth lesions.

CONTROL AND TREATMENT. There are unconfirmed reports that the disease can be controlled by feeding potentiated sulfonamides.

Salmonid Rickettsial Septicemia or Piscirickettsiosis

A rickettsia-like organism, the recent subject of reviews (Fryer and Lannan 1996; Almendras and Fuentealba 1997), has been shown to be the cause of a severe septicemia in pen-reared salmon in Chile (Branson and Nieto Diaz-Munoz 1991; Cvitanich et al. 1991; Garcés et al. 1991). The name proposed by Cvitanich et al. (1991) for the disease in Chile is salmonid rickettsial septicemia. Following the naming of the causative organism as *Piscirickettsia salmonis* by Fryer et al. (1992), the disease has also been widely referred to as piscirickettsiosis. In British Columbia, an essentially identical disease was first observed in seawater-reared pink salmon, held for experimental purposes, at the Pacific Biological Station in 1970. Because the causative organism involved in Chile and British Columbia proved morphologically, serologically, and culturally indistinguishable, it was concluded that the same organism was involved in both locations. More recently, infections of Atlantic salmon with rickettsia-like organisms have been reported from Norway (Olsen et al. 1997), Ireland (Rodger and Drinan 1993), Atlantic Canada (Jones et al. 1998) and Scotland (European Association of Fish Pathologists, undated). A PCR method developed by Mauel et al. (1996) for detecting and identifying the pathogen showed that the isolates from Norway, Ireland, Canada, and Chile were likely all *P. salmonis*, although it was clear that at least two variants of the pathogen occurred in Chile. The Scottish isolate was not included in the above PCR study but it may be a different rickettsia, because unlike the isolates from the other countries, it apparently does not cross-react with sera prepared against *P. salmonis*. House et al. (1998) showed that the strain from Chile was more pathogenic than those from British Columbia and Norway, which is consistent with field observations.

After the first record of the disease in 1970, we observed the condition in pink salmon (1978), in chinook (1983, 1984, 1986), and coho (1984), but always incidental to more important disease problems. Until an outbreak of the disease in 1991 in a production farm in British Columbia (Brocklebank et al. 1992), the disease was regarded as of academic interest only in British Columbia. At this time, the disease has been detected at in chinook and Atlantic salmon at several netpen farms in British Columbia. In this region, the infection is usually coincidental to other infectious diseases (e.g., BKD) in the population, but may occasionally cause epizootics in which it is the primary cause of mortality.

In contrast, piscirickettsiosis is the most important infectious disease of pen-reared salmonids in Chile, where it caused about US$ 48 million losses in 1995 . In Chile, the disease was at first most problematic in coho salmon, but now is also common in both rainbow trout and Atlantic salmon. Several outbreaks of the disease may occur in the same population of fish during its seawater grow out, particularly with coho salmon.

Information to date on the epizootiology of the organism suggests that it is normally acquired in sea water from a marine source. However, a marine reservoir has yet to be identified, although certain salmon ectoparasites, such as *Caligus* sp. and *Ceratothoa gaudichaudii*, may be involved in the transmission, perhaps serving as vectors (Garcés et al. 1994). Furthermore, Cvitanich et al. (1991) found possible evidence of the organism in crustaceans and molluscs around netpens based on histology and serology. Regardless if vectors or non-salmonid fishes reservoirs are involved with the disease, it can be easily transmitted directly from fish to fish in sea water (Cvitanich et al. 1991; T.P.T. Evelyn unpubl. data). Many organisms occur in the intestine of infected salmon, suggesting that the agent is released in the feces (Cvitanich et al. 1991), and the gills and gut appear to be the portal of entry for the infection (Almendras 1996; Almendras et al. 1997). *Piscirickettsia salmonis* survives well in sea water, which may be important for its transmission in netpens (Almendras 1996).

The disease may also occur in brackish water with low salinity, and the infection has recently been reported in rainbow trout and coho salmon held in fresh water (Bravo 1994; Cvitanich et al. 1995; Gaggero et al. 1995). However, *P. salmonis* survives very poorly in fresh water (Lannan and Fryer 1994). Nevertheless, *P. salmonis* can be transmitted by co-habitation in fresh water (Almendras et al. 1997). Bustos et al. (1994) conducted field trials that suggested that vertical transmission may occur in the natural condition, and Larenas et al. (1996) detected the infection in 10% of fertilized ova from infected fish. This may explain its occurrence in fresh water. However, the relative poor survivability of the organism in fresh water may explain the rarity of the infection seen before fish are introduced to sea water.

Husbandry practices (such as grading and net changes), storms and rapid temperature changes predispose fish to outbreaks of the infection (V. Palma, Chile, pers. comm.). In addition, infections by other pathogens apparently predispose salmon to the infection. In Chile, mixed infections with *R. salmoninarum* in both fresh water and sea water have been reported (Cvitanich et al. 1991; Gaggero et al. 1995; Smith et al. 1995). Mixed infections with *Nucleospora salmonis* have been seen in Chile (Enríquez 1997) and in British Columbia.

CLINICAL SIGNS AND GROSS PATHOLOGY. Clinical and gross pathological changes associated with *P. salmonis* infections have been outlined by Cvitanich et al. (1991), Branson and Neito Diaz-Munoz (1991) and Brocklebank et al. (1992). Affected fish are lethargic, anorexic, exhibit pallor of the gills due to anemia, are dark in color, and may swim near the surface. There are marked differences in clinical signs between salmonid species. For example, with rainbow trout it is hard to find "slow swimmers" at the surface, while many dead fish are collected from the bottom of the pens. In Atlantic and coho salmon the nervous system is often affected, with flashing and side swimming being common in the former. Multiple, small white spots and petechiae occur in the skin. Ulcerations often occur on the skin with coho salmon and rainbow trout, whereas this is rare with Atlantic salmon in Chile. However, Atlantic salmon with the disease from Norway occasionally showed skin lesions - e.g., raised nodules or white spots (Olsen et al. 1997).

Hallmark internal lesions of the disease are found in the liver. The liver of affected fish usually exhibit large, whitish or yellow, multifocal, coalescing, granulomatous nodules (Fig. 5-11). These lesions often rupture, resulting in shallow crater-like cavities in the liver. Internal examination also reveals ascites, an enlarged spleen and a grey, enlarged kidney. The spleen is extremely enlarged in infected pink salmon. Pallor (suggesting anemia) and petechiae are observed in the visceral organs and muscle, and a whitish pseudomembrane may cover the heart.

MICROSCOPY. Giemsa-stained or Gram-stained imprints of infected tissues, especially the kidney and liver, reveal pairs or aggregates of basophilic coccoid organisms, about 0.5 - 1.5 µm in diameter (Fig. 5-3c). The organism is Gram-negative and is frequently found in the cytoplasm of macrophages.

Cvitanich et al. (1991) and Branson and Nieto Diaz-Munoz

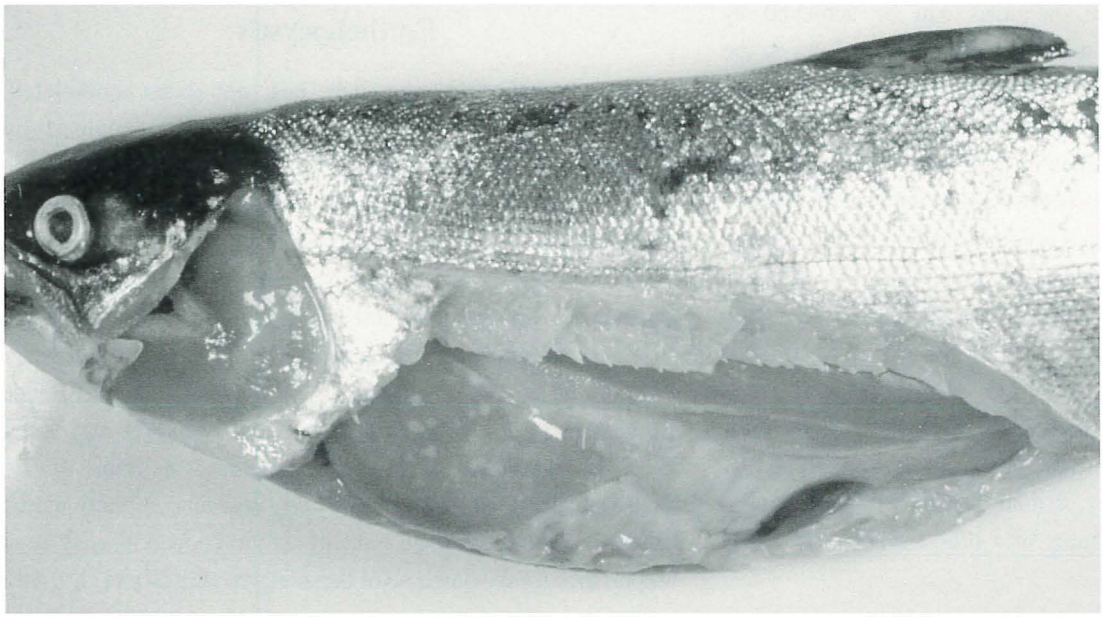


Figure 5-11. Salmon with *Piscirickettsia salmonis*. Note white, focal lesions in the liver.

(1991) described the histopathological changes in infected coho from Chile, and these changes are essentially the same as those seen in salmon from British Columbia (Brocklebank et al. 1993). The disease causes prominent tissue damage, and the liver, kidney, and spleen are the most severely affected organs. The liver exhibits large foci of necrosis and inflammation, and vasculitis and thrombi. High magnification reveals aggregates of the organism in the cytoplasm of degenerated hepatocytes and in macrophages, including melano-macrophages. Infected macrophages are usually hypertrophied and replete with cellular debris. The kidney interstitium exhibits generalized necrosis and prominent infiltration of macrophages. Vasculitis and thrombi are often found in the kidney. As with the liver, high magnification reveals the rickettsia in macrophages (Fig. 5-12). These macrophages occur in large accumulations in the thrombi, or more diffusely throughout the kidney interstitium. The spleen exhibits similar histological changes as those seen in the kidney. In tissue sections stained with hematoxylin and eosin, the organism appears as basophilic or amphophilic spheres. Special stains showed that the organism is Gram, acid-fast, and PAS-negative, and they stain blue with Machiavello's and toluidine blue (Brocklebank et al. 1993). We have found that the methylene blue stain is the best stain for showing the organism in tissue sections.

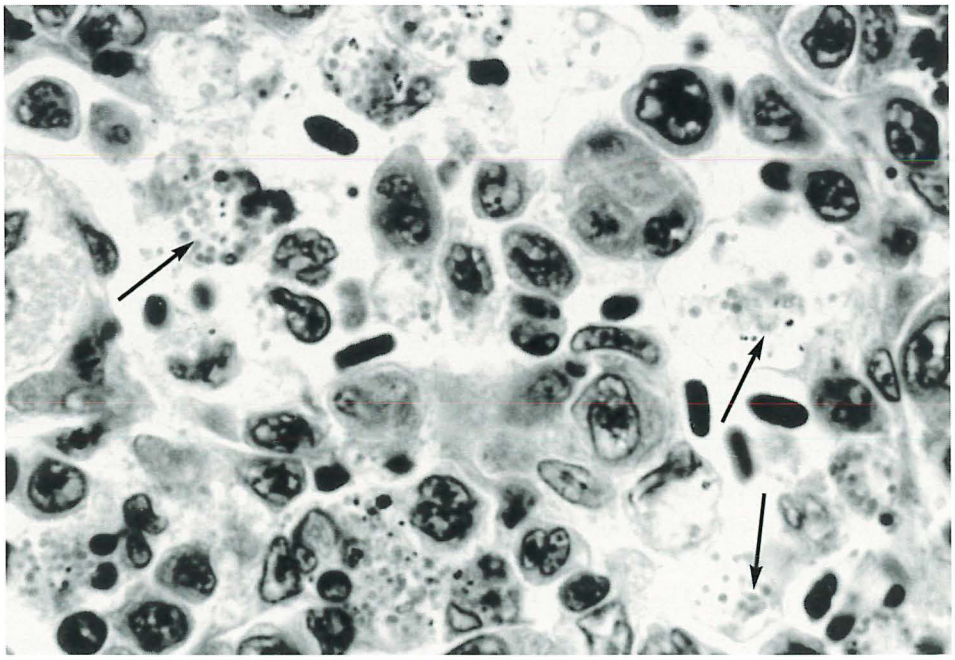


Figure 5-12. Kidney of chinook with *Piscirickettia salmonis* infecion. Macrophages are enlarged and the spherical organism (arrows) are found in the cytoplasm. H & E.

DIAGNOSIS. Presumptive diagnosis can be achieved by observing the distinctive crater-like lesions and nodules in the liver. Reasonably definitive diagnosis can be achieved by observing the organism within phagocytic cells in Giemsa, Gram or methylene blue-stained imprints of the liver or kidney, or in macrophages in tissue sections along with the distinctive histological changes described above. Acridine orange-stained tissue smears are also useful for demonstrating the organism (Lannan and Fryer 1991). Confirmatory diagnosis is based on isolation of the organism in cell culture using CHSE-214 cells (Fryer et al. 1990; Cvitanich et al. 1991), by an indirect fluorescent antibody test (Lannan et al. 1991) or by PCR (Mauel et al. 1996). A commercial ELISA test developed by Microtek Ltd-Bayer (Sidney, British Columbia) has been used extensively by Chilean farmers in a brood stock segregation program.

CONTROL AND TREATMENT. Various antibiotics, such as oxolinic acid, flumequine, and oxytetracycline, have been used in attempt to control the infection with limited success. In extreme cases some Chilean farmers have resorted to employing injectable treatments with fluoroquinolones with some success. Control of salmonid rickettsial septicemia is handicapped by the uncertainties regarding the source of the infection and its mode of spread. This is compounded by the intracellular nature of the organism, which in part, may explain why antibacterial agents, administered with the feed, have been less than satisfactory in controlling the disease. To compound the difficulties, the pathogen has exhibited an obvious ability to develop resistance to the antimicrobial agents used in its control.

Ideal control would be by means of a vaccine (Smith et al. 1995). However, the likelihood of a vaccine for controlling the pathogen appears a long way off. For one thing, the pathogen has to be grown in tissue culture, thus vaccine production by this means would be very expensive. For another, little is known about the virulence factors that might be used as the basis of a vaccine. Thus the outlook for a vaccine based on a genetically engineered virulence factor or based on "injected" DNA coding for such a factor is presently rather remote. For the time being, it appears that rearing at lower densities, fallowing of farms in a given region, and the holding of single year classes on any given site within a region might offer a degree of control. In addition, if ectoparasites are involved as reservoirs and /or vectors of the pathogen, measures to control the parasites might be worthwhile. Finally, although the role of vertical transmission is unknown, the techniques used for preventing vertical transmission of BKD (e.g., brood stock screening) is being employed in Chile.

Epitheliocystis

Although Koch's postulate has not been completed, epitheliocystis is characterized as a condition caused by rickettsia- or chlamydia-like organisms. The severity of the disease may be variable and there is uncertainty whether several species are involved or not. The first description of the disease is by Plehn (1920) and the disease through the years has been described in several wild and farmed fishes. The infection has also been observed in salmonids from both fresh and salt water (Rourke et al. 1984; Carvajal et al. 1990a; Bruno and Poppe 1996: Kent et al. 1998). The term epitheliocystis was introduced by Hoffman et al. (1969). In salmonids, the most severe consequences of infection have been seen in sea-water reared Atlantic salmon in Europe. Concurrent viral infections may be of importance for the outcome of the infection (Bradley et al. 1988).

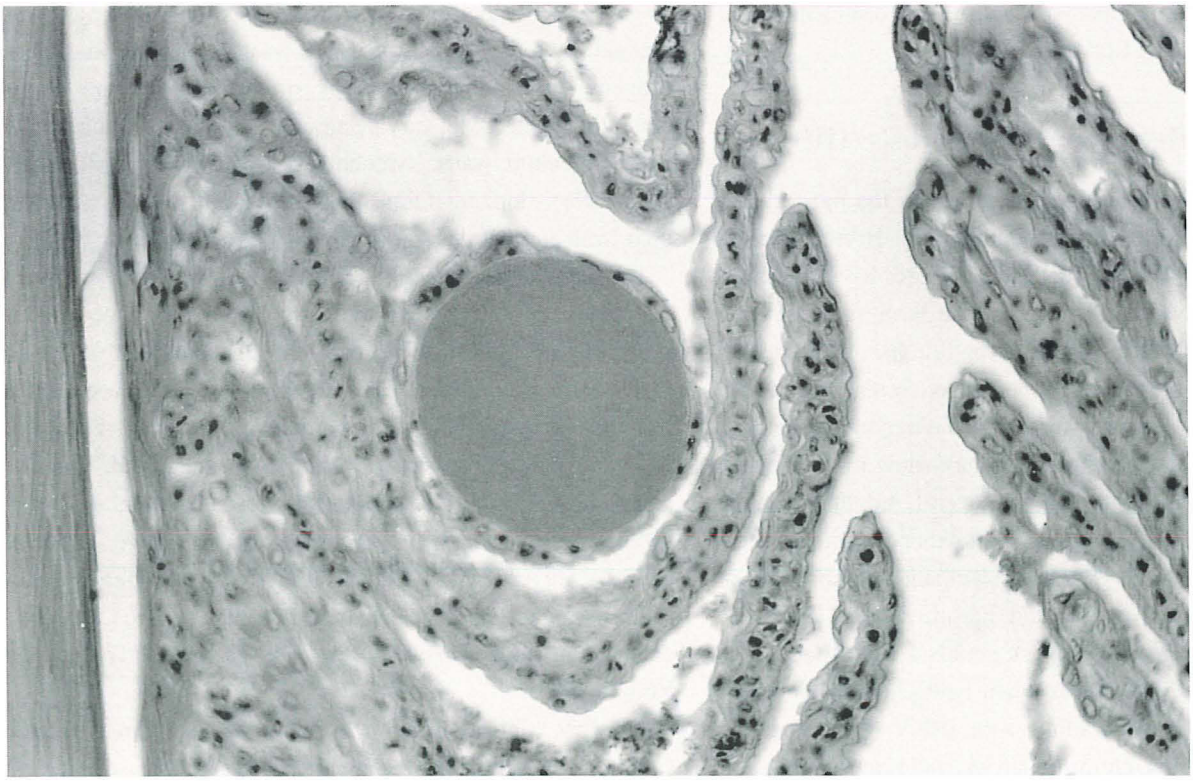
CLINICAL SIGNS AND GROSS PATHOLOGY. The clinical signs may be unspecific, but may include emaciation, respiratory stress, dark coloration and increased susceptibility to secondary infections, e.g., IPN virus. In Atlantic salmon post-smolts mortality may reach 10% per cage per day. Gross lesions are few, but may include flared opercula and pale gills.

MICROSCOPY. Typical lesions are granulated, basophilic cytoplasmic inclusions in epithelial cells where the nucleus is flattened and peripherally located (Fig. 5-13). Although most infected cells are found in the gill epithelium, inclusions may also be found in the skin. Infected cells are hypertrophic and the inclusions constitute the majority of the cell volume. Heavily infected cells will degenerate and necrotize, thereby releasing the microorganisms. The response in surrounding tissue may be variable, in post-smolts there is often extensive hyperplasia and fusion of secondary lamellae.

DIAGNOSIS. The diagnosis is based on observation of the characteristic cytoplasmic colonies and a peripherally dislocated and flattened/crescent-shaped nucleus in the lamellar epithelium of the gills. These may have some similarities to colonies of *Aeromonas salmonicida* subspecies *salmonicida* in furunculosis (McArdle et al. 1986, Turnbull et al. 1989), but these colonies are usually located deep in the pillar cells and vessels and will be easily identified using immunohistochemistry techniques. Using this technique, Groff et al. (1996) demonstrated epitheliocystis organisms from various fish species react positively with antibodies to chlamydia. Presently, there is no method available for culture of the causative organism.

CONTROL AND TREATMENT. As the reservoir of the organism(s) is unknown, little can be done to avoid outbreaks of the disease. Experience from Norwegian sea-farms indicates that mortalities may be kept low if concurrent infections can be kept under control. Although the organism(s) probably are susceptible to oxytetracycline, most treatments have been ineffective and no clinical experiments have been done. Minimalization of stressors and a good environment are important factors for reducing the mortality associated with epitheliocystis.

Figure 5-13. Gill with epitheliocystis inclusions. H & E.



VIRAL DISEASES

G. S. Traxler, M. L. Kent, and T. T. Poppe

Several viruses are important pathogens of salmonid fishes, particularly during the early development of the fish in fresh water (Wolf 1988a). Viral diseases of fishes have historically been of great concern to fish health managers because they can cause high mortality. In contrast to bacterial diseases, there is only one commercially-available vaccine for viruses of salmon (i.e., IPN virus), and no drugs are available for their control. In addition, the mere presence of certain viruses in a population may be an economic hardship due to restrictions on transfer or sale of these fish. At least six viral diseases are of serious concern for pen-reared salmon: infectious hematopoietic necrosis (IHN), infectious pancreatic necrosis (IPN), salmon pancreas disease (SPD), infectious salmon anemia (ISA), salmonid herpesvirus 2 infections, and erythrocytic inclusion body syndrome (EIBS). The erythrocytic necrosis virus has the potential to infect salmon in sea water, but has yet to be recognized as a serious problem. Plasmacytoid leukemia (PL), also known as marine anemia, of chinook salmon is another infectious disease that has caused significant losses in netpens in the Pacific Northwest. This disease may be caused by an oncogenic retrovirus, the salmon leukemia virus (Eaton and Kent 1992; Kent and Dawe 1993), but the etiology of PL has not yet been firmly established. Therefore, PL, along with other neoplastic diseases of possible viral etiology, are dealt with under Neoplastic Diseases and Related Disorders.

Infectious Hematopoietic Necrosis (IHN)

Infectious hematopoietic necrosis is one of the most costly viral diseases of cultured salmon and trout in North America (Pilcher and Fryer 1980; Wolf 1988a, b). Losses have also been reported in wild populations of fish. The virus is capable of causing extensive losses in young susceptible fish, with most of the losses occurring within days of onset. Initial losses can occur within 4 days post exposure, with the majority occurring within the next 10 days. Larger fish may show more chronic losses occurring over a period of several months. Natural salmonid hosts for IHNV are sockeye/kokanee, chinook , and Atlantic salmon, and rainbow/steelhead trout. Other susceptible species are chum, and masou salmon. Whereas coho salmon are considered resistant to IHNV, adults have been reported as carriers of the virus when being held at the same facility as chinook salmon adults with IHNV (LaPatra et al. 1989a). Experimentally, cutthroat, brook, and brown trout have been demonstrated to be susceptible to IHNV. Recently the virus has caused high mortalities in pen-reared Atlantic salmon in British Columbia.

The virus is enzootic along the Pacific coast of North America. It has been transported to several central and eastern states in the USA through shipments of fish or eggs, but fortunately was quickly eradicated following its detection. The virus has also been transferred to Asia and more recently to Europe, where it has spread from France to Italy and Germany. It has become established in Japan where it causes losses among wild fish stocks. In British Columbia, sockeye fry migrating from spawning channels have suffered high mortality due to IHNV (Traxler and Rankin 1989). The losses observed in the fry do not have a high correlation with the virus titers measured in the spawning adults. Other factors such as density and environmental stressors seem to be an important influence in determining losses in the fry.

Certain aspects of the epizootiology of the IHN virus are still unresolved, including the possibility of vertical or egg-associated transmission. There are years of data from sockeye production in Alaska were IHN occurrences in fry can only be attributed to vertical transmission (Meyers et al. 1990). In spite of other anecdotal information implying vertical transmission, no controlled laboratory experiment has yet confirmed this method of viral transmission. Virus was not detected in eggs or progeny from random matings of sockeye salmon adults naturally infected with varying amounts of IHN virus and treated with fish culture water, iodophore, or added IHN virus (Traxler et al. 1996). Horizontal transmission of IHNV readily occurs in both fresh and salt water (Wolf 1988 a,b; Traxler et al. 1993).

The infrequent detection of the virus in wild stocks of salmonids, except during the susceptible fry stage and at spawning, may be due to a carrier state or subclinically infected fish. Various hypothesis such as the virus being reactivated from asymptomatic carriers, viral latency, and the existence of defective interfering particles have been suggested. There is also some evidence to support the view that uninfected fish become re-infected with IHNV at a later life stage (Amos et al.1989; Meyers 1998). The possibility of a seawater reservoir for IHN virus has been suggested since the virus has been detected in wild sockeye salmon in sea water (Traxler and Roome 1993).

The first confirmed report of IHNV in pen-reared Atlantic salmon in British Columbia occurred in 1992 (Armstrong et al.

1993; Traxler et al. 1993). Since this initial finding, IHN has been reported at numerous netpen farm sites and has become a major disease concern on Atlantic salmon farms in British Columbia. Affected sites tend to be concentrated in the Campbell River/Quadra Island area of the Province. No confirmed cases of IHN affecting Atlantic salmon have been reported in the northern or western region of Vancouver Island where there is also extensive salmon farming. The infection has not been detected in Atlantic salmon freshwater hatcheries, and field observations suggest that the Atlantic salmon in netpens may become infected in sea water.

In addition to infection of the kidney, spleen, and intestinal submucosa, the virus is found in high titer in the mucus and feces of IHN infected fish. The presence of high levels of virus in the mucus of farmed Atlantic salmon indicates a possible mechanism of rapid and efficient transmission during handling whenever fish are concentrated and fish to fish contact occurs

A concern is the existence or establishment of marine hosts or reservoirs of IHNV that may serve as sources of the virus at grow-out sites. To determine whether fish that inhabit areas in and around net pens can become virus reservoirs, the susceptibility of three such marine fishes to IHNV was tested (Traxler and Richard 1996)). Tubesnout, shiner perch, and Pacific herring were susceptible when injected with the virus, with losses in excess of 50% occurring in all species of fish tested. When exposed by immersion, herring were the most susceptible, with 25% of the exposed fish dying due to IHNV. Furthermore, Traxler and Richard (1996), found high titers of the virus in tubesnout and shiner perch collected at a fish farm undergoing an IHN epizootic. We have also isolated IHN virus from one Pacific herring that was collected well away from fish farms. However, how long the virus can persist in these species and the role they play as marine reservoirs for the infection is unknown.

Using polyclonal antisera, only a single serotype of IHN virus has been identified. However, several electropherotypes have been recognized based upon the molecular weights of the structural proteins (Leong et al. 1981). Several strains were identified and tended to be from distinct geographical regions and have a degree of species specificity. Monoclonal antibodies have also been used to differentiate between isolates from different locations (Winton et al. 1988; Ristow & Arnzen 1989). Kurath et al. (1995) developed a RNAse protection assay capable of determining genetic diversities between isolates of IHNV. This technique will be very useful for understanding the relatedness of IHNV isolates from different organs, individuals, watersheds, host species, and geographic areas.

CLINICAL SIGNS AND GROSS PATHOLOGY. In netpens, swimming behavior can vary from lethargy to rapid erratic flashing motions. Externally, affected fish are usually dark and exhibit hemorrhages at the base of the fins. The gills and liver may be pale, indicating anemia, and affected fish show ascites, and petechial and ecchymotic hemorrhages throughout the viscera (Fig 5-4a). The digestive tract may be filled with a yellowish mucus-like fluid. Certain chronic affects, such as scoliosis, have been attributed to the virus (Amend et al. 1969).

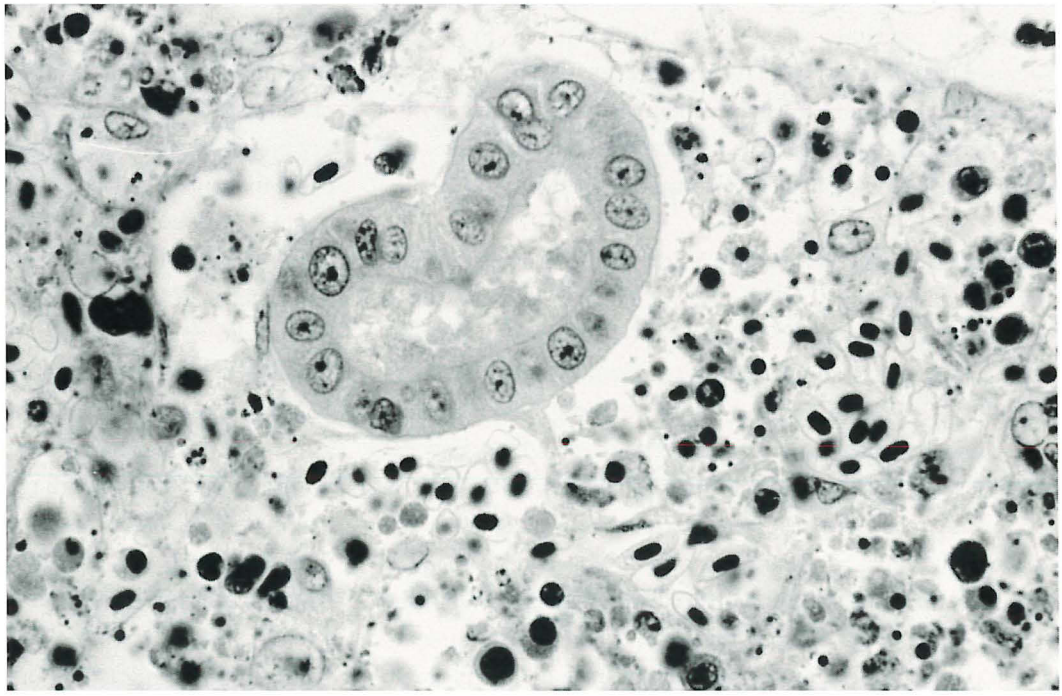


Figure 6-1. Tissue section of the kidney of a sockeye salmon with IHN. Note severe necrosis in the hematopoietic cells in the kidney interstitium. H & E

MICROSCOPY. The hematopoietic tissues of the kidney and the spleen are the most severely affected (Yasutake 1970). In the early stages of infection these tissues exhibit focal necrosis, which progresses to severe, diffuse necrosis and degeneration of these tissues (Fig. 6-1). The gills, pancreas, liver, and submucosa of the alimentary tract may also exhibit diffuse necrosis.

DIAGNOSIS. Presumptive diagnosis of IHN can be achieved by observing severe, diffuse necrosis in the hematopoietic tissue, which can be detected in histological sections or tissue imprints (Yasutake 1978). The standard method of detecting the virus relies upon isolation of the virus using cell cultures derived from fish tissues (Anonymous 1984; Amos 1985). Cell cultures inoculated with IHN virus usually show characteristic cytopathic effect (CPE) within 10 days at 15 °C. The CPE is fairly distinctive with margination of the chromatin and rounding of the cells into clusters which retract from the center of the plaque. Frequently used confirmatory tests include virus neutralization, fluorescent antibody tests, ELISA (enzyme linked immunosorbent assay), and the DNA probe (Winton 1991). A polymerase chain reaction (PCR) test with sensitivity approaching that of cell culture is now being more widely used as a method to detect the presence of the viral genome (Arakawa et al. 1990). The use of serological tests to detect the presence of humoral antibodies to fish viruses may become a useful technique for determining past exposure to viral pathogens and in epizootiological studies (LaPatra 1996).

The gross pathological changes in Atlantic and sockeye salmon infected with IHN virus in sea water (Fig. 5-4a) are essentially indistinguishable from those caused by Gram-negative bacterial septicemias (Traxler et al. 1991b). Therefore, diagnosticians should consider conducting virus assays on fish with generalized internal hemorrhaging that are not consistently infected with known bacterial pathogens.

CONTROL AND TREATMENT. In freshwater hatcheries, IHN is controlled by avoiding the infection. This is usually accomplished using eggs from IHN virus-free females, disinfecting eggs with iodophores, and rearing eggs and fry in IHN virus-free water (Winton 1991). Rearing Atlantic salmon and chinook in netpens in freshwater lakes before seawater entry has been employed by some fish farmers in the Pacific Northwest. The virus is prevalent in sockeye salmon and kokanee in some of these lakes, which could be a potential source of transmission to pen-reared fish. Therefore, until the risk of transmission of IHN virus is assessed in this situation, we suggest that these lakes be avoided or that fish reared in such pens be tested for the presence of the virus before they are transferred to sea water. In addition, only smolts free of the virus should be transported to netpen sites because the disease can be transmitted in sea water.

Circumstantial evidence suggest that a marine reservoir is the primary source of the infection for outbreaks in seawater netpens. If this is the case, then avoidance of the infection in netpens would be very difficult. Marine-phase chinook salmon may harbour the virus for several months with no signs of the disease, and the virus has been found in healthy chinook reared at netpen farms that have experienced IHN outbreaks in Atlantic salmon (S. St. Hilaire, Pacific Biological Station, Nanaimo, British Columbia, pers. comm.). Therefore, chinook salmon may act as a subclinical reservoir for the virus when they are reared with Atlantic salmon.

Several types of vaccines have been developed that are effective in experimental situations (see review by Winton 1997) , but the efficacy of these vaccines in netpen situations has yet to be clearly demonstrated. Anderson et al. (1996) developed a DNA vaccine against IHN for rainbow trout, which also protects Atlantic salmon.

Infectious Pancreatic Necrosis (IPN)

The viruses (several serotypes) responsible for causing infectious pancreatic necrosis (IPN) in cultured salmonids belongs to the family Birnaviridae. Viruses in the Birnaviridae have a widespread geographic and host range with isolations reported from numerous species of freshwater and marine fishes, and from many species of marine invertebrates (McAllister 1983). However, IPN-like virus isolates from non-salmonids are usually non-pathogenic for salmonids. The classification of this group of viruses is complex and their organization is dependent upon the methods used for comparison (Caswell-Reno et al. 1989; Hill and Way 1995).

The most susceptible species of fish appear to be rainbow trout, brook trout, brown trout, and Atlantic salmon. Younger fish usually suffer acute infections resulting in losses often exceeding 90%. Fish over 6 months old are usually more resistant to IPN infections. Survivors of IPN outbreaks may become carriers, shedding virus throughout their life and acting as a source of re-infection. Although IPN has been considered a typical fry and fingerling disease, the virus now causes tremendous problems in sea-water netpens as well.

The infection is prevalent in pen-reared Atlantic salmon in Norway (Krogsrud et al. 1989) and has caused problems in Scotland (Smail et al. 1992, 1995). For many years, the virus was extremely widespread in Norwegian netpen farms (Melby

et al. 1991) without causing clinical disease. In recent years, however, clinical IPN has become an increasing problem in sea-farmed Atlantic salmon. Most commonly, outbreaks occur a few weeks up to a couple of months after transfer to sea water (Jarp et al. 1995), but outbreaks up to one year post sea-transfer also occur frequently (Smail et al. 1992). The disease may be the most important infectious disease of farmed fish in Norway, accounting for losses of approximately NOK 400 million/year (Christie 1996). In Scotland, significant mortality has been associated with the infection, particularly in combination with salmon pancreas disease. Vertical transmission of IPN virus has been well documented (Wolf et al. 1963; Bullock et al. 1976; Fijan and Giorgetti 1978), and has occurred in groups of eggs that had been treated with iodophores.

In 1989 a birnavirus was isolated from a group of Atlantic salmon post-smolts from a seawater netpen site in British Columbia. Tests conducted to identify the isolate concluded that the virus was most similar to, but not identical with, a strain of IPN virus isolated from brook trout in British Columbia (Kieser et al. 1989). This isolate, referred to at the time as IPN virus, proved to be non-pathogenic to fry of chinook, coho, and Atlantic salmon, and rainbow trout (Traxler and Evelyn 1991). In view of the failure of the isolate to cause IPN in the tested salmon, the virus would more appropriately have been identified as a birnavirus than an IPN virus.

CLINICAL SIGNS AND GROSS PATHOLOGY. In fry and fingerlings, the initial sign of IPN are often a sudden increase in mortality. Moribund fish may be unable to swim against the current, are usually dark in color, and show abdominal distension and exophthalmos. Post-smolts may show very few clinical signs, but stop feeding and show nervous symptoms. The most significant losses may sometimes be attributed to the long-term effects of reduced or completely ceased feeding. Internal organs are often pale, and the digestive tract may be devoid of food and filled with a whitish mucus due to sloughing of the intestinal epithelium (Wolf 1988b). In older fish (e.g., post-smolt Atlantic salmon), hyperemia and petechial hemorrhage in the visceral fat and in the pyloric caeca are common findings.

MICROSCOPY. As the name implies, IPN virus causes necrosis of the exocrine pancreas. Intracytoplasmic inclusions have been reported (Yasutake 1970). Lesions have also been reported in the hematopoietic and excretory tissues of the kidney, the mucosal cells of the digestive tract, and the liver parenchymal cells (Wolf 1988b).

DIAGNOSIS. A definite diagnosis of IPN is based upon isolation of the virus in tissue culture and a neutralization test, along with observation of the described pathological changes (Bruno and Poppe 1996). The cell lines of choice are RTG-2 and CHSE-214 and they should be incubated at a temperature of 15 ^{o}C. The use of two or more dilutions of the inoculum are recommended in order to quantify the virus present. This approach allows the diagnostician to determine whether carrier or lethal levels of the virus are present. It also helps to reduce toxicity of the tissue extracts for the cell cultures and helps to avoid inhibition of IPN virus due to high concentrations of fish tissue homogenates (Dixon 1987). Positive identification of the virus is accomplished by serological methods such as serum neutralization or fluorescent antibody techniques.

Salmon pancreas disease is an important differential diagnosis for IPN, and the two diseases can be difficult to distinguish, both clinically and pathologically. Characteristic lesions that react positively with IPN antisera using immunohistochemistry techniques are strongly indicative of IPN, while chronic lesions may be impossible to separate as the virus disappears from the lesions.

CONTROL AND TREATMENT. An epidemiological study of IPN in post-smolts has shown that the risk of clinical disease was related to the mixing of smolts from several suppliers at the same sea site (Jarp et al. 1995). A consequence of this would therefore be to buy smolts from as few producers as possible. It has been shown that smolts with no history of IPN in fresh water, but with specific humoral immunity against IPNV prior to smoltification, were protected against clinical IPN up to 4 months after transfer to sea water (Jarp et al. 1996).

Because IPN virus can be vertically transmitted and infected fish can excrete virus for the rest of their life, the only effective control method is avoidance. The use of IPN virus-free broodstock, rearing progeny in virus-free water, and restricting the movement of fish are measures that can reduce the spread of IPN virus.

A multivalent vaccine, which includes *E. coli*-expressed IPNV proteins, protected presmolt Atlantic salmon against natural exposure to IPN (Christie 1996). This vaccine is now licensed in Norway, and results from the 1996 season are very promising as mortalities due to IPN have been reduced considerably.

Salmon Pancreas Disease (SPD)

Salmon pancreas disease of pen-reared Atlantic salmon is an important disease in Scotland, Ireland, and Norway (Munro et al. 1984; Ferguson et al. 1986; McVicar 1987; Menzies et al. 1996). The disease has also been observed in pen-reared Atlantic salmon in Washington State (Kent and Elston 1987), and has occurred rarely in British Columbia. The cause of the disease has been controversial. Laboratory transmission studies indicate that the disease is caused by an infectious agent, probably a virus (McVicar 1990; Pringle et al. 1992; Raynard and Houghton 1993; McLoughlin et al. 1995, 1996), whereas the clinical and histopathological changes observed in affected fish were suggestive of a vitamin E - selenium deficiency (Ferguson et al. 1986). Bell et al. (1987) concluded that the depletion of vitamin E and selenium observed in affected fish is probably an effect of the disease rather than its cause.

Nelson et al. (1995) isolated a toga-like virus from fish with SPD, and McLoughlin et al. (1996) experimentally reproduced the disease with the virus. Therefore, the evidence is essentially conclusive that the cause of the disease is this virus, referred to as Salmon Pancreas Disease Virus (SPDV). McLoughlin et al. (1998) suggested that the virus may occur in all major salmon producing countries in Europe.

Salmon pancreas disease only occurs in salmon after they have been transferred to sea water and has not been linked to the freshwater environment or stock origin. Based on these observations, McVicar (1987) concluded that SPD is purely a marine disease. Fish usually start to exhibit clinical SPD about 6 to 12 wk after introduction to netpens, but fish that have been in pens as long as two years may be affected (McVicar 1987). Often close to 100% of a population is affected, but most fish recover and return to normal feeding and growth in a few months (Munro et al. 1984; McVicar 1987). In these cases, overall mortality due to SPD is usually low, but surviving fish may grow poorly and may be more susceptible to other diseases (McVicar and Munro 1987). In other cases, mortality as high as 50% has been attributed to the disease (Wheatley 1994).

CLINICAL SIGNS AND GROSS PATHOLOGY. Affected fish are anorexic, lethargic and hang listlessly at the sides of cages near the surface. Fish with SPD are usually emaciated . Internal examination often reveals hemorrhages in the pancreatic tissue and fat between the pyloric caeca (Fig. 5-4b), or the tissue between the pyloric caeca may be severely atrophied.

MICROSCOPY. Fish with SPD exhibit acute and generalized necrosis of the pancreatic acinar cells. The pancreatic tissue is markedly reduced and may be replaced by a marked increase in cellularity, suggestive of inflammation, stromal condensation, and fibrosis (Ferguson et al. 1986). Some fish may also exhibit degenerative changes in the heart, with coagulative necrosis of the ventricular myocardium (Ferguson et al. 1986). Lesions in the skeletal muscle may also occur about 3-5 wk after infection (McCoy et al. 1994). In the post-acute (recovery) phase, fish may exhibit islets of regenerating acinar tissue amongst fibrotic tissue (Munro et al. 1984).

DIAGNOSIS. Hemorrhages in tissues associated with the pyloric caeca in emaciated Atlantic salmon smolts, along with the absence of other infectious agents (e.g., IHN or IPN viruses, *A. salmonicida* or *Vibrio* spp.), is indicative of SPD. Confirmation of the disease is based on observation of the histological changes described above, or by isolation of SPDV from affected fish. The latter can be achieved by co-cultivation of kidney tissues on CHSE-214 cells at 15 ^{o}C in which cultures are blind passed after 28 days (Nelson et al. 1995). In the second passage, CPE may be observed after about 10 days. A significant decrease in *p*-aminobenzoic acid may also be useful for diagnosing SPD (Pringle et al. 1992).

CONTROL AND TREATMENT. No treatment is known for SPD. Reports from Scotland indicate that reducing stressors (e.g., transport and handling) during the acute phase of the disease may enhance recovery. In addition, some farmers have reported that keeping fish on a smaller pellet size lessens anorexia and reduces the overall mortality associated with the disease. Recovered fish exhibit strong protection to reinfection (Houghton 1994), which provides hope that a vaccine could be produced against the virus.

Infectious Salmon Anaemia (ISA)

The first case of the disease today known as infectious salmon anaemia (ISA) occurred in a smolt farm in southwestern Norway in 1984. During the following 10 years, the disease spread to most fish farming areas along the coast, but only seawater farms or freshwater farms that used some sea-water (to increase temperature, reduce stress during smoltification or as a buffer in acidified areas) have experienced natural outbreaks (Thorud and Djupvik 1988). However, the disease can be experimentally transmitted in fresh water as well. Although a viral etiology has been suspected from the beginning, it was not until 1995 that a virus

was isolated and classified as the causative agent of ISA by Dannevig et al. (1995). Recent experiments indicate that the virus resembles the orthomyxovirus group. Virus is shed from infected carriers before they develop clinical signs of the disease through skin mucus, urine and feces. Electron microscopy has revealed that early colonization of the causative virus occurs in the pillar cells of the gills and the endocardium indicating that the gills are the most likely port of entry (Totland et al. 1996). Natural outbreaks have occurred in Atlantic salmon only, but other salmonids may harbour the virus (Nylund and Jakobsen 1995). A virus very similar to the ISA agent has recently been isolated from salmon in Atlantic Canada with hemorrhagic kidney disease (see below), and ISA has recently been observed in Scotland (Rodger et al. 1998). Extensive measures have been taken to eradicate the disease from Norwegian aquaculture and presently (1997) only a few farms are affected by the disease.

CLINICAL SIGNS AND GROSS PATHOLOGY. Most clinical cases have occurred during rapid temperature increments in the spring, but outbreaks may also occur in the late autumn. Initially, the fish go off the feed, are listless and tend to sink to the bottom or rest near the edges of the cages. These are different strains of the virus and thus the manifestation of the disease may be variable (see hemorrhagic kidney disease). Mortality may vary from 15 to 100%. An outbreak may last for several months, but is normally of shorter duration if temperatures are above 10-12°C. Macroscopically, distended abdomen, exophthalmos, skin edema and hemorrhage are typical findings. The gills and heart may be extremely pale. Congestion in internal organs (e.g., liver, spleen and foregut), punctate hemorrhage in perivisceral fat and on peritoneal surfaces are typical lesions. In some cases the liver may appear extremely congested and almost black in colour (Fig. 5-4c). Hematocrit values may drop to as low as 1% or less in severely affected fish.

MICROSCOPY. In the liver, light microscopical lesions include multifocal hemorrhagic necrosis, dilatation and congestion of the sinusoids (Fig. 6-2). The liver lesions typically develop into anastomosing, confluent haemorrhagic necrosis with a characteristic pattern leaving the area closest to the central veins intact. There is often pronounced congestion of the foregut with diffuse hemorrhage to the stratum proprium (Evensen et al. 1991a, b).

DIAGNOSIS. The diagnosis is based upon characteristic gross pathology and light microscopical changes, negative bacteriology and anaemia (low hematocrit values). The isolation and propagation of a causative agent in salmon head kidney (SHK-1) cells will allow the production of specific antibodies and diagnostic kits based on an indirect fluorescent antibody test (IFAT) (Falk and Dannevig 1995). A PCR test for the virus is also available (Mjaaland et al. 1997).

CONTROL AND TREATMENT. No treatment is known for ISA. Nevertheless, the eradication program for ISA has been successful indicating that the viral pathogen is not very invasive in the marine environment. After the implementation of several measures to reduce the impact and spread of the disease, a steady decrease in new outbreaks has been recorded. These measures include mandatory health control in smolt farms, disinfection of sea water used in freshwater farms, disinfection of processing water from slaughtering facilities, and isolation of infected sites and fallowing sites after slaughtering of infected stocks.

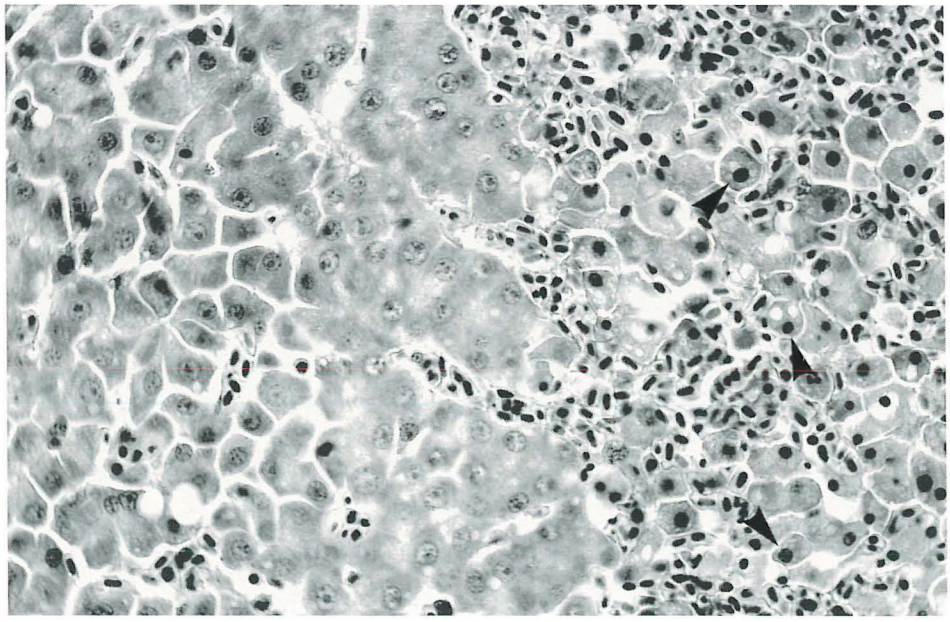


Figure 6-2. Liver of Atlantic salmon with ISA. Note areas of anastomozing, zonal hemorrhage and necrosis. Arrowheads = necrotic hepatocytes.

Hemorrhagic Kidney Disease (HKD)

A newly-recognised disease of pen-reared Atlantic salmon has been observed in Atlantic Canada. The disease, referred to as "mystery disease" or "hemorrhagic kidney disease" was first observed in 1996. HKD has been devastating at some farms, causing up to 5% mortality/day. Some farms have experienced concurrent BKD problems with HKD, but the pathologic changes are distinctly different in these two diseases. The disease is clearly transmissible, and a virus very similar to ISA has been isolated from affected fish (Mullins et al. 1998). Therefore, the consensus among most fish pathologists at this time is that HKD is caused by a variant of the ISA virus.

CLINICAL SIGNS AND GROSS PATHOLOGY. Affected fish exhibit an enlarged kidney and ascites.

MICROSCOPY. Byrne et al. (1998) described the pathology of HKD. The hallmark of HKD is massive, diffuse hemorrhage of the kidney, resulting in massive pooling of erythrocytes in the renal interstitium (Fig. 6-3). Renal tubule necrosis is also common. In the spleen we have seen severe congestion, and Byrne et al. (1998) reported that the spleen may also exhibit erythrophagocytosis, ceroid accumulation and other changes. Other organs may show minor pathological changes. As with typical ISA, some fish may exhibit liver necrosis.

DIAGNOSIS. Diagnosis is based on histological examination and observation of severe hemorrhage in the kidney interstitium.

CONTROL AND TREATMENT. Because there is compelling evidence that the disease is caused by a virus (or viruses), control measures typically used for viral (e.g., eradication of infected stocks) have been implemented.

Viral Erythrocytic Necrosis (VEN)

Viral erythrocytic necrosis (VEN) has been reported in various species of marine and anadromous fishes throughout the world (Appy et al. 1976; Walker and Sherburne 1977). Along the Pacific coast of North America, natural occurrences of VEN have been documented in chum, pink, coho and chinook salmon, steelhead trout, and Pacific herring (Bell and Traxler 1985; Meyers et al. 1986; Rohovec and Amandi 1981).

The viruses responsible for VEN have been tentatively placed in the family Iridoviridae. Virions observed from fish have been icosahedral with a diameter ranging from 140-350 nm. Most of the virions found in Pacific herring and salmon fall within the 140-210 nm size range, whereas those reported from Atlantic cod ranged from 310-360 nm in diameter (Appy et al. 1976).

The widespread range of VEN viruses and susceptibility of salmonids to at least one of these viruses suggest that the virus poses a threat to netpen-cultured salmonids. Viral erythrocytic necrosis was frequently observed among pink and chum

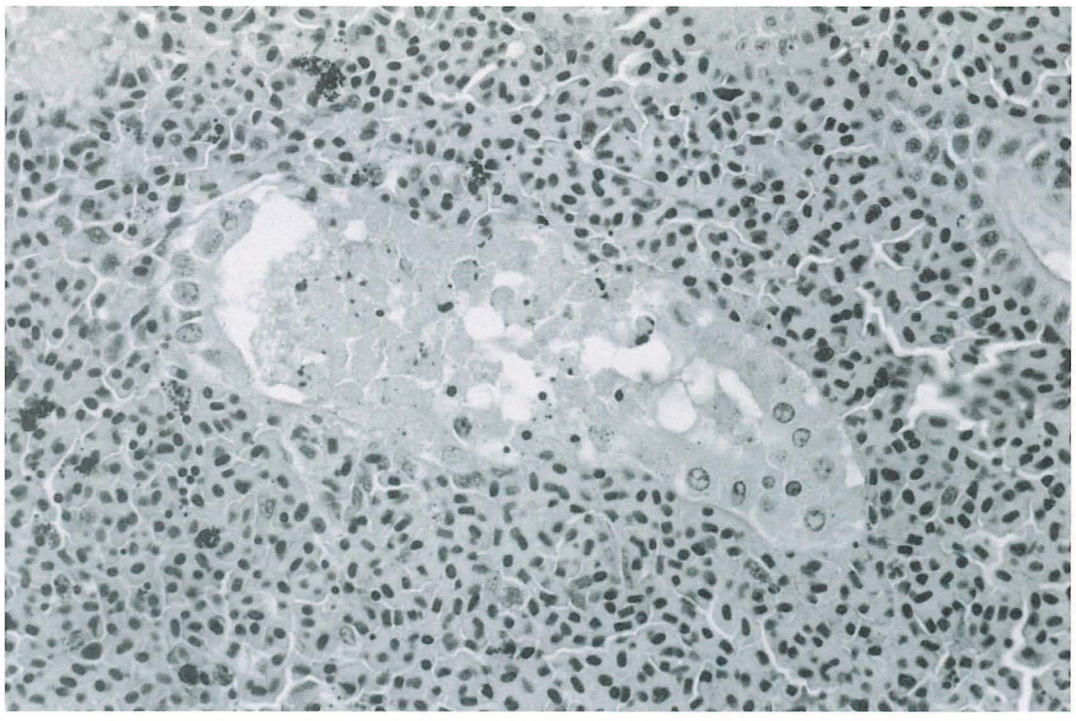


Figure 6-3. Kidney section of an Atlantic salmon with hemorrhagic kidney disease. Note the interstitium is replaced by red blood cells and the tubules are necrotic. H & E.

salmon being farmed in netpens located at Departure Bay, British Columbia but coho, chinook, and sockeye salmon raised at the same site did not appear to acquire the disease (Evelyn and Traxler 1978). However, a survey of anadromous salmonids in Oregon revealed that 25% of the populations tested were positive for VEN virus (Rohovec and Amandi 1981). Positive species were spawning adult coho, chinook, and chum salmon, and steelhead trout.

Natural occurrences of VEN in Pacific herring in Alaska resulting in high losses were reported by Meyers et al. (1986). The VEN virus from Pacific herring has been transmitted to chum, pink, and Atlantic salmon using intraperitoneal injection, and the virus in the salmon was associated with anaemia and death (Evelyn and Traxler 1978; MacMillan and Mulcahy 1979; Traxler et al. 1991a). Preliminary evidence from transmission studies conducted in our laboratory also indicate that VEN virus from Pacific herring is transmittable to Atlantic salmon by cohabitation with infected herring in sea water.

CLINICAL SIGNS AND GROSS PATHOLOGY. In heavily infected fish, the most significant clinical sign is severe anemia, indicated by the pallor of the gills and visceral organs. The virus infects erythrocytes, resulting in reduced hematocrit values (Evelyn and Traxler 1978). Some fish may exhibit unilateral or bilateral exophthalmos (MacMillan et al. 1989). Infection with VEN virus usually results in chronic, low level losses. A significant side-effect of VEN is reduced resistance to other pathogens and environmental stressors (MacMillan and Mulcahy 1979; MacMillan et al. 1980).

MICROSCOPY. Histological signs of VEN are not pathognomonic. The kidney may show increase in hematopoiesis. The characteristic cytoplasmic inclusions in erythrocytes that accompany the infection are not frequently observed in tissue sections. Blood smears stained with Giemsa typically reveal eosinophilic or amphophilic intracytoplasmic inclusions (0.8 - 4.0 µm in diameter) in erythrocytes (Fig. 5-3a). In chum salmon, erythroblasts are also infected as the disease progresses (MacMillan et al. 1989). Affected cells usually contain a single round or oval inclusion in the cytoplasm. Transmission electron microscopy of infected erythrocytes reveals large, spherical viroplasms and pentagonal or hexagonal virions (about 150 - 200 nm) in the cytoplasm (Fig. 6-4). The viroplasm likely represent the inclusions observed in Giemsa-stained blood smears.

DIAGNOSIS. The viruses responsible for VEN have not been grown in cell culture. A strong presumptive diagnosis is made by finding the characteristic intracytoplasmic inclusions in erythrocytes. Confirmation is accomplished by observing the characteristic virions by electron microscopy (Fig. 6-4).

CONTROL AND TREATMENT. There are no known control measures. Horizontal transmission has been well documented, making avoidance difficult given the widespread marine distribution of the virus. There is evidence that individual fish are able to recover from VEN. Maintaining affected stocks in conditions of low stress may reduce losses.

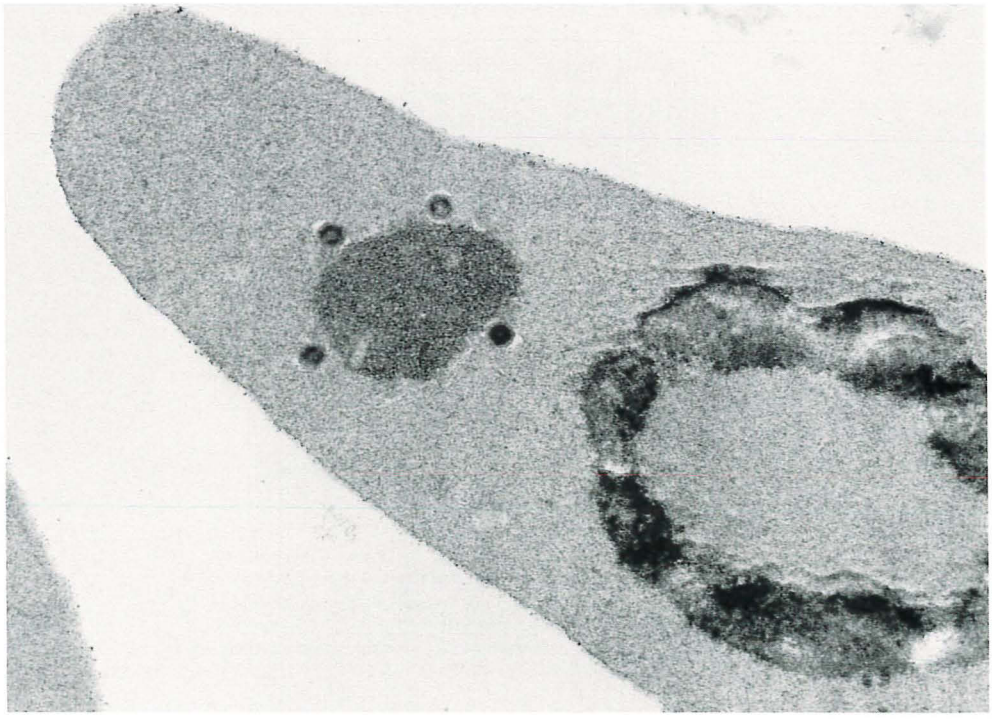


Figure 6-4. Electron micrograph of erythrocyte of Atlantic salmon infected with VEN virus (arrows). VP = viroplasm, N = nucleus.

Erythrocytic Inclusion Body Syndrome

Erythrocytic inclusion body syndrome (EIBS), caused by a togavirus, is a common disease in salmonids reared in freshwater in the Pacific Northwest, particularly in coho salmon (Leek 1987; Piacentini et al. 1989). Epizootics of EIBS have also been reported in pen-reared coho salmon in Japan (Takahashi et al. 1992), and infections associated with variable mortality have been observed in pen-reared Atlantic salmon in Ireland (Rodger et al. 1991), Scotland (Rodger and Richards 1998), and Norway (Lunder et al. 1990). As with VEN virus, the EIBS virus infects erythrocytes and causes anemia. Mortalities in netpens due to EIBS have been as high as 23%, and peak at lower temperatures (e.g., 8-10 °C). However, field observations of Atlantic salmon from Scotland showed little correlation of the infection with anemia and clinical disease. In laboratory studies, Piacentini et al. (1989) demonstrated that the disease is more severe at 12 °C. Horizontal transmission of the virus in water has been demonstrated (Piacentini et al. 1989), but it is unknown if vertical transmission occurs. Fish may recover from the infection, and are then resistent to reinfection (Piacentini et al. 1989; Takahashi et al. 1992).

CLINICAL SIGNS AND GROSS PATHOLOGY. Affected fish are lethargic and swim near the surface. Consistent with anemia, the principle sign of the disease is pallor of the gills and liver. Fish may also exhibit splenomegaly and hyperemia of the intestine. Moribund fish exhibit extremely low hematocrit values, often below 10%.

MICROSCOPY. Blood smears reveal an increase in immature erythrocytes, often as high as 80%. Multiple inclusions within erythrocytes are characteristic of the disease (Fig. 5-3b).

DIAGNOSIS. Strong presumptive diagnosis can be obtained by observing multiple inclusions about 0.8-2.0 µm in the cytoplasm of erythrocytes in blood smears stained with either Giemsa (Fig. 5-3b) or stained with pinacynol chloride (Yasutake 1987). EIBS virus inclusions differ from those of VEN in that the latter are larger and usually singular. In contrast to VEN, EIBS inclusions are often difficult to see in blood smears. Confirmatory diagnosis can be made by visualizing viral particles (75-100 nm) in the cytoplasm of erythrocytes (Fig. 6-5).

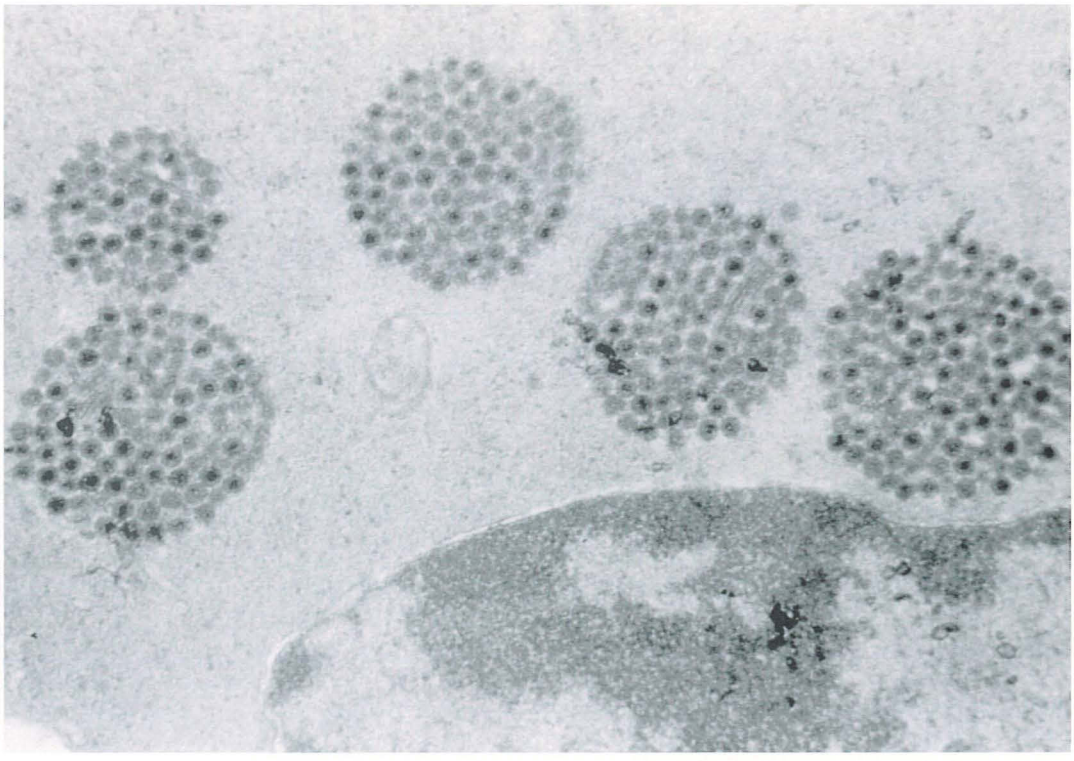


Figure 6-5. Electron micrograph of erythrocyte of coho salmon infected with EIBS virus. (Courtesy of J. Morrison).

Salmonid herpesvirus 2 infections

Several members of the family Herpesviridae are well recognized pathogens of fishes (Wolf 1988a, b). In Japan, a herpesvirus type 2 (SH-2) infection has caused up to 30 % mortality in pen-reared coho salmon (Kumagai et al. 1994). The disease affects fish from less than 100 g to 1 kg, and epizootics usually last from 30-80 days.

Certain strains of salmon herpes virus 2 (e.g., *Oncorhynchus masou* virus [OMV] and yamame tumor virus [YTV]), cause liver damage in young fish, and fish that survive the infection may later develop epithelial tumors (Sano et al. 1983; Kimura et al. 1981 a,b; Kimura and Yoshimizu 1991; Yoshimizu et al. 1995).

CLINICAL SIGNS AND GROSS PATHOLOGY. Affected fish are dark in color, and exhibit skin ulcers and erosion of the fins. The liver exhibits focal pale areas, and the intestinal tract show erythema. Surface tumors appear as whitish papillomatous masses around the mouth, eyes, fins, or gills (Fig. 6-6). Renal tumors occasionally are observed, which appear as solid, well-defined white masses.

MICROSCOPY. Histological examination reveals severe, multifocal acute necrosis of the liver parenchyma. Histology of the tumors reveals that they consist of epitheloid cells (Kimura et al. 1981 b).

DIAGNOSIS. Focal necrosis of the liver in coho salmon reared in Japan is presumptive diagnosis for the disease. Confirmatory diagnosis is acheived by isolation of the virus from affected livers on CHSE-214 or RTG-2 cell lines. Syncytia formation occurs in the latter.

CONTROL AND TREATMENT. As with other viral diseases, the best method to control the infection is avoidance. Kumagai et al. (1997) linked outbreaks in seawater netpens with previous infections at freshwater hatcheries, and that pen to pen transmission in sea water was negligible. They also reported that rainbow trout may act as subclinical reservoirs for the infection. Based on the their findings, Kumagai et al. (1997) recommended the following to control the infection: 1) do not rear other salmonids with coho salmon; 2) disinfect facilities after out-planting stocks; 3) avoid smolts from contaminated hatcheries; 4) examine fish for virus shortly after seawater introduction.

Kimura et al. (1983) reported that daily immersion of chum salmon in the anti-viral compound acyclovir suppressed the growth of OMV-associated tumors. These authors also found that oral treatment with another anti-viral drug, IUdR, decreased mortality due to the infection. Surface tumors are often removed manually at harvest from fish before they are sent to market.

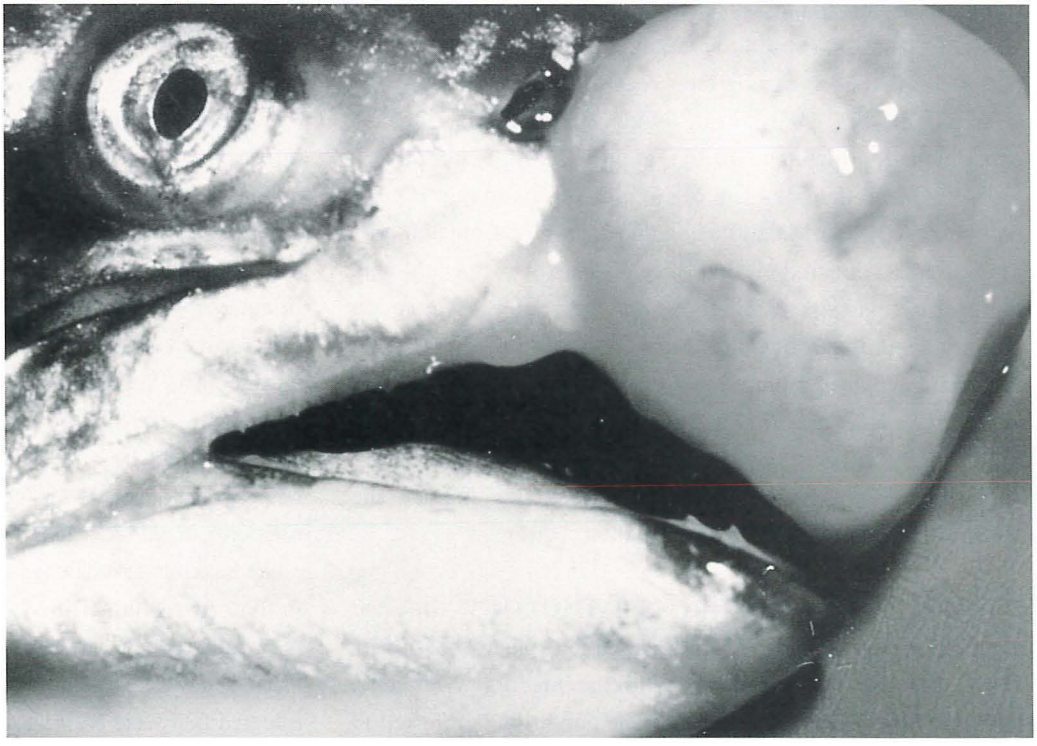


Figure 6-6. Epithelial tumor on pen-reared coho salmon with OMV virus infection (Courtesy of T. Kimura)

FUNGI AND RELATED ORGANISMS

M. L. Kent and T. T. Poppe

Two fungus-like organisms, the rosette agent and *Ichthyophonus* (presumably *I. hoferi*), and a true fungus, *Exophiala* sp., have been recognized as causes of disease in pen-reared salmon.

Rosette Agent

A severe infectious disease of chinook reared in netpens occurred repeatedly at the U.S. National Marine Fisheries Service experimental station in Manchester, Washington, USA (Elston et al. 1986; Harrell et al. 1986). The disease is caused by an unclassified intracellular protistan parasite, called the "rosette agent". Based on ribosomal DNA data Kerk et al. (1995) reported that the rosette agent is most closely related to choanoflagellates (subphylum Choanozoa), an unusual group of protists evolutionary between fungi and animals. Using similar sequence comparisons, Ragan et al. (1996) demonstrated that the rosette agent, *Dermocystidium salmonis* of salmon gills, and *Ichthyophonus hoferi* form a distinct group, and Cavalier-Smith and Allsopp (1996) assigned this group to a new class, the Ichthyosporea, within the subphylum Choanozoa.

The rosette agent primarily infects macrophages in the spleen and kidney, but it may occur in other organs in heavy infections. Mortality due to the disease is highest in the summer and fall, and losses of over 90% have occurred in some years. Epizootics caused by the pathogen have only been observed in chinook during their second summer in sea water (Harrell et al. 1986), but younger chinook can be experimentally infected (Elston et al. 1986). Elston et al. (1986) isolated the organism in culture and found that it grew only in the CHSE-214 cell line, which is derived from chinook. The authors concluded that the organism is an obligate intracellular parasite of chinook, but the source of infection was not determined. In California a similar, probably identical organism, has been reported in Atlantic salmon reared in fresh water (Hedrick et al. 1989) and in chinook salmon in sea water (Arkush 1997). The parasite infects seawater-reared S1 and S2 Atlantic salmon smolts as well as market-size fish on the Atlantic coast of Canada (Cawthorn et al. 1991).

CLINICAL SIGNS AND GROSS PATHOLOGY. Infected chinook are anemic, and exhibit an enlarged spleen and kidney. Pen-reared Atlantic salmon often with the infection exhibited a marked black appearance, and thus the condition is referred to as "black smolt syndrome" in Atlantic Canada. Atlantic salmon smolts may exhibit no gross changes to hypertrophy of liver, kidney and spleen with yellow to white nodules. Petechiae may occur on affected organs.

MICROSCOPY. Histological examination of heavily infected spleens and kidneys reveals numerous eosinophilic spherical organisms in phagocytic cells associated with multifocal necrosis and chronic inflammation. The organism can readily be identified from heavily infected fish in Giemsa-stained imprints of the spleen and kidney. In imprints, the parasites appear as 3-7 µm spheres surrounded by a distinct clear halo around the exterior (Fig. 5-2f). They are often found in clusters or rosettes within macrophages, hence the name rosette agent. The organism is Gram positive, and thus stains blue to black in Gram-stained imprints (Fig. 5-2e).

DIAGNOSIS. The infection is diagnosed by detecting the parasite in either histological sections or Giemsa-stained imprints of the kidney or spleen.

CONTROL AND TREATMENT. There are no known treatments for rosette agent infections.

Ichthyophonus hoferi

Ichthyophonus hoferi is a common pathogen of many species of wild marine fishes (McVicar 1982; Sinderman 1990). The infection is very prevalent in some species, and the organism has caused severe disease and mortality in some fishes, such as Atlantic herring (Sinderman 1990) and plaice (McVicar 1982). The fungus also causes disease in freshwater species. Infections have been reported in freshwater-reared rainbow trout, which were apparently the result of feeding infected marine fish. Salmonids are very susceptible to the infection (Miyazaki and Kubota 1977), and McVicar (1982) warned that salmon would be vulnerable to the infection when reared in netpens. The organism is spread from fish to fish by ingestion of spores or infected fish.

We have observed heavy infections in Atlantic salmon smolts in their first summer in sea water. It is unclear how and when the smolts became infected. However, Atlantic salmon smolts often feed heavily on natural marine organisms, and the infected fish were feeding on calinoid copepods about a month

before the infection was detected. Some have suggested that the organism is spread in infected copepods, and it is possible the smolts became infected by feeding on infected copepods or another marine organism.

CLINICAL SIGNS AND GROSS PATHOLOGY. Infected Atlantic salmon smolts from netpens in British Columbia were lethargic and extremely emaciated, clinical signs that have also been reported in other fish infected with *I. hoferi* , which include curvature of the spine (McVicar 1982). Dissection of heavily infected fish reveals focal, white granulomatous lesions in the visceral organs, particularly the heart. Infections of the skeletal muscle may reduce flesh quality. Although poor flesh quality has not been associated with the infection in salmon, this condition has been observed in non-salmonid fishes (McVicar 1982).

MICROSCOPY. Squash preparations or histological sections of infected organs reveal the resting spores with thick walls (Fig. 7-1), which is the most commonly observed developmental stage in fish. The spores are variable in size and may reach 100-200 µm (McVicar 1982). The spores are Periodic acid-Schiff (PAS) positive and are multinucleated. After the host dies, the spores produce a germination tube and branched hyphae (Fig. 7-2). In plaice and haddock, the spores germinate 15-30 min after death at 20°C (McVicar 1982).

The tissue reaction to *Ichthyophonus* infections is characterized by granuloma formation around the resting spores (Fig. 7-2). Infections are often most severe in the heart, but essentially any visceral organ, the skeletal muscle, and the brain may be infected.

DIAGNOSIS. The infection can be presumptively diagnosed in squash preparations by observing the spherical bodies of varying size ranging up to 200 µm. Confirmatory diagnosis can be achieved by observing post mortem sporulation of the resting spores or by observing the multinucleate resting spores, with a thick fibrous capsule, in histological sections. *Ichthyophonus* has been cultured in vitro, but this is not required for identification because the large resting spores are distinct from other fungi and related pathogens of fishes.

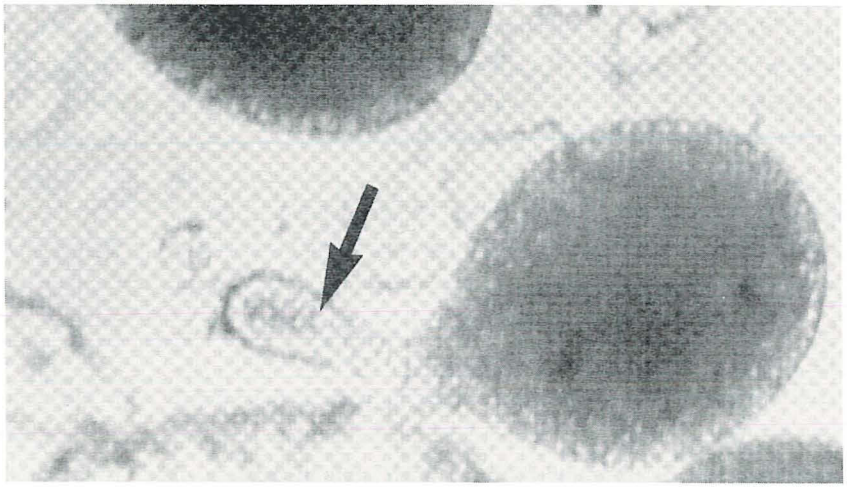


Figure 7-2. Wet mount preparation of *Ichthyophonus* spore. Arrow = germination tube. (Courtesy of the Registry of Study Materials of the Charles Louis Davis, D.V.M. Foundation for the Advancement of Veterinary & Comparative Pathology).

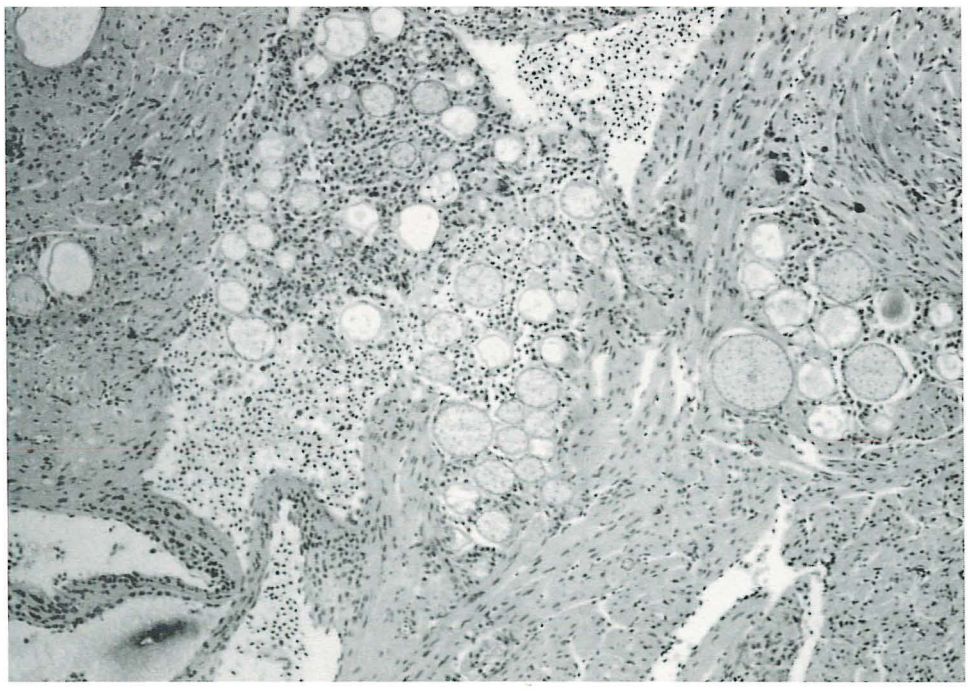


Figure 7-1. Multinucleate *Ichthyophonus* spores in the heart of an Atlantic salmon. H & E.

CONTROL AND TREATMENT. Van Dujin (1956) indicated that fungicidal drugs such as phenoxyethanol might be effective for treating early infections. However, there are no commercially available therapeutants for *Ichthyophonus* infections, and the disease is generally considered incurable. Salmon in netpens may become infected by feeding on natural marine biota (e.g., Pacific herring or copepods). If this is indeed the case, it would be very difficult to avoid the infection in the netpen environment.

Systemic Mycosis (*Exophiala* spp.)

Several systemic fungal diseases are described in fish, but in salt-water farmed salmonids, those caused by *Exophiala* spp. seem to be the most commonly occurring (Richards et al. 1978; Blazer and Wolke 1979; Pedersen and Langvad 1988;). These fungi are widespread in soil and deteriorating organic material, and may also be found in fish feeds kept under damp conditions. The most commonly described species are *E. salmonis*, *E. pisciphila* and *E. psychrophila*.

CLINICAL SIGNS AND GROSS PATHOLOGY. Clinical signs may be variable depending on the severity and location of lesions but frequently involve nervous symptoms like erratic swimming and whirling or circling movements. Affected fish are usually dark and may exhibit abdominal distention and exophthalmos. At necropsy, the posterior part of the kidney may be grossly enlarged and more or less fill the rear part of the abdominal cavity thereby pushing other abdominal organs cranially. The enlarged kidney consists of granulomas of variable size and age mixed with necrotic areas. Granulomas may also be found in liver and spleen.

MICROSCOPY. The lesions are characterized by multiple granulomas where centrally located multinucleate giant cells containing fungal hyphae dominate (Fig. 7-3). There is often considerable inflammatory response in the adjacent areas with massive infiltration of lymphocytes and macrophages. Septate and branching hyphae are usually easily seen in the granulomas and in the giant cells (Fig. 7-3). These are even more visible with PAS-staining. The growth of the fungus is highly infiltrative and may spread rapidly from the kidney to most other organs including heart, brain, muscle and nervous tissue where similar lesions as in the kidney may be found. Healing lesions are typically dark and consisting of fibrous tissue and melanomacrophages.

DIAGNOSIS. The diagnosis is based on demonstration of PAS-positive septate and branching hyphae in smears from affected tissue or histologically in granulomas and multinucleate giant cells. The fungus may also be cultivated on Sabouraud's agar at 25 °C, where it develops characteristic dark grey to black colonies. Identification to species level may be difficult.

TREATMENT AND CONTROL. Although the antifungal compound Natamycin has shown good effect in *Exophiala*-infected fish, treatment is not practical and will be too expensive under farming conditions. Avoidance through strict hygienic measures including cleaning of automatic feeders is the best preventive measure. The use of soil-based biological filters in smolt farms have also been a source of infection in Atlantic salmon and should be monitored carefully.

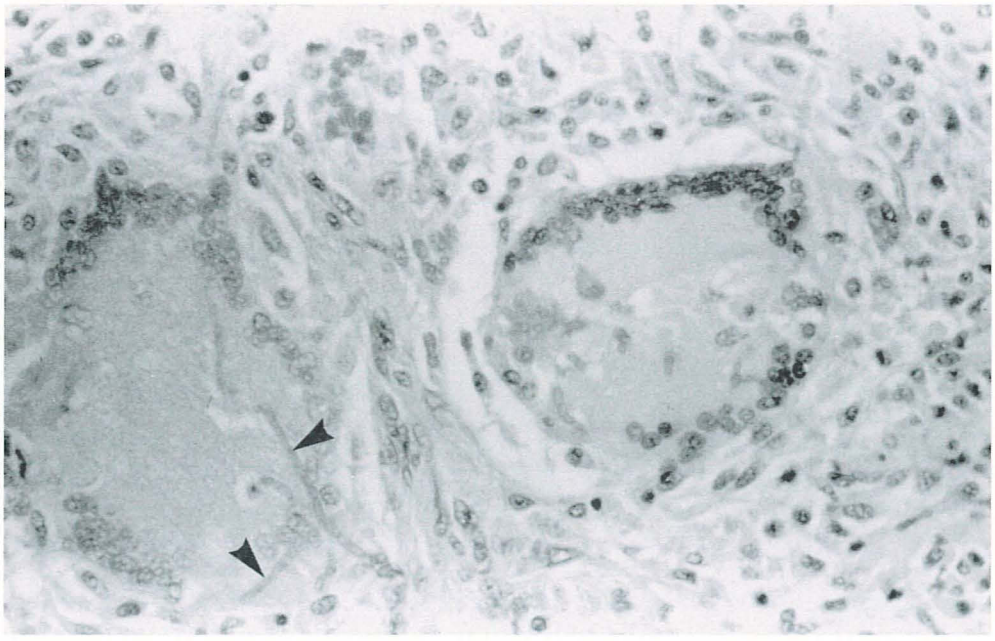


Figure 7-3. Chronic inflammatory response to *Exophiala* infection. Note multinucleate giant cell reaction and fungal hyphae (arrowheads). H & E.

PROTOZOA AND MYXOZOA

M. L. Kent

Protozoans and myxosporeans (phylum Myxozoa) are important pathogens of pen-reared salmonids (Fig. 8-1). An amoeba, *Paramoeba pemaquidensis*, a flagellate, *Ichthyobodo* (= *Costia*) sp., and *Trichodina* infect gill surfaces of salmon. Systemic infections by *Cryptobia salmositica* and a diplomonad flagellate, similar to *Hexamita salmonis*, have caused disease in chinook salmon in British Columbia. Another diplomonad (*Spironucleus barkhanus*) has caused extra-intestinal infections in Atlantic salmon in Norway. Four myxosporeans (*Parvicapsula* sp., *Myxobolus aeglefini*, *Kudoa thyrsites*, and *Chloromyxum truttae*) and three microsporidians (*Loma salmonae*, *Nucleospora salmonis*, and *Microsporidium cerebralis*) infect internal organs. Although recent reports assigned the myxosporeans (phylum Myxozoa) to the Metazoa (Smothers et al. 1994; Siddall et al. 1995), we include them here with the protozoa to remain consistent with other texts on fish pathology. Furthermore, microsporeans are considered by many taxonomists to be separate from the Protozoa, and are now placed in the ancient kingdom Archizoa.

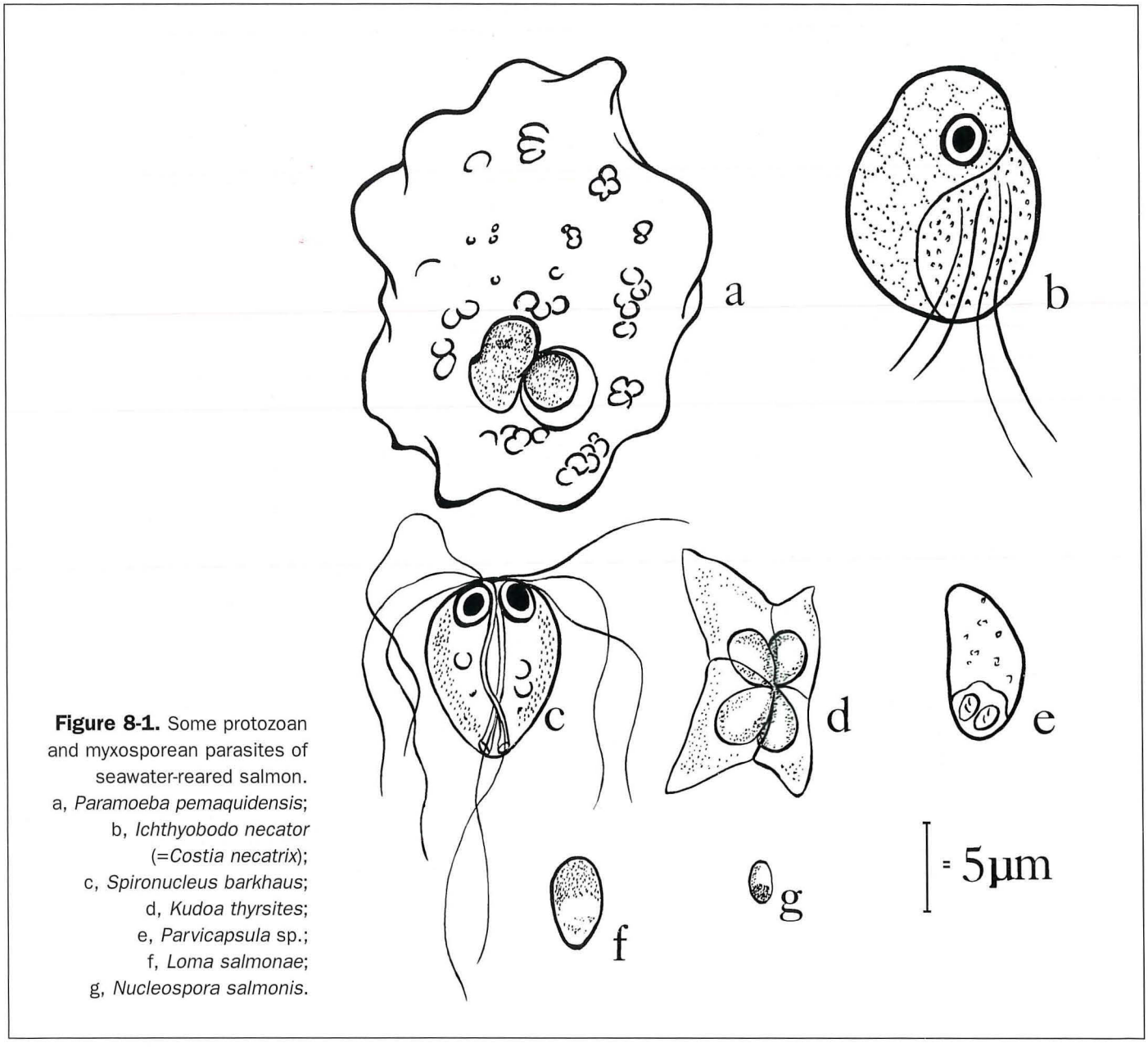


Figure 8-1. Some protozoan and myxosporean parasites of seawater-reared salmon. a, *Paramoeba pemaquidensis*; b, *Ichthyobodo necator* (=*Costia necatrix*); c, *Spironucleus barkhaus*; d, *Kudoa thyrsites*; e, *Parvicapsula* sp.; f, *Loma salmonae*; g, *Nucleospora salmonis*.

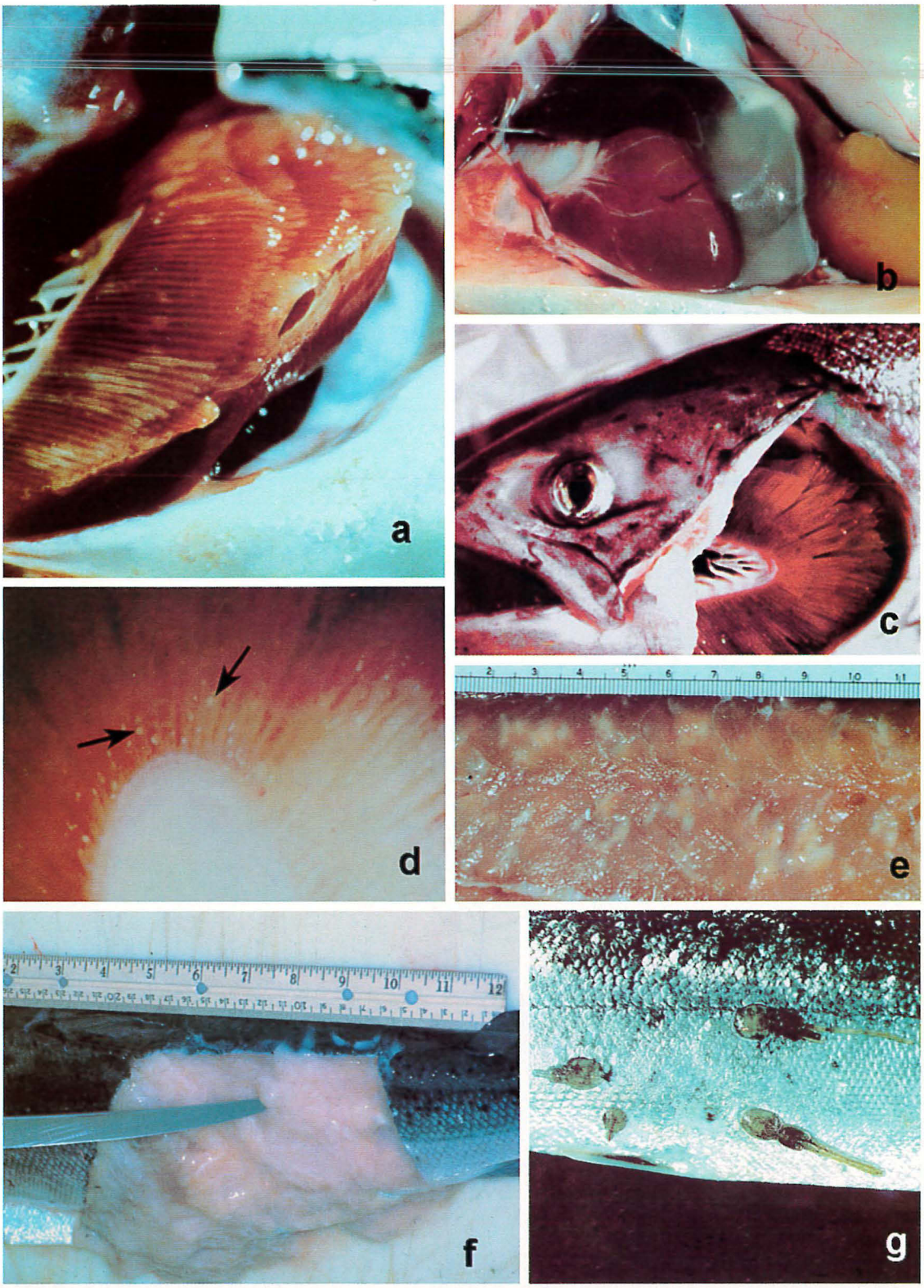


Figure 8-2. Parasite infections in pen-reared salmon
a. Rainbow trout with *Paramoeba* gill infection. Note white areas in gill filaments. (Courtesy of C.K. Foster). b. Chinook salmon with systemic diplomonad infection. Note chicken fat clots in pericardium. c, d. Chinook salmon with *Loma salmonae* infection. Examination of gills with a dissecting microscope (d) reveals numerous xenomas (arrows). c. Note appearance of gills with no obvious xenomas as seen with the naked eye. e, f. *Kudoa thyrsites* infection in Atlantic salmon. e. Multifocal white patches in smoked Atlantic salmon fillet. f. Severe myoliquefaction in an Atlantic salmon that was held on ice for 6 d. g. Sea lice *Lepeophtherius salmonis* infection on Atlantic salmon. Note small, shallow ulcer associated with the infection.

Gill Amoebiasis

Paramoeba pemaquidensis (Sarcomastigophora: Paramoebidae) has been associated with severe gill disease in coho reared in netpens in Washington State and land-based seawater tanks in California (Kent et al. 1988c). A similar, possibly identical, amoeba has caused devastating losses in pen-reared rainbow trout and Atlantic salmon in Tasmania (Munday et al. 1990, 1993; Roubal et al. 1989). *Paramoeba pemaquidensis* is an opportunistic pathogen that is normally a free-living amoeba in sea water. Intensity and prevalence of the amoeba on fish gills has varied from year to year, with infections being most prevalent in the late summer and fall. At one farm in Washington, 25% mortality was attributed to the amoeba in 1985. The exact environmental conditions or health status of the fish that allow the organism to proliferate on fish gills are unknown. Presumably fish already compromised by other diseases are more susceptible to the infection, and at times heavy infections are observed on fish debilitated by pre-existing disease or smoltification problems.

CLINICAL SIGNS AND GROSS PATHOLOGY. Consistent with respiratory problems of fish, heavily infected fish are lethargic, accumulate at the surface, and have flared opercula. Excessive mucus is often observed on heavily infected gills. Focal, whitish patches may be observed on heavily infected fish (Fig. 8-2a).

MICROSCOPY. Floating and transitional forms of the amoeba on the gills are 20-30 µm in diameter and have several digitiform pseudopodia (Fig. 8-3). Careful observation reveals movement in the amoebae. In wet mounts, amoebae will attach to the slide after about an hour, resulting in a locomotive form measuring about 20 X 25 µm. *Paramoeba* spp. contain a unique structure, called a parasome or Nebenkörper, which is adjacent to the nucleus. The parasome can be observed in wet mounts of locomotive forms, and is readily visible with Feulgen DNA stains (Fig. 8-3). Histological sections of affected gills reveal prominent epithelial hyperplasia and fusion of the distal portion of secondary lamellae, which often results in formation of large interlamellar vesicles (Fig. 8-3). The amoebae are usually confined to the gill surface and rarely penetrate the epithelium.

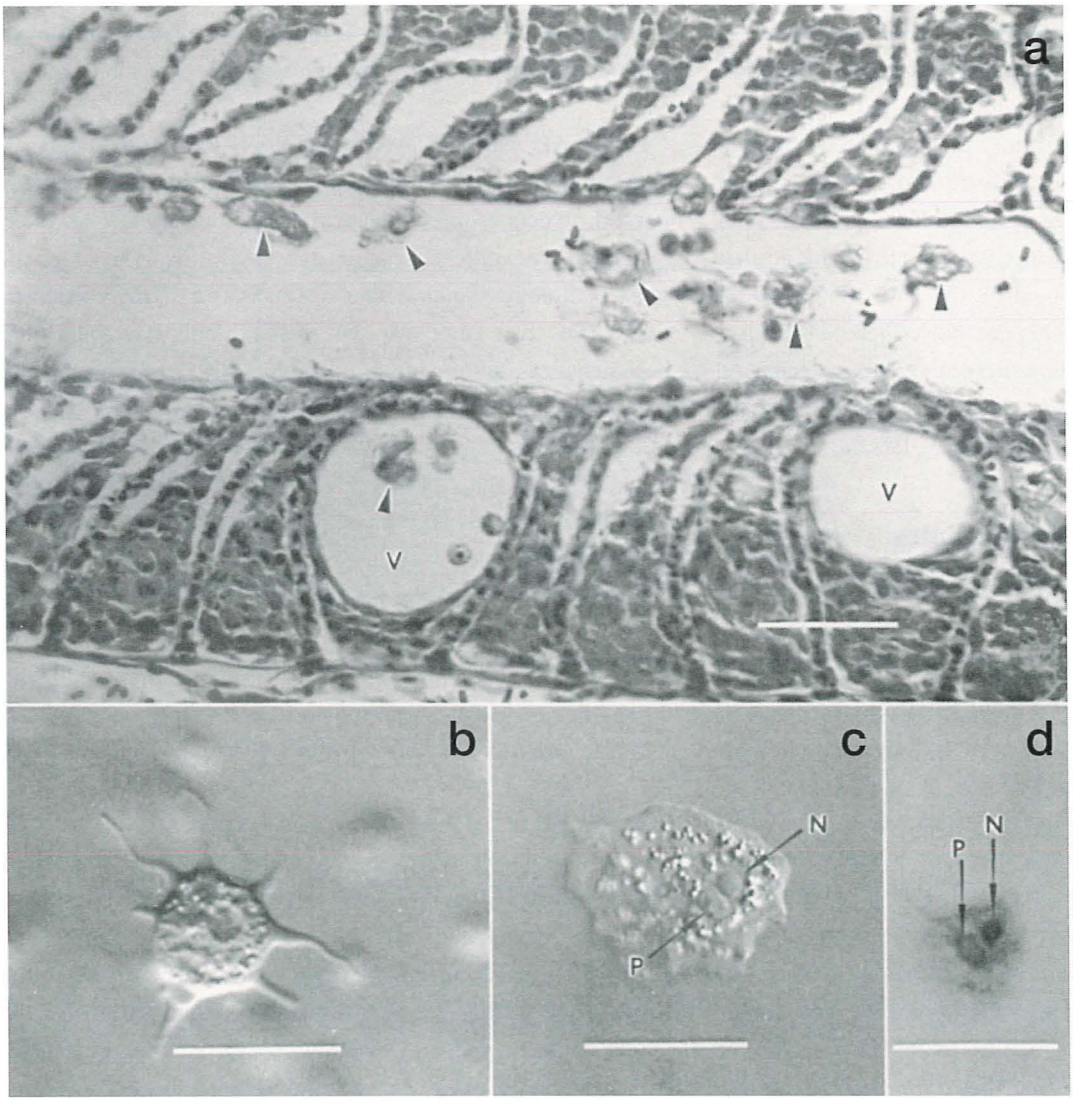


Figure 8-3. *Paramoeba pemaquidensis* from coho salmon gills. a. Epithelial hyperplasia, fusion of the secondary lamellae and interlamellar vesicles (V) associated with amoebae (arrowheads). Wet mounts of transitional form (b) and locomotive form (c), Nomarski phase contrast. d. Feulgen stain of amoeba. N = nucleus, P = parasome. (Courtesy of Dis. Aquat. Org.).

DIAGNOSIS. Paramoebiasis of salmon is diagnosed by the detection of large numbers of the amoebae on the gills. The organisms are best identified in fresh wet mount preparations of the gills. Amoebae can also be identified on gill surfaces in histological preparations, but many detach from the gill surfaces during processing. The amoebae can also be identified with specific polyclonal antibodies in tissue sections or imprints (Howard and Carson 1993a).

TREATMENT AND CONTROL. Munday et al. (1993) found that most compounds typically used as external treatments (e.g., formalin, chelated copper, diquat, malachite green and chloramine T) did not eradicate the disease, but they found that the amoeba is quickly eradicated from fish gills with freshwater bath treatments. The organism requires sea water for growth and survival, and grows poorly at salinities below 10 ppt (Kent et al. 1988c). Cameron (1993) reported that reducing seawater concentrations to 4 ppt was needed for effective treatment. Reducing the salinity has been effective for eradicating infections in fish held in land-based tanks, but this treatment is usually difficult to apply and impractical in netpens. Cameron (1993) reported that hydrogen peroxide bath treatments at concentrations between 200-400 ppm were moderately effective at controlling the infection, and Howard and Carson (1993b) reported that 100 ppm hydrogen peroxide for 2 h caused total killing of the organism. However, Cameron (1994) found that hydrogen peroxide did not control the infection in field situations, even when used at 300 ppm. Nevertheless, there is a narrow safety window for treating fish with hydrogen peroxide because it may be toxic, particularly when applied at higher temperatures (Cameron 1993; Johnson et al. 1993).

Diplomonad Flagellates

Severe systemic infections by a diplomonad flagellate (family Hexamitidae) resembling *Hexamita salmonis* caused close to 50% mortality in chinook at one netpen site in the Sechelt area, British Columbia (Kent et al. 1992). The fish were introduced to sea water in the spring of 1990 and showed unusual mortality starting in September 1991. Interestingly, about the same time, extraintestinal infections by a similar parasite was reported in post smolt to adult Atlantic salmon reared at various netpen farms in northern Norway (Mo et al. 1990; Poppe et al. 1992). *Hexamita salmonis* is a common parasite of the intestinal tract of salmonids reared in fresh water. Most infections do not cause disease, but some reports have attributed anorexia, emaciation, poor growth and mortality in salmon fry to the infection (Davis 1961; Sano 1970; Becker 1977). Other diplomonad parasites infect strictly marine fish (Poynton and Morrison 1990), and Lom (1984) suggested that *H. salmonis* may persist in salmonids after they migrate to sea water. Systemic infections by diplomonad parasites in fish are rare and we are aware of only one other report of such infections; Ferguson and Moccia (1980) reported a similar disease in Siamese fighting fish. Although the flagellates observed in pen-reared chinook salmon were morphologically indistinguishable from the relatively non-pathogenic *H. salmonis* that infects the intestinal tract of salmonids in fresh water, it may represent a new, highly invasive strain or species. Streud et al. (1997a) recently named the organism from pen-reared Atlantic salmon and grayling and Artic char from fresh water as *Spironucleus barkhanus*, and Sterud et al. (1997b) suggested that wild Artic char may be source of the infection for pen-reared Atlantic salmon in Norway.

In both freshwater and seawater aquaria, we could readily reproduce the systemic disease in chinook by water-borne exposure of the fish to infected blood and viscera, or by cohabitation with infected fish. However, the parasite of Atlantic salmon was not easily transmitted.

CLINICAL SIGNS AND GROSS PATHOLOGY. In chinook salmon from British Columbia, infected fish appeared normal except some fish exhibited a distended, swollen abdomen. The gills were pale due to anemia. The hallmark gross pathologic change of the disease is an extremely enlarged liver. The liver may also be mottled, and have petechial hemorrhages and whitish, friable areas. Affected fish consistently exhibited serosanguineous ascites and blood clots in the visceral cavity. The clots were often pale and translucent – i.e., "chicken fat clots" (Fig. 8-2b). The spleen and kidney were moderately enlarged, and petechiae occurred throughout the skeletal muscle.

In Atlantic salmon, the infection differs in that the parasite causes large, multifocal, white, lesions in the musculature, liver, spleen, and kidney (Poppe et al. 1992). Yellow or white cysts filled with the parasite may also occur in the fins, and infected fish often exhibit exophthalmia (Poppe and Mo 1993).

MICROSCOPY. Wet mount preparations of the visceral organs reveals massive numbers of highly motile flagellates that are 10 X 5 µm (Fig. 8-4a). The parasites are also readily detected in Diff-Quick or Giemsa-stained imprints (Fig. 8-4b), where they appear as dark-staining, oval bodies and two clear bands, representing the flagellar pocket, running through the length of the organism. Depending on the staining technique, two nuclei at the anterior end of the parasite may be visible.

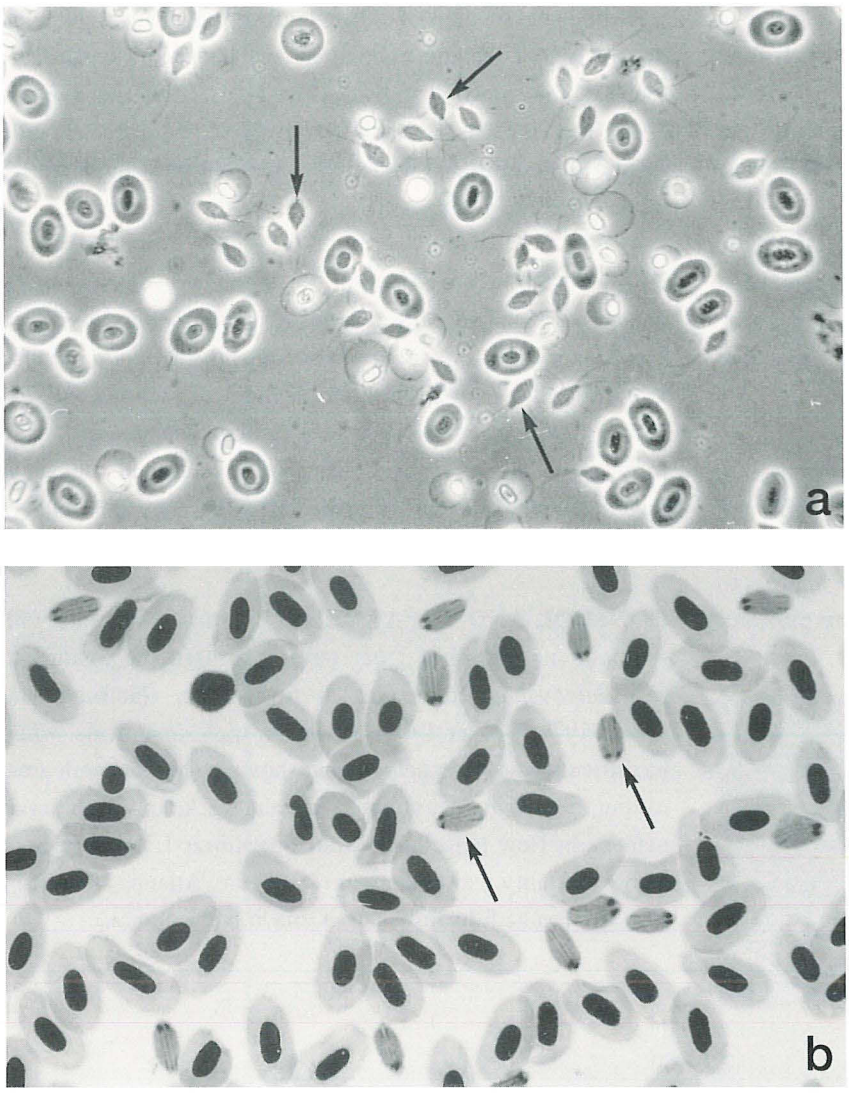


Figure 8-4. Systemic diplomonad flagellates (arrows) from chinook salmon. a. Phase contrast wet mount. b. Blood smear, Giemsa stain.

Histological examination of infected chinook reveals massive numbers of the parasites in blood vessels of essentially all organs, with particularly high concentrations in the liver and the lamina propria of the lower intestine. The liver is edematous and shows diffuse infiltration of inflammatory cells resembling lymphoblasts and plasmablasts in the sinusoids. The renal interstitium is hyperplastic. Although many parasites are observed in blood vessels of the lamina propria and submucosa of the gut, the epithelium is usually intact. With Atlantic salmon from Norway, Poppe et al. (1992) reported focal areas of coagulative and caseous necrosis, and in some cases the lesions were also comprised of substantial chronic inflammation and fibrosis. The parasite also infects the brain and is associated with encephalitis and suppurative meningitis.

DIAGNOSIS. The infection is identified by wet mount preparations or stained imprints of the gut or visceral organs. Because the parasite is highly motile, it may be easier to identify the parasites in wet mounts.

CONTROL AND TREATMENT. Several drugs, most of which are added to the diet, have been recommended for the control of *H. salmonis* infections in the gut of salmonids (Yasutake et al. 1961; McElwain and Post 1968; Becker 1977; Hoffman and Meyer 1974; Tojo and Santamarina 1998). Nitroimidazoles (e.g., metronidazole) and other compounds such as albendazole and diethylcarbamazine) are effective for controlling these infections. However, at this time, none of these compounds are approved for treatment of food fish in Canada. Although we have determined that the disease is transmissible in sea water, we have yet to determine if the chinook contracted the infection in sea water, or were subclinically infected when they were transferred to netpens. The same is the case with the disease in Norway. At present, the best guess is that both infections were contracted in sea water. In addition, Poppe et al. (1992) suggested that fish could become infected by exposure to untreated water from processing facilities. An understanding of the source of the infection would be helpful for implementing effective control strategies or prophylactic treatments.

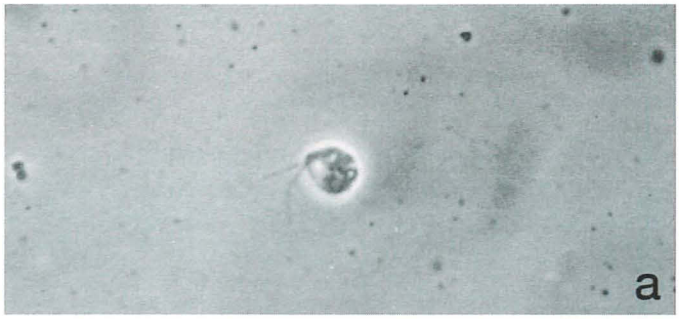
Ichthyobodo (=Costia) gill infections

The bodonid flagellate *Ichthyobodo necator* is a common ectoparasitic pathogen of fishes reared in fresh water (Becker 1977). *Ichthyobodo* infections have been observed on seawater-reared Atlantic salmon in Europe, and it was suspected that the fish acquired the infections in fresh water (Ellis and Wootten 1978; Roubal et al. 1987). However, *Ichthyobodo* has also been observed on strictly marine fishes, such as flatfishes and haddock (Cone and Wiles 1984; Bullock and Robertson 1982; Morrison and Cone 1986; Diamant 1987). Using cross infection studies, Urawa and Kusakari (1990) determined that *Ichthyobodo* sp. of Japanese flounder was a different species than *I. necator* of salmonids, and they showed that the parasite from fresh water can survive and proliferate on fish transferred to sea water. We have occasionally observed

heavy *Ichthyobodo* infections associated with gill damage in pen-reared chinook from a few netpen sites in British Columbia. The parasite is transmitted directly from fish to fish. Multiplication is rapid, and untreated fish in confined environments can develop very heavy infections in a few days.

CLINICAL SIGNS AND GROSS PATHOLOGY. Macroscopic changes in affected Pacific salmon have not been described. Heavily infected Atlantic salmon in Scotland were emaciated, anorexic, and swam near the surface (Ellis and Wootten 1978).

MICROSCOPY. Wet mount preparations of the gills and skin reveal numerous, motile parasites (Fig. 8-5a). The parasites are small (about 10-15 µm long), and free-swimming forms are oval with two pairs of uneven flagella. *Ichthyobodo* appears to swim with a jerky, spiral pattern. Attached forms are pyriform with obscure flagella.



Histological sections of affected gills reveal diffuse, epithelial hyperplasia of the gill epithelium and attached parasites are found on the epithelial surfaces (Fig. 8-5b). Ellis and Wootten (1978) reported that heavily infected gills of pen-reared Atlantic salmon were infiltrated with melanomacrophages and that there was an increase in goblet cells.

DIAGNOSIS. The infection is identified by observing numerous flagellate parasites with the morphology described above on the gills or skin in wet mounts. *Ichthyobodo* can also be identified on gill surfaces in histological sections, but wet mounts are preferred because parasites may become dislodged from the gills during processing of tissues, and the small flagellates are more easily identified when they are actively motile.

CONTROL AND TREATMENT. External treatments with formalin and malachite green have been used successfully to treat *Ichthyobodo* infections in fresh water (Hoffman and Meyer 1974; Becker 1977). However, these treatments would be difficult to apply in netpens and should be applied with great caution. For example, attempts at treating Atlantic salmon in netpens in New Brunswick with formalin at 1:10,000 caused heavy mortality within an hour (D. Speare, Atlantic Veterinary College, Prince Edward Island, Canada, pers. comm.).

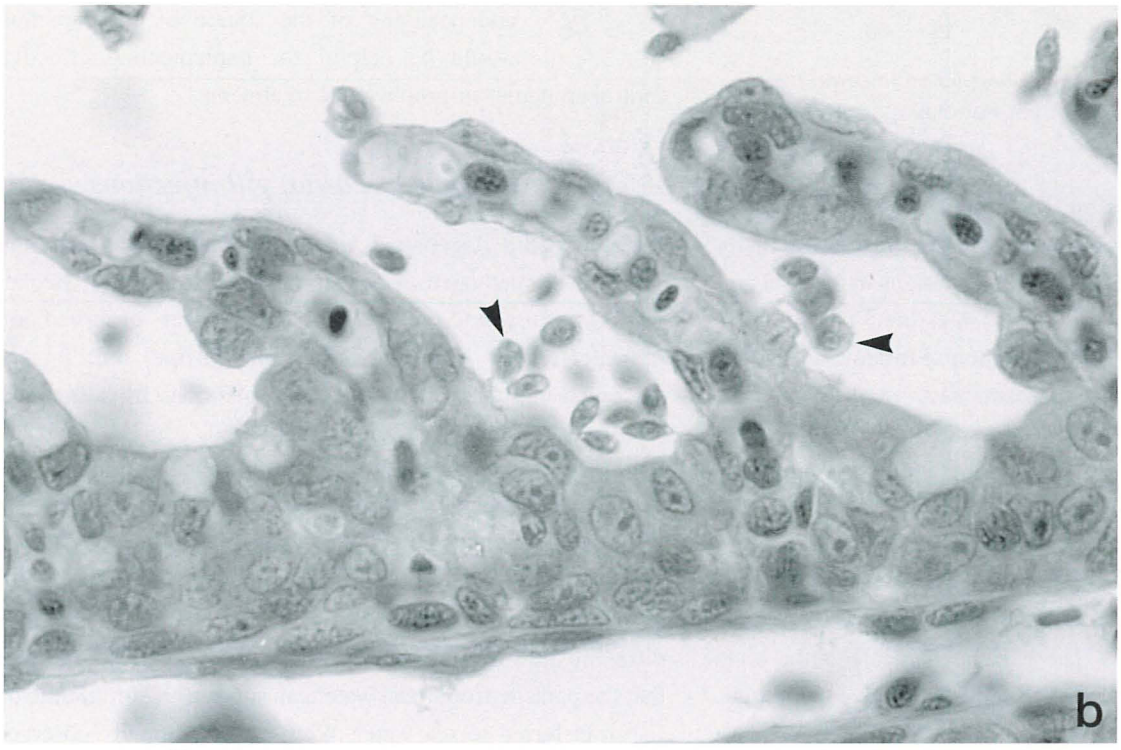


Figure 8-5.
Ichthyobodo necator from the gill surface of a chinook salmon. a. Wet mount preparation, phase contrast. b. Tissue section of gill, arrowhead = parasites. H & E.

Cryptobiosis

A flagellate identified as *Cryptobia salmositica* caused severe disease in adult chinook salmon at one netpen farm in British Columbia. This flagellate is common in salmonids from fresh water throughout the Pacific Northwest where the leech vector (*Piscicola salmositica*) is present. Although the parasite is usually transmitted with leeches, direct fish to fish transmission also occurs when fish are held in crowded culture conditions (Bower and Margolis 1983b). In wild fish, the infection is usually seen in sexually-mature salmon that have returned to fresh water to spawn. However, juveniles are also susceptible to the infection, and the parasite can persist in fish after they are transferred to sea water. See Woo and Poynton (1995) for an excellent review on *Cryptobia* and related flagellates.

Cryptobiosis was observed in chinook salmon at a netpen site which had previously recieved smolts infected with *C. salmositica* at the time of sea water transfer. The infection was only detected in fish undergoing sexual maturation, where more than 50% of this population died due to the infection.

CLINICAL SIGNS AND GROSS PATHOLOGY. Clinically infected fish were extremely anemic, exhibited bilateral exophthalmia, very swollen spleens and a moderately swollen kidney.

MICROSCOPY. When clinical disease is present, the parasite is readily seen in blood smears (Fig. 8-6). In fresh wet mounts of blood, the parasites are actively motile, exhibiting an undulating motion. The parasite is about 10 X 5 µm. Histological examinations do not reveal any pathognomonic changes. Infected fish exhibit generalized chronic inflammation.

DIAGNOSIS. The infection is best diagnosed by examination of blood smears stained with Giemsa or Diff Quik or in wet mount preparations of blood. In subclinical infections, the infection is often missed in standard blood smears. Examination of buffy coat from blood in a hematocrit tube will enhance the ability to detect light infections (Woo 1969; Bower and Margolis 1983a).

CONTROL AND TREATMENT. At this time there are no commercially-available drugs to treat cryptobiosis. Adapting fish to 20 °C has been shown to greatly enhance survival with the infection in experimental conditions (Bower and Margolis 1985; Bower 1995). However, this is dangerous and impractical in most production situations. An attenuated live vaccine shows promise for protecting fish from the infection (Li and Woo 1997). This vaccine showed protection for at least 24 mo. after vaccination (Li and Woo 1995). There is a significant difference in strain and species susceptibility to the infections (Bower et al. 1995), and selection of resistant salmonid strains for culture would probably reduce the problem where the infection is unavoidable.

CILIATES

The only ciliate that has been recognized to cause disease in seawater-reared salmon is a *Trichodina* sp. Trichodinid ciliates are well-recognized skin and gill parasites of salmonids reared in fresh water, and McArdle (1984) observed gill lesions and mortality associated with heavy infections in yearling rainbow trout and maturing Atlantic salmon reared in netpens in Ireland. In Norway, we observe concurrent gill infections by trichodinid ciliates and *Ichthyobodo*. In these cases, *Ichthyobodo* is found on the secondary lamellae, while the ciliates are found between the primary lamellae at the base of the gill filaments.

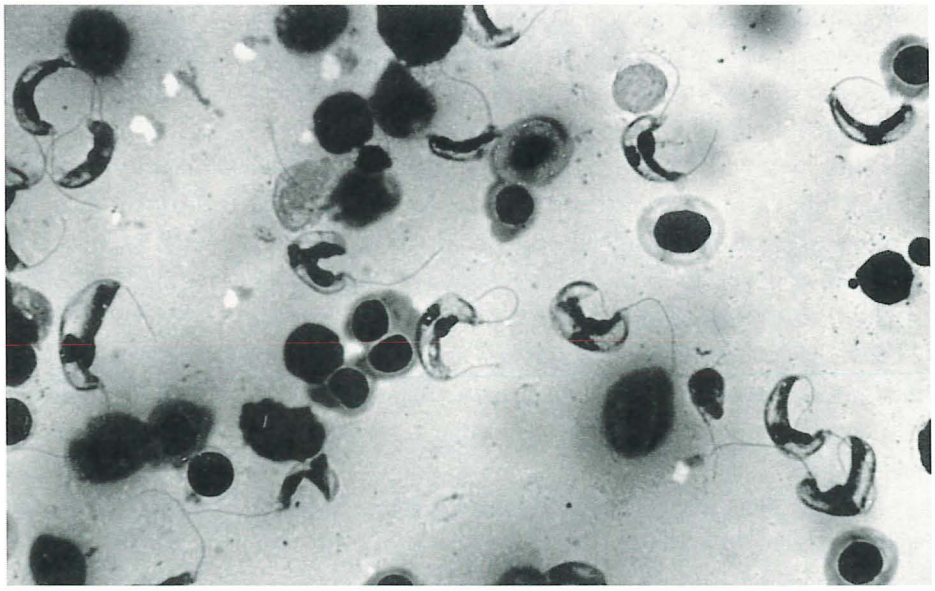


Figure 8-6. *Cryptobia salmositica* in the blood of chinook salmon. Diff Quik.

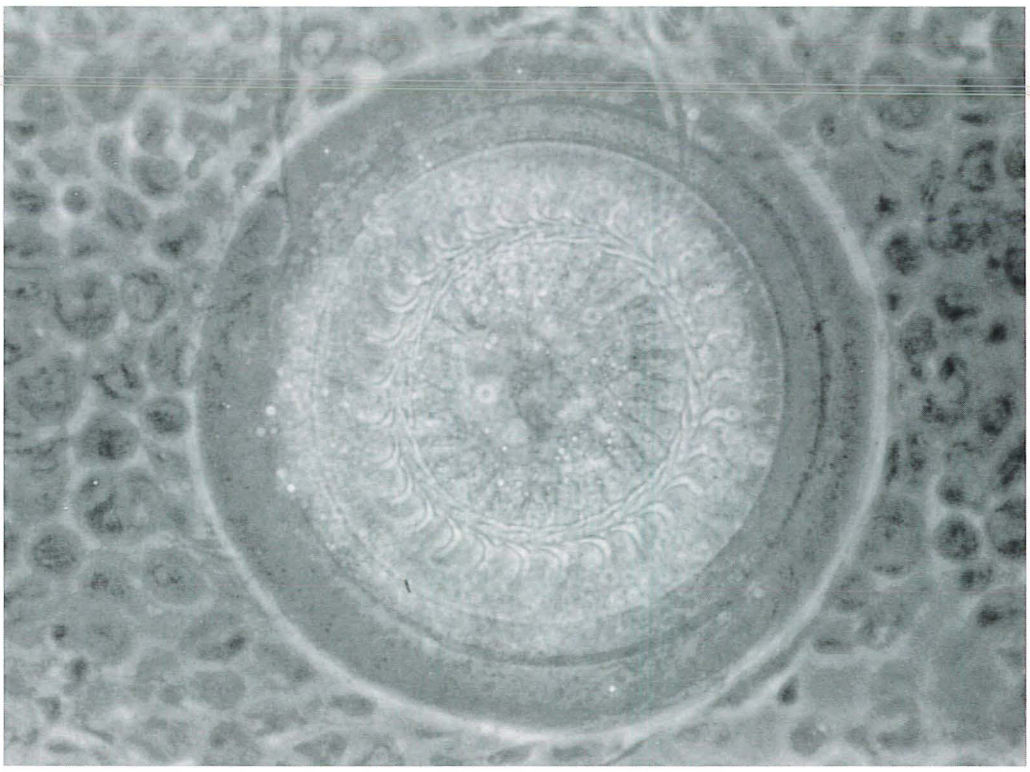


Figure 8-7. *Trichodina* from the gills of salmon.

DIAGNOSIS. Trichodinid infections are identified by examining gills or skin scrapings in wet mount preparations, which readily demonstrate the actively moving, discoid ciliates (Fig. 8-7).

CONTROL AND TREATMENT. McArdle (1984) reported that treatment with formalin at a concentration of 1:2000 or 1:4000 for 1/2 h was effective for eradicating the parasite.

MYXOSPOREANS

Myxosporean parasites (phylum Myxozoa, class Myxosporea) are common parasites of cold-blooded vertebrates, particularly fishes, and hundreds of species have been described (Lom and Noble 1984; Lom 1987). Traditionally the Myxozoa have been classified with the Protozoa. However, analysis of small subunit ribosomal DNA revealed that the Myxozoa rooted within the kingdom Animalia (Smothers et al. 1994). Siddall et al. (1995) subsequently reported that, based on ribosomal DNA sequence and morphological features, the Myxozoa are most closely related to the Cnidaria. Affinities of the Myxozoa with the Cnidaria have been suggested for many years (Lom 1989), particularly due to similarities of polar capsules with nematocysts.

Most myxosporeans are relatively non-pathogenic. Histozoic species usually form small, confined white cysts with little associated tissue damage. However, when these cysts are numerous in vital organs, such as the gills or heart, they can cause disease. Furthermore, heavy infections of histozoic myxosporeans in the flesh may lower the market value of the affected fish. Pathogenic coelozoic species generally cause more diffuse infections without macroscopically visible cysts. Several species are recognized pathogens of salmonid fishes reared in fresh water (e.g., *Myxobolus cerebralis*, *Ceratomyxa shasta*, and the PKX myxosporean). Four myxosporeans (*Kudoa thyrsites, Chloromyxum truttae, Myxobolus aeglefini* and *Parvicapsula* sp.) cause problems in seawater pen-reared salmon.

The life cycle of myxosporeans is complicated (Fig. 8-8). They contain several vegetative stages (trophozoites) and development in the fish culminates in the formation of multicellular spores. Spore morphology is the primary criterion used for identification of myxosporeans. The mode of transmission of most myxosporeans is poorly understood. At least for many freshwater myxosporeans, development in an aquatic oligochaete is required to complete the life cycle (Wolf et al. 1986; El-Matbouli and Hoffmann 1990; Kent et al. 1990b; Ruidisch et al. 1991; Yokoyama et al. 1991). The forms found in oligochaetes were originally considered to be different parasites, which were assigned to the class Actinosporea. Because these stages are not separate taxa from myxosporeans, the taxonomy of the phylum Myxozoa has been revised (Kent et al. 1994a). In brief, the class Actinosporea, the order Actinomyxidia, and all families in the Actinosporea (except Tetractinomyxidae) were suppressed. We propose that

actinosporean generic names be treated as collective group names, and thus they do not compete in priority with myxosporean generic names.

Fumagillin. There are no commercially available drugs for treating myxosporean diseases of salmonids. However, fumagillin DCH has been shown to be efficacious for treating some myxosporean diseases, including those affecting salmonids (e.g., whirling disease and proliferative kidney disease) (Molnár et al. 1987; Hedrick et al. 1988; Wishkovsky et al. 1990; Yokoyama et al. 1990; El-Matbouli and Hoffmann 1991; Sitjà-Bobadilla and Alvarez-Pellitero 1992; Higgins and Kent 1996). Fumagillin is an antimicrobial agent used primarily for treating *Nosema apis* (phylum Microspora) infections in honey bees. The drug apparently acts by inhibiting RNA synthesis (Jaronski 1972). Kano and Fukui (1982) reported that fumagillin was effective against the microsporean *Pleistophora anguillarum* in eels (*Anguilla japonica*). Molnár et al. (1987) was the first to demonstrate the effectiveness of the drug for the control of a myxosporean disease - i.e., sphaerosporosis of carp (*Cyprinus carpio*).

Various concentrations of the drug were employed in these studies. Based on these reports, 3-10 mg fumagillin/kg fish/day for about 2 wk is the recommended dose for salmonids. Higher concentrations or prolonged treatment (e.g., 30-60 days) may cause anorexia, poor growth, anemia, renal tubule degeneration and atrophy of hematopoietic tissues in salmonids (Laurén et al. 1989; Wishkovsky et al. 1990). We have conducted extensive field trials with fumagillin for the treatment of PKD in hatchery-reared coho salmon (Higgins and Kent 1996). A 2 wk treatment at 3 mg drug/kg feed reduced the prevalence of PKX infections and was not associated with toxic side effects or a reduction in growth.

The drug is not heat stable. Therefore, it is recommended that the feed be coated with the drug, instead of incorporation of the drug into the feed during milling. Fumagillin is available in Canada as Fumadil-B, a relatively dilute mixture of the drug used for treating *Nosema* in bees. However, in our experience we have found that it was difficult to coat the feed with this compound due to the high amount of inactive carrier. Therefore, we recommend using the parent compound, which is about 60-70% active. The drug is not very soluble in water,

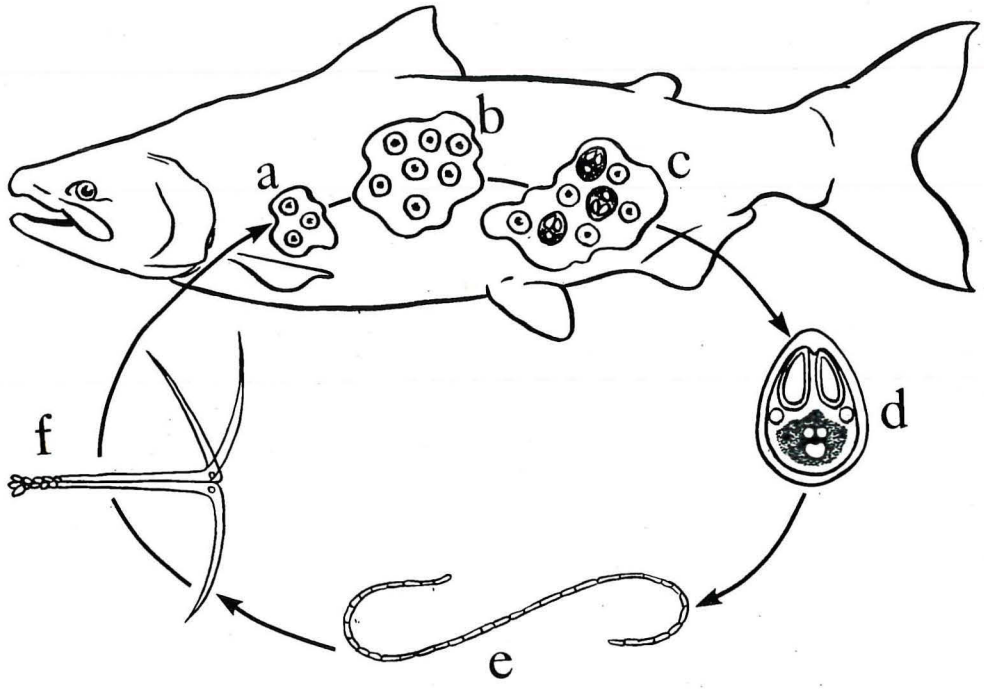


Figure 8-8. The life cycle and development of myxosporean parasites of fishes, examplified by *Myxobolus* spp.: a and b, vegetative development results in formation of a multinucleated plasmodium with many daughter cells; c, daughter cells develop into multicellular spores; d, spores are released from the fish to complete development and transmission of the parasite - spores are released after death for most histozoic species, whereas spores are released in feces or urine in coelozoic species; e, development of the parasite after release from the host is unknown for most myxosporean genera, but for *Myxobolus* spp. an aquatic oligochaete worm is apparently a required intermediate host in which the spores develop into actinosporean stages; f, actinosporean stage is released from the worm and infects the fish host to complete the life cycle.

but is very soluble in alcohol. In most studies, fumagillin was mixed with alcohol, sprayed on the feed, and then the feed was coated with oil. In Canada, an Experimental Drug Certificate or Emergency Drug Release from the Canadian Bureau of Veterinary Drugs would be required if the parent compound is used for treating fish destined for human consumption because the parent compound does not have a DIN number.

Drs. D. Speare (Atlantic Veterinary College, Prince Edward Island) and E. Athanassopoulou (Veterinary Research Centers, Paraskevi, Athens, Greece) (pers. comm.) have found that a water soluble form of fumagillin, Fumagilin-B (Medivet Pharmaceuticals, High River, Alberta), was relatively easy to apply to fish feed. We have also found that an analog of fumagillin, TNP-470 (Takeda Chemical Co., Japan), is effective for controlling certain microsporean infections in salmon (Higgins et al. 1998).

Parvicapsula sp.

Parvicapsula sp. infects the kidney of salmon reared in netpens, and in the early 1980s it was reported to be the cause of severe disease in pen-reared coho in Washington State (Hoffman 1984; Johnstone 1984). However, concurrent infections with *Renibacterium* and *Vibrio* often occurred, and the role of the parasite in the overall mortality was unclear.

Johnstone (1984) also observed *Parvicapsula* in chinook, Atlantic, and masou salmon, and cutthroat trout. A *Parvicapsula* sp. was observed in Pacific cod that were collected near pens containing infected coho. The parasite in Pacific cod appeared similar to the *Parvicapsula* of salmon, and Johnstone (1984) suggested that Pacific cod may be the reservoir for infections. In British Columbia, we have detected the parasite in coho at one netpen site, and in wild sockeye salmon and coho salmon. The organism from wild sockeye salmon was described as *Parvicapsula minibicornis* by Kent et al. (1997a). However, it is possible that the *Parvicapsula* sp. from pen-reared coho salmon represents a separate species.

CLINICAL SIGNS AND GROSS PATHOLOGY. There are no pathognomonic clinical changes associated with parvicapsulosis. However, infected fish often are dark and lethargic, and exhibit kidney hypertrophy. *Parvicapsula* is a coelozoic myxosporean and macroscopically visible cysts containing the parasite are not observed.

MICROSCOPY. Histological sections of heavily infected kidneys show numerous trophozoites and developing spores in the epithelium and lumina of tubules. The epithelium is often necrotic and displaced by developing parasites (Fig. 8-9b). Trophozoites also occur in the blood and kidney interstitium and may cause interstitial nephritis. Spores are elongate, 7-10 X 6-5 µm (measured from tissue sections) and have two tiny polar capsules at the anterior end (Fig. 8-9a).

DIAGNOSIS. *Parvicapsula* spores are very small and identification of this parasite is usually based on histological examinations. Positive identification is based on observation of the spore, which are readily visible in histological sections stained with Giemsa. Spores can also be detected in Gram stained kidney smears (Fig. 8-9a).

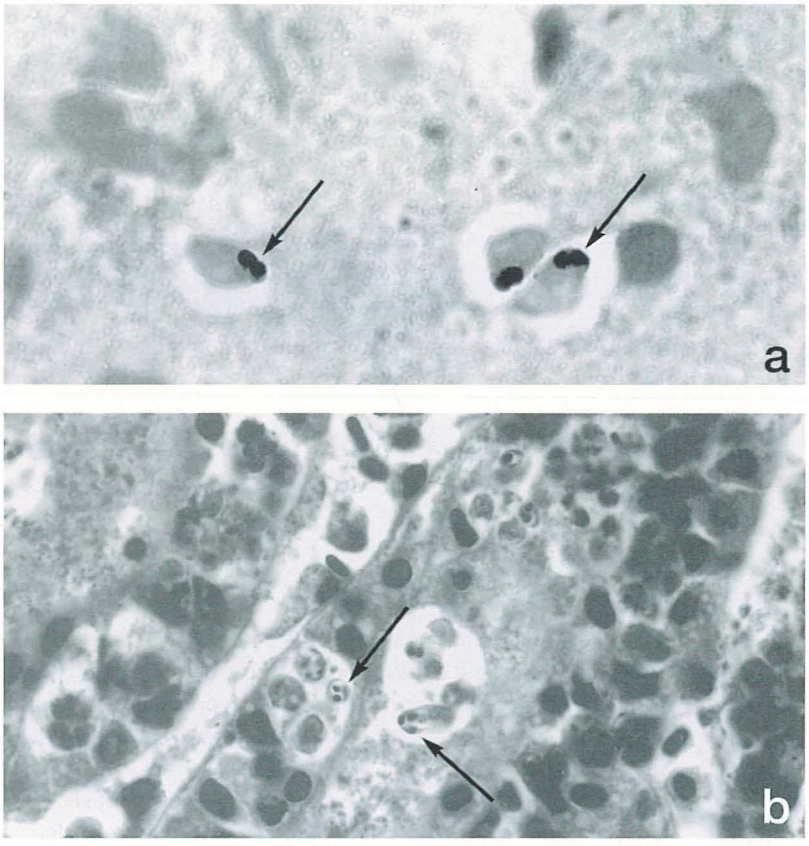


Figure 8-9. *Parvicapsula* sp. from coho salmon kidneys. a. Gram stain of *Parvicapsula* sp. spores. Arrows = polar capsules. b. Tissue section of coho kidney with *Parvicapsula* sp. infection. Parasites (arrows) cause necrosis and degeneration of the epithelium of renal tubules. Giemsa.

CONTROL AND TREATMENT. No drugs are available to treat the infection. See discussion on fumagillin (see page 57). Pacific cod may be a reservoir for infection, but control of these fish is impractical in the netpen situation.

Kudoa thyrsites

Myxosporeans of the genus *Kudoa* and related genera infect the muscle of many marine fishes, and heavy infections can cause unsightly white cysts or soft texture in fillets (Kabata and Whitaker 1981; Patashnik et al. 1982). These parasites are therefore of concern because they can lower the market value of the infected fish, although they seldom cause morbidity. *Kudoa thyrsites* is a cosmopolitan parasite that infects many species of marine fish (Whitaker et al. 1994). Infections in pen-reared Atlantic salmon have been reported from the Pacific Northwest (Whitaker and Kent 1991), Spain (Barja and Toranzo 1993), and Ireland (Palmer 1994). In one instance, Harrell and Scott (1985) attributed mortalities in Atlantic salmon smolts to this parasite. More importantly, heavy infections have been associated with soft flesh and the unsightly white patches in pen-reared Atlantic salmon that are either held on ice for 3-6 days or smoked (Fig. 8-2 e,f). *Kudoa thyrsites* infections and associated soft flesh have also been observed in farmed coho salmon (Whitaker and Kent 1992) and brown trout (Baudin-Laurencin and Bennassr 1993).

St-Hilaire et al. (1998) found that the infection was much more prevalent in Atlantic salmon grilses or reconditioned grilses than in market-size fish that had not undergone sexual maturation. For example, the prevalence of the infection in farmed Atlantic salmon examined on the processing line in the spring and winter was, on average, 13 times greater in grilses and reconditioned fish than in those that had not undergone sexual maturation. Infection prevalence in grilses may reach as high as 70%, whereas immature fish usually show infections below 10%.

There is also a positive correlation between intensity of infection and severity of soft flesh in Atlantic salmon held on ice (St-Hilaire et al. 1997a). Heavily infected fish always showed soft flesh, whereas lightly infected fish (i.e., fewer than 20,000 spores/g) usually show no signs of the condition. The condition is unnoticed on the processing line, and only becomes apparent after fish are held for about 3 to 6 days on ice or when fillets are smoked. In the investigation of *K. thyrsites* infections in Pacific hake, it was found that the flesh softening was caused by a proteolytic enzyme produced by the parasite (Tsuyuki et al. 1982). This enzyme remains active at temperatures below 70 °C. Therefore, tissue breakdown will continue through most smoking processes, which are normally conducted at 50 °C or less. In contrast to early reports, Seymour et al. (1994) suggested that the flesh degredation was due to cathepsin L from the host inflammatory response to the parasite, instead of a proteolytic enzyme from the parasite.

Very little is known about development and transmission of *K. thyrsites* in fish. By experimentally exposing fish at a netpen site where the infection is indigenous, Moran et al. (1998a) found that it takes about 5-6 months (i.e., about 2,000 degree-days) after infection before spores are detected in the flesh. A high prevalence of infection occurs in post-smolts, with as high as 60-70 % infection after the first 5 mo. in sea water. As the infection progresses in Atlantic salmon, pseudocysts in the muscle fibers enlarge and ultimately rupture. A prominent inflammatory response is associated with ruptured pseudocysts, and fish exhibit recovery after about a year in sea water (Moran et al. 1998). It is not known if the high prevalence of the infection in grilses is due to reinfection, or proliferation of a cryptic infection that originally occurred when the fish were first transferred to sea water. An infectious stage of the parasite occurs in the blood, and Moran and Kent (1998b) found that fish injected with blood from infected fish and then held in fresh water developed the infection. This experiment also demonstrated that once a fish is exposed the parasite can complete its development even if fish are transferred to fresh water. These authors also found that direct *per os* exposure of Atlantic salmon with heavily-infected tissue did not cause infections.

Hervio et al. (1997) conducted studies using ribosomal DNA (rDNA) sequence of *Kudoa* and other myxosporeans to determine their specific identity and relationship to one another. Ribosomal DNA is very useful for taxonomic comparisons because portions of the molecule are species specific, thus allowing researchers to distinguish *K. thyrsites* rDNA from other myxosporeans, as well as from fish rDNA. Analysis using small subunit rDNA suggests that *Kudoa* species are phylogenetically very different from the other myxosporean genera examined thus far (i.e., *Myxobolus*, *Henneguya*, and *Myxidium*), and that *K. thyrsites* in Atlantic salmon is indistinguishable from that infecting tube-snout (and probably other marine fishes in the Pacific Northwest). Furthermore, we have sequenced the small subunit rDNA of *K. thyrsites* from snoek collected off South Africa and found this sequence to be 99.4% similar to that of the Pacific Northwest isolates. This suggests that *K. thyrsites* from around the world represents the same species.