WASHINGTON SEA GRANT PROGRAM

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SALMONID DISEASES

A WORKSHOP SUMMARY

April 17, 1974

UNIVERSITY OF WASHINGTON
DIVISION OF MARINE RESOURCES
UNIVERSITY OF WASHINGTON 98195

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A WASHINGTON SEA GRANT REPORT

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WORKSHOP ON SALMONID DISEASES

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April 17, 1974
UNIVERSITY OF WASHINGTON

CHAIRMAN: *Ernest L. Brannon*
EDITORS: *Terry Y. Nosho, Ernest L. Brannon*

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FOREWORD

This report summarizes proceedings of the second in a series of workshops on salmonid aquaculture, sponsored by the Washington Sea Grant Marine Advisory Program and the College of Fisheries at the University of Washington. The first, held September 21, 1973, encompassed all aspects of rearing salmon, including saltwater pen-rearing and ocean ranching, with related disease and economics questions. The second workshop, covered in this report, was held April 17, 1974, and dealt with the subject of salmonid diseases. A third workshop, on ocean ranching in Washington, was held December 19, 1974. Copies of all three workshop reports are available from Sea Grant Communications, Division of Marine Resources, University of Washington, Seattle, WA 98195.

ACKNOWLEDGMENTS

Special thanks go to each of the panel members and to their respective organizations for the time and effort expended in participating in this workshop.

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PREFACE

As a general background to the disease workshop, I want to comment briefly on the occurrence of disease in fish cultural operations. The concept that disease does not occur in wild populations simply is not true. Diseased wild fish are seldom observed because predators take immediate advantage of the increased vulnerability of sick fish. Mortalities from bacterial diseases among wild adult sockeye populations have been reported as high as 50 and 90 percent. Similarly, natural populations of sockeye fry have been isolated as carriers of a virus disease, with subsequent mortality attributed to that agent. Every disease known in fish culture has had its genesis in wild populations. Representative organisms of most endemic diseases can be found on healthy wild fish. Whether or not the fish culturist has real problems with disease, therefore, is related more to his fish cultural practices than to the presence or absence of disease organisms.

Stress on the fish--created by overcrowding, low water flows, or buildup of pond filth--greatly increases the probability of disease problems. Care to prevent such conditions is routine in most fish cultural operations. There are several other measures that can be taken, however, to minimize disease outbreaks. Sanitation is often overlooked, but is an important consideration. Sanitation involves the food source and the water source as well as routine disinfection and cleaning operations. A few years ago, diseases were spread extensively by infected fish used in fish diets. This is not a problem now since commercially prepared diets are largely used. Water source, however, can still be a major problem. Scrap fish are carriers of several diseases infectious to salmon, and if their presence in the water supply can be reduced or eliminated, the number of disease-causing organisms will be reduced.

Diet is one of the most important factors in maintaining healthy fish. Poor diets will cause nutritional problems and increase the susceptibility of fish to disease. Moreover, the method of feeding even a good diet can influence fish health. Starting the fish feeding is critical, since delay will result in reduced fish health and increased disease susceptibility. Overfeeding often results in disease outbreaks because excess food provides a substrate on which disease organisms can concentrate, and it increases the particulate organic matter in the water, irritating the gills. Good food distribution helps minimize size variability and also reduces the potential for disease outbreaks among the otherwise small and unhealthy fish.

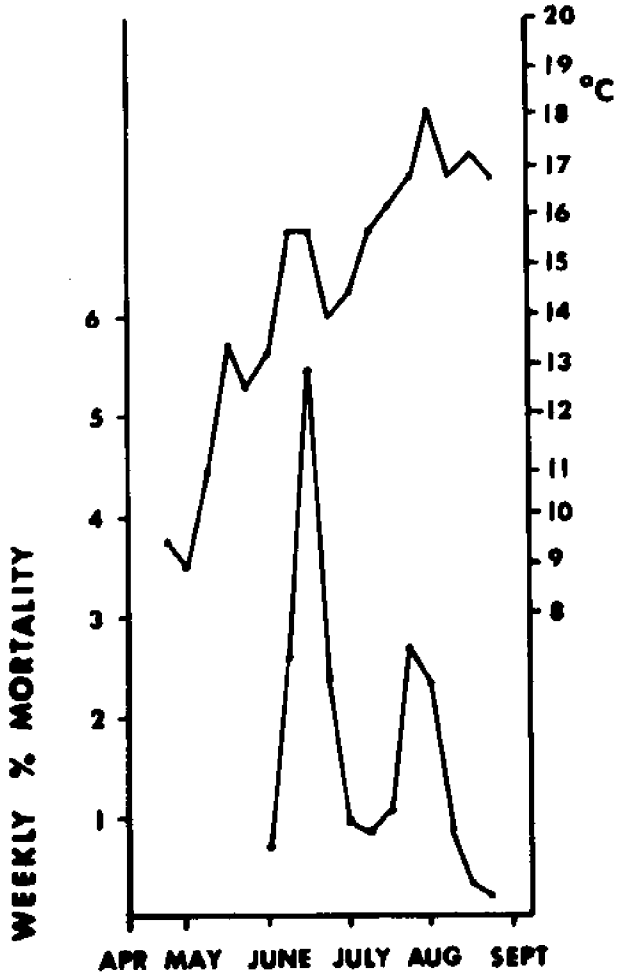
As we proceed with the disease workshop, therefore, let us remember that the most important treatment is prevention, and the most important preventative treatment is good fish cultural practices.

Ernest L. Brannon
August 14, 1975

EPIDEMIOLOGY OF FURUNCULOSIS IN PACIFIC SALMON

*George W. Klontz**

Furunculosis disease has been an annually recurring problem in juvenile hatchery-reared salmon (*Oncorhynchus* sp.). Four hatcheries in Washington State were selected for the following observations on the occurrence of furunculosis: season, water temperature before and during the epidemics, mortality patterns with and without chemotherapy, presence and fate of post-epidemic carrier fish, antigenic relationships of the *Aeromonas salmonica* isolates, and the disease-transmitting potential of fish residing in the hatchery water supply. Data were gathered over a three-year period from 12 natural epidemics in the study hatcheries and 25 experimental epidemics in the laboratory.



SUMMER FURUNCULOSIS

Four types of furunculosis epidemics were recorded. The classical late spring-early summer epidemic was the most common. The other three occurred in 1) middle summer and late winter; 2) late fall and early winter; 3) late winter and early spring. Each of the four types of epidemics was caused by a serologically distinct *A. salmonicida*.

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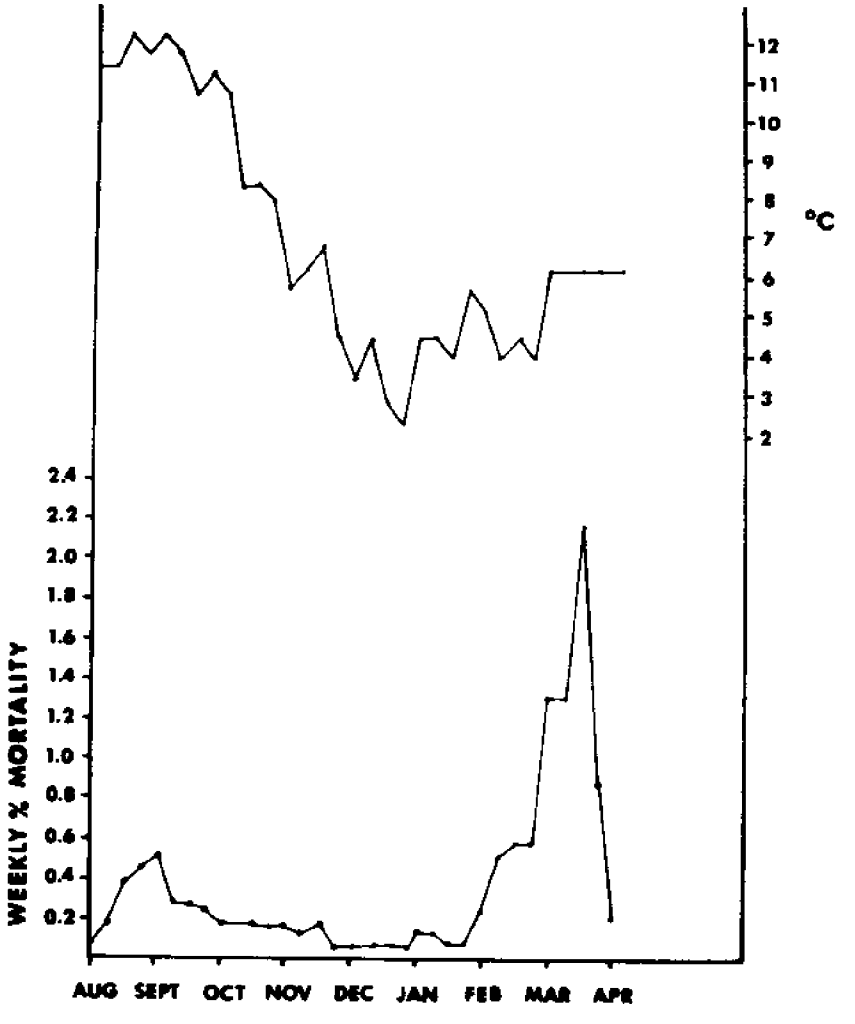

Summer - Winter Furunculosis

WINTER FURUNCULOSIS

RESPIRATORY DISEASES OF PACIFIC COAST SALMONIDS

*George W. Klontz**

Respiratory diseases of fish in intensive culture systems are a significant factor contributing to the high cost incurred by the aquaculture industry. In a 1973 survey (Klontz, 1973) of all federal, state and private fish-raising facilities in Idaho, respiratory diseases were listed by the respondents as being a major diesease problem (Table 1).

Table 1: Annual mortality of fish in Idaho, 1972

Agency	Number Reporting	Mortality		Fish size
		Res. dis.[1]	Total[2]	
Private	13/24	5-35%	15.5%	1-3'
	10/24	1-10%	6.5%	3-6'
State	10/13	1-40%	22.6%	1-3"
	8/13	1-6%	8.3%	3-6"
Federal	2/3	5-10%	10.5%	1-3"
	2/3	5-10%	25.0%	3-6"

[1] Respiratory diseases.

[2] The average total mortality from all causes.

Causes of respiratory diseases of fish may be classified as: 1) Non-infectious - e.g., genetic, physical, chemical, nutritional, neoplastic, or idiopathic; 2) Infectious - e.g., bacterial, viral, fungal, or parasitic (Table 2). Concern for their seriousness should be based on the fact that any agent in the water, whether it be animate or inanimate, which affects the gill epithelium affects the exchange of respiratory products. The effects of altering the exchange of respiratory products are various. They may range from being noted only as a decreased rate of growth to being noted as significant numbers of dead fish. In the majority of cases, their effects are very likely attributed to nonrespiratory problems. In any event, the net result is the same--namely, an increased cost of production.

The 110 state and federal hatcheries in Oregon, Washington and Idaho release nearly 400 million salmon smolts annually. Nearly half of these are chinook salmon with a majority of these being fall chinook (BFSW, 1968). The State of Idaho releases 8.8 million spring and summer chinook smolts each year. Post-release smolt migration studies have recorded that as few as 5% of the hatchery fish released into the Salmon River

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enter the Columbia River (Raymond, 1970). Other smolt migration studies indicate that nearly 30% of the fall chinook smolts released from lower Columbia River hatcheries enter the ocean (Burrows, personal communication).

It is a generally accepted fact by fishery scientists that between 4 and 6% of the released salmon smolts can be accounted for as adults in the commercial and sport fisheries and less than 1% at the hatcheries of origin (Parrish et al., 1973; Washington Department of Fisheries, 1970). The factors contributing to this high degree of unaccountability are not understood because of their complexity (Royce, 1972). Burrows (personal communication) postulates from studies on the stamina potential of hatchery-raised chinook smolts that improved fish husbandry techniques should increase the returns to the fisheries significantly.

Table 2: Direct causes of pathological changes in gill tissues.

Infectious Agents	*References*
Bacteria	
Myxobacteria	Bullock, 1972
Pseudomonas sp.	Snieszko and Axelrod, 1971
Fungi	
Saprolegnia	Reichenbach-Klinke and Elkan, 1965;
Branchiomyces	Pauley, 1967
Protozoa	
Trichodina	Davis, 1956; Meyer, 1966; Sindermann,
Chilodon	1969; Hoffman, 1967
Scyphidia	
Epistylis	
Amphileptus	
Trichophrya	
Oodinium	
Glossatella	
Bodomonas	
Tripartiella	
Henneguya	
Trematodes	
Dactylogyrus	Davis, 1956; Meyer, 1966; Sindermann
Gyrodactylus	1969; Hoffman, 1967
Cleidodiscus	
Monocoelium	
Urocleidus	
Diplozoon	
Magecraeiodes	
Crustacea	
Ergasilus	Snieszko and Axelrod, 1971
Lernaea	
Achtheres	

Noninfectious Agents	*References*
Genetic Malformations	Halver, 1972; Reichenbach-Klinke and Elkan, 1965
Physical	
Silting	Ellis, Westfall, and Ellis, 1948
Chemical Precipitation or Flocculation	
Chemical	
ph (below 6.0 and above 9.0)	Spotte, 1970; Herman, 1970; Ashley, 1972; Burrows and Combs, 1968
Ammonia	
Chemotherapeutic agents dispensed in water	
Copper	
Thiourea	
DDT	
Aflatoxins	
Detergents	
Neoplastic	Mawdesley-Thomas, 1972
Nutritional	
Vitamin C deficiency	Ashley, 1972
Pantothenic acid deficiency	
Vitamin E deficiency	
Nicotinic acid deficiency	
Vitamin A excess	
Idiopathic	
Sporadic telangiectasis	Klontz, unpublished

Table 3: Indirect causes of pathological changes in gill tissues

Infectious Agents	*References*
Bacteria	
Corynebacterium	Yasutake, unpublished; Klontz and Wood, 1972; Klontz, unpublished; Anderson and Conroy, 1968
Aeromonas salmonicida	
Aeromonas liquefaciens	
RM bacterium	
Vibrio anguillarum	
Viruses	
Infectious hematopietic necrosis	Yasutake, 1970; Ghittino, 1965
Viral hemorrhagic septicemia	

Indirect causes of pathological changes in gill tissues (cont.)

Infectious Agents	*References*
Protozoa	
Cryptobia Trypanosoma Ceratomyxa Myzobolus Plistophora Dermocytisdium	Davis, 1956; Sanders et al., 1970; Wood, 1968
Trematodes	
Cardicola Clinostomum Nanophyetus	Holway, unpublished; Klontz, unpublished; Farrell, R. D., personal communication
Cestodes	
Proteocephalus	Klontz, unpublished
Noninfectious Agents	*References*
Chemical	
Nitrates Nitrogen	Smith, unpublished; Rucker, 1972
Nutritional	
Starvation Essential amino acid deficiency Vitamin B complex deficiency	Ashley, 1972
Neoplastic	
Metastatic malignancies	Mawdesley-Thomas, 1972

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CONTROL OF VIBRIOSIS AND CORYNEBACTERIAL KIDNEY DISEASE IN CULTURED FISHES

*G. L. Bullock, R. L. Garrison, J. Rohovec and J. L. Fryer**

Introduction

The intent of this paper is to present pertinent information concerning clinical signs, severity, host and geographic range, and control of vibriosis and corynebacterial kidney disease. However, the main emphasis will be placed on control measures for each disease, since this is most probably the main interest of this group.

Vibriosis

Fish diseases caused by members of the genus *Vibrio* have been recognized for a long time, possibly since the 1700's. *Vibrio*-caused diseases of saltwater eels (*Anguilla vulgaris*) are known as "red pest" and "red boil" and these diseases have been responsible for large annual losses in eels for many years. Vibriosis typically affects fishes in the marine and estuarine environment, although serious outbreaks have been reported in salmonid fishes in freshwater. At least in some of the disease outbreaks in freshwater, affected fish had been fed ground-up, unpasteurized carcasses of *Vibrio*-infected marine fish.

Gross symptoms and pathology associated with *vibrio* infections vary with the species affected and are well defined in species such as juvenile turbot (*Rhombus maximus*). The pathology produced in salmonids is similar to that seen in furunculosis and vibriosis has been referred to as "saltwater furunculosis". Typically, vibriosis in salmonids is characterized by red, necrotic lesions of abdominal musculature and erythema at the bases of fins and also at the mouth. Hemorrhages are often seen in gills, intestines, and body wall. In all species affected, the disease develops as a generalized septicemia in which the causative bacterium can be isolated from blood and most internal organs.

Correct diagnosis of vibriosis depends on isolation and identification of the causative agent. Although other species have been described, the organism most closely associated with vibriosis of fish is *Vibrio anguillarum*. Serologically, three types have been described. Type one includes isolates from Pacific Northwest salmonids; type two contains isolates from fish from European waters; type three is comprised of strains from Pacific Northwest herring (*Clupea pallasi*). All isolates are gram negative rods 1-3 microns long by 0.5-1.0 micron wide, which are motile by a single polar flagellum. Biochemically, *V. anguillarum* is cytochrome oxidase-positive, ferments sugars with production of acid only, requires sodium chloride for growth, and is sensitive to the vibriostat 0/129 and Novobiocin.

**Respectively, Eastern Fish Disease Laboratory, Kearneysville, W. Va.; Oregon Wildlife Commission, Corvallis, Oregon; and Rohovec and Fryer are both at Dept. of Microbiology, Oregon State University, Corvallis, Oregon.*

Vibriosis occurs throughout the world's estuarine and marine waters, and while some species such as the eel or herring may serve as reservoirs of infection, *V. anguillarum* occurs naturally in salt water. Transmission may, therefore, occur by fish to fish contact or merely through water. Of the propagated salmonids, the chum (*Oncorhynchus keta*) and pink salmon (*O. gorbuscha*) are the most susceptible species. Disease outbreaks occur most commonly when water temperatures are over 50° F. and severity of disease increases with temperature with most severe outbreaks occurring near 60° F. The importance of vibriosis among fishes cultured in salt water cannot be overemphasized. Wood (1968) stated "any attempt to raise fish in saltwater impoundments, subject to considerable warming, should consider vibrio disease as a factor possibly limiting the success of the project."

Control of vibriosis as with most infectious fish diseases can be accomplished by prevention or treatment. Preventative measures for vibriosis are similar to those employed in other bacterial fish diseases. Incidence and severity of vibriosis can be reduced by eliminating environmental stress conditions such as low dissolved oxygen levels. Other conditions also predispose fish to *vibrio* infections such as infections with other agents, or nutritional imbalance. Since the transition from fresh to salt water is physiologically demanding, fish should be in the best condition possible before transfer to salt water to reduce the risk of infection with *Vibrio*. Prophylactic chemotherapy during critical months of warm water temperature has been suggested with sulfamethazine given at the rate of 2 grams per 100 pounds of fish per day. However, this treatment cannot be used on fishes meant for human consumption since it has not been cleared for use by the Food and Drug Administration.

The most recent attempt at prevention of vibriosis among cultured fishes has been oral immunization. These studies were begun at Oregon State University in 1968 by Dr. J. L. Fryer, his students, and members of the Oregon Wildlife Commission. A vaccine consisting of a lyophilized sonicate of *V. anguillarum* cells was fed to 200 chinook salmon at the rate of 300 micrograms of vaccine per fish given over a period of 14 days. After vaccination, these fish were kept in freshwater an additional 20 days and then transferred to the Lint Slough saltwater rearing facilities at Waldport, Oregon, where they received a natural challenge of *V. anguillarum*. After 70 days in salt water, 45% of the vaccinated fish had died of vibriosis while 98% of unvaccinated fish died.

These studies have been continued with modification and the following facts determined. Formalin-killed wet packed cells of *V. anguillarum* can be used in place of the lyophilized sonicate for vaccination. Control of vibriosis with wet packed cells was achieved with 2-mg wet packed cells per gram of food (Oregon Moist Pellet) fed for 14 days. Feeding the 2-mg level up to 45 days did not increase survival over that obtained with the 14-day feeding. Fish orally immunized for 14 days at the 2-mg level have a limited period of protection if not exposed to *V. anguillarum*. Fish should be challenged to *V. anguillarum* within 30 days after the last day of vaccine administration. Protection against vibriosis has also been effectively achieved by parenteral injection of a mixture of *V. anguillarum* cells in Freund's adjuvent.

It is important to emphasize that research on oral vaccination of fish against vibriosis has been on a laboratory scale using small groups of 100-200 fish. The usefulness of an oral vaccine for control of this disease will have to be determined using pilot scale studies and ultimately hatchery trials. There may well be problems encountered with pilot or hatchery scale trials which were not encountered in laboratory studies. Among the questions yet to be answered in pilot trials are the need for booster feedings and the influence of different serotypes of *V. anguillarum* on efficacy of vaccination.

Treatment procedures for vibriosis among cultured fishes include the following:

Terramycin at 2.5-3.5 grams/100 lbs. of fish/day for 10 days
Sulfamerazine at 8-12 grams/100 lbs. of fish/day for 10 days
A combination of 3 grams sulfamerazine and 2 grams NF 180/100 lbs. of fish/day for 10 days.

It should be noted that none of the above treatments have been cleared by the Food and Drug Administration for treatment of vibriosis of fish. However, attempts are being made at present to supply the necessary data to clear Terramycin.

Corynebacterial Kidney Disease

Corynebacterial kidney disease which is known as "Dee Disease" in the British Isles is a chronic to subacute disease of salmonid fishes. It was first reported in the British Isles in 1933 and in the United States in 1935. As far as it is known, the disease affects only salmonid fishes and occurs in North America, the British Isles, and recently has been reported in Japan. Among the Pacific salmon, chinook (*O. tshawytscha*), coho and sockeye (*O. kisutch*) are the most susceptible species while brook trout (*Salvelinus fontinalis*) is the most susceptible of the trouts. Brown trout (*Salmo trutta*) are intermediate in resistance, and rainbow trout (*S. gairdneri*) are resistant to kidney disease.

Kidney disease is a septicemic condition which begins when the causative bacterium enters the fish through the gut or a break in the skin. The disease develops slowly with incubation period dependent on water temperature. Losses in Pacific salmon usually begin 30-35 days after exposure in a water temperature of 52° F., but incubation period is 60-90 days for the same species in 45-50° F. water. Also, water hardness has been shown to affect the severity of kidney disease epizootics in that the disease is far more severe in soft waters (below 50 ppm total hardness). However, whether the relationship of disease severity and water hardness is one of direct cause and effect has not been determined.

The gross pathology seen in salmonids with kidney disease varies with the species affected. Externally, exophthalmos may be present, and small blebs or blisters containing blood cells and cells of the kidney disease bacterium may be seen on the sides of diseased fish above the lateral line. The blebs may ulcerate forming deep lesions. Hemorrhages may occur at the base of the fins. Internally, liver, kidney, spleen, and heart may contain local foci of infection. In advanced stages the kidney is

generally grossly swollen which results in anemia and production of ascitic fluid. Hemorrhages often occur in the viscera and body wall.

Diagnosis of kidney disease is based on symptoms and microscopic examination of stained tissue smears for the presence of gram positive diplobacilli characteristic of the kidney disease bacterium. Usually no attempt is made to isolate the causative organism in routine diagnosis of kidney disease, because the bacterium grows slowly, often requiring one to three weeks for initial isolation. While diagnosis of kidney disease epizootics by microscopic examination of stained tissue smears is satisfactory, this method is not useful for detecting low level infections. Recently an immunodiffusion test using tissues from kidney diseased fish and rabbit anti-kidney disease serum has been put into practice and this promises to be more sensitive in detecting low level kidney disease infections. Since the causative agent of kidney disease grows slowly and requires cysteine for growth, only limited studies on its morphological, biochemical, and serological characteristics have been carried out. Based on present information, this bacterium is presently classified in the genus *Corynebacterium*.

Present evidence suggests that the kidney disease bacterium, unlike *V. anguillarum*, does not occur naturally in water and probably requires fish or other aquatic animals to serve as a reservoir of infection. Effective control of kidney disease can, therefore, be best achieved by preventative measures. As will be discussed, no effective treatment is presently available for kidney disease, but the following preventative measures have been used in controlling kidney disease. Disease incidence in Pacific salmon was substantially reduced by discontinuing the practice of feeding raw carcasses and viscera from infected adult salmon to young salmon. It was found that salmon fed a diet containing corn gluten suffered a much higher incidence of kidney disease than salmon fed a diet containing cottonseed meal. Therefore, it appeared that substituting cottonseed meal for corn gluten would reduce incidence of kidney disease. Recently published results showed that the incidence of kidney disease was the same in groups of salmon fed either corn gluten or cottonseed meal, but the non-specific stress caused by the chronic nature of the disease was more severe in fish fed the diet containing corn gluten, causing increased mortality in these groups.

Among the trouts, effective prevention of kidney disease is accomplished by obtaining fertilized eggs from broodstock free of the disease and hatching and raising the fish at hatcheries whose water source is free of fish which may harbor the kidney disease bacterium.

Prophylactic chemotherapy with sulfamerazine at 2 grams/100 lb. of fish has been used to control kidney disease in young salmon. Feeding of prophylactic levels was continued until the salmon were released.

Because many of the kidney disease bacteria occur intracellularly in diseased fishes, treatment of kidney disease is difficult. Reports of treatment of outbreaks with sulfonamides at 8-12 grams/100 lbs. of fish/day up to 14 days all indicate that treatment was effective only as long as the drug was given and that mortalities rose once treatment was stopped. The most effective drug in treating outbreaks of kidney disease has been Erythromycin

given at the rate of 4.5 grams/100 lbs. of fish/day for three weeks. However, there have been several instances where this drug has failed to control outbreaks and also there have been reports of toxicity with Erythromycin thiocyanate, the form usually used. It should be noted that no drugs have been cleared for use in treating kidney disease in cultured fishes. It would appear that if drugs are going to be effective in controlling kidney disease, they would have to be used before the disease became well established in the population.

All of the preceding information has been obtained with salmonids raised in freshwater. Control of kidney disease in species raised in salt water can at the present time only be based on information obtained from fishes in freshwater. For example, experience has shown that salmonids harboring the kidney disease bacterium in freshwater and then brought to salt water can suffer severe epizootics of kidney disease. However, information concerning transmission of kidney disease in salt water is lacking. Therefore, it would seem at the present time, control of kidney disease in salmonids raised in salt water would be essentially those procedures used for the freshwater environment: obtaining fertilized eggs from broodstock free of the disease and raising the fish in freshwater free of the kidney disease bacterium, before transfer of fish to salt water.

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VIRAL DISEASES OF SALMONIDS IN NORTH AMERICA

*Donald F. Amend**

There are two viral diseases of salmonids in North America which can cause significant mortalities and both are potential threats to aquaculture and mariculture on the Pacific Coast. Infectious pancreatic necrosis (IPN) is a highly contagious disease of brook trout fry and fingerlings, but rainbow trout are also susceptible. Other species of trout appear to be less susceptible but the virus has been detected in carrier coho salmon. Infectious hematopoietic necrosis (IHN) is a disease of sockeye salmon, chinook salmon and rainbow trout fry and fingerlings. Coho salmon are resistant to IHN but the susceptibility of other species is not yet known.

Infectious Hematopoietic Necrosis

Clinical signs: the first signs to be observed include abdominal distension and accumulation of ascitic fluid in the peritoneal cavity, darkening of the body, exophthalmos, anaemia, and petechiae at the base of the fins; internally the anterior kidney, spleen, liver and pancreas show signs of severe necrosis; the digestive tract is often filled with fluid, and petechiae are frequently found in the perivisceral fat in the mesentery.

Histopathology: stained sections of the anterior kidney and spleen show marked structural changes in the hemaotpoietic tissues, and necrosis of the granular cells of the *stratum compactum*; the histopathological findings are of value in the presumptive diagnosis of cases of IHN.

Identification of the virus: any presumptive diagnosis of IHN on a basis of clinical signs and histopathological findings must be confirmed in the laboratory by isolation of the virus. This requires isolation of the virus on cell culture and serological identification.

Geographical distribution: IHN is prevalent along the Pacific Coast from Alaska to California. It is most prevalent in sockeye salmon, but IHN is a serious problem in chinook salmon and steelhead in the Sacramento River in California and in isolated areas where rainbow trout are reared. It is a problem in both hatchery and wild populations of fish. Sporadic outbreaks have occurred outside the Pacific Coast due to the shipment of fry and eggs from infected brood stocks. Recent outbreaks in chinook salmon in Columbia River tributaries indicate potential local problems.

Transmission: transmission occurs from fish to fish through the water, with contaminated feed, and with contaminated eggs.

Carrier status: Carrier fish are the reservoir of infection. Once a fish becomes infected it can become a lifetime carrier, but virus is shed mostly

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at or shortly after spawning.

Prevention and control: IHN is a cold-water disease. The epizootic potential is highest at 10° C. (50° F.) and the disease has not been recorded above 15° C. (60° F.). The use of temperature to control epizootics has been used successfully to control the disease in sockeye and chinook salmon hatcheries, but this does not eliminate the carrier state.

Disinfection of eggs with iodophors successfully eliminates the virus, but the eggs and fry must be reared in virus free water to completely prevent the disease. Acriflavin and merthiolate do not destroy IHN virus. If fish ever become infected, there is no known way of eliminating the virus from the fish. The best prevention of IHN disease is to obtain fish certified free of IHN virus or to obtain disinfected eggs.

Potential threat: Particular attention should be taken to prevent the disease in chinook, sockeye, and rainbow trout because of possible serious mortality and because of the potential risk of exposing natural populations. Coho salmon are resistant to the disease and there is no evidence that coho can transmit the virus. The disease has been described only in fresh water; the threat in salt water is not known.

Infectious Pancreatic Necrosis

Clinical signs: the first signs to be observed include abnormally high mortalities (often higher than 80 percent) during the first feeding stages; the diseased fish show signs of distress, and swim in a characteristic spasmodic spiralling or whirling manner, followed by periods during which they remain quiescent on the bottom of the tanks in an exhausted state; complete darkening of the body, abdominal distension, and possibly moderate exophthalmos are other external signs which may be present; internally the spleen and liver are pale in color, and the gall bladder is frequently filled with an abnormal amount of whitish-colored mucoid material; the anterior visceral mass and pyloric caeca (among which the diffuse pancreas lies) show petechial hemorrhages.

Histopathology: stained sections of the exocrine pancreas show signs of marked necrosis of the pancreatic tissue, and the presence of inclusion bodies; certain strains of IPN virus produce necrotic changes affecting the hematopoietic tissue and glomeruli of the kidney.

Identification of the virus: any presumptive diagnosis of IPN on a basis of clinical signs and histopathological findings must be confirmed in the laboratory by isolation of the virus. This requires isolation of the virus on cell culture and serological identification.

Geographical distribution: IPN is found throughout all major trout-producing areas in the world. Because rainbow trout are reared more than any other trout, IPN has been found in this species over a greater geographical area. However, the brook trout appears to be most susceptible to the disease. IPN has been found in all Pacific states except Alaska and British Columbia. Recent isolation along the Columbia River and in various areas in Oregon indicates a potential growing problem.

Transmission: the virus is transmitted from fish to fish, with feed, and with eggs. The primary means of spreading the virus around the world has been with eggs from carrier brood stock.

Carrier status: carrier fish are the reservoir of infection. Recent publications indicate that various species of fish can carry the virus, and transmission does not necessarily come from parent fish. Once fish become infected, they become lifetime carriers and shed virus intermittently throughout their life.

Prevention and control: the epizootic potential is greatest between 50° and 60° F. but rearing fish at other temperature ranges does not necessarily eliminate the disease. The only known method of preventing the disease is to obtain fish or eggs certified free of IPN and to rear them in virus-free water. Once fish become infected, there is no known cure or method of eliminating the virus from the fish. Complete eradication of all carrier fish, disinfection of the water supply and hatchery, and restocking with virus-free fish is the only known way of eliminating the virus. There is no advantage in disinfecting eggs because recent evidence has shown that IPN can still be transmitted with eggs disinfected with iodophors.

Potential threat: the threat is greatest to brook trout. The risk of disease in rainbow trout is variable. In some localities mortalities up to 90% have occurred and in other localities no mortalities occur even though most fish carry the virus. Japanese workers have shown that sockeye salmon are susceptible to IPN and other species of salmon are known to carry the virus. However, the risk of disease to salmon is unknown, but it must be considered a potential threat. Furthermore, the potential problem in the marine environment is not known.

In conclusion, there are only two viral diseases on the Pacific Coast known to be a potential problem to aquaculturists or mariculturists. So far, none of these viruses have been known to cause problems in marine waters, but in some instances have caused considerable problems in freshwater. There is a potential risk and I advise precaution in introducing any of these viruses into your operation. In my opinion the least problems (strictly from a viral point of view and with our current knowledge) would occur by rearing coho salmon and the most problems would occur by rearing sockeye salmon.

NUTRITIONAL RELATIONSHIPS WITH DISEASE

*Clarence L. Johnson**

Nutritional diseases are defined as those which can be attributed to deficiency, excess or improper balance of components present in the available food. Such diseases usually have a gradual onset because

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symptoms do not appear until one or more of the components of the diet drop below the critical level of body reserves.

One of the major difficulties confronting the commercial growers is the diagnosis of nutritional diseases. Invariably the general health of the fish drops because of suboptimal or insufficient diet; their resistance to infectious disease is lowered with the result being that the nutritional disease is masked by the onset of a noningested-infectious disease. Two recent publications summarize the symptoms of nutritional deficiencies and will be of benefit to the fish grower: (1) Nutrient Requirements of Trout, Salmon and Catfish, National Academy of Sciences, 2101 Constitution Avenue, N.W., Washington, D. C. 20418. (2) Fish Nutrition, Academic Press, Inc., 111 Fifth Avenue, New York 10003.

Most fish growers should avoid the mixing or preparation of feeds. The biggest danger in indiscriminate mixing of dietary ingredients is the development of an imbalanced or deficient diet. Feed manufacturers are highly competitive and want to provide good consistent, high quality feeds at the least cost and have the resources and technical knowledge to prepare them.

Gross protein requirements of fish vary with water temperature, water quality, fish size and species. Preliminary studies suggest that the requirements for salmonids reared in sea water will be nearly the same as those reared in fresh water. It is essential that a proper balance of the amino acids be maintained since an imbalance will inhibit growth. The major symptom of a deficiency of the essential amino acids is a reduction in growth. To date, all symptoms of an amino acid deficiency are reversible, with the exception of tryptophan.

Fish have difficulty in effectively utilizing hard, high melting-point fats. In contrast, liquid fats are readily digested and used by fish. By using fat as an energy source, the protein is spared and can be used to build and maintain tissues. Oxidation of fats during storage destroys essential fatty acids and produces oxidative rancidity. Only the linolenic series of fatty acids have been shown to be essential for rainbow trout and probably are required for salmon. A high level of polyunsaturated fatty acids in the diet causes difficulty in maintaining fat stability during storage.

No carbohydrate requirements have been established for fish. Carbohydrates may supply up to 20% of the available calories in the ration. This will spare protein since less protein will be used for energy. Overfeeding or high levels of carbohydrate may cause excessive storage of glycogen in the liver, which can result in death.

Vitamin needs of fish have been recognized for over forty years. Considerable work has been done to determine requirements for each vitamin. Symptoms for each vitamin are described in both the Nutrient Requirements of Trout, Salmon and Catfish and Fish Nutrition. A vitamin supplement is commonly added to fish rations. A modest excess of vitamins is necessary for several reasons: losses during processing and storage, oxidation, exposure to direct sunlight and temperature. Hypervitaminosis caused by excessive supplementation with water-soluble vitamins seldom

occurs because of the cost of B-vitamins. Fat-soluble hypervitaminosis can occur and should be avoided.

Inorganic elements, as nutrients, are difficult to study. Most research on minerals in fish has been limited to toxicity and osmoregulation studies. All minerals essential to adequate nutrition of higher animals should be considered essential for fish until proved otherwise.

In summary, it has been stated that every disease has a nutritional aspect. I have tried to cover some of the principal factors influencing nutritional diseases. The pathology that occurs in fishes incident to oral ingestion of foods or gill absorption may be termed nutritional as opposed to pathology induced by noningested bacteria, viruses, parasites or other non-nutritional entities. However, the effects of suboptimal food intake, environmental stress and resistance to infectious diseases are all interrelated. The influence of each is variable--disease breaks out only if there is sufficient relationship between them.

PREVENTATIVE MEDICINE: STATUS OF THE LEGAL USE OF VACCINES*

*Anthony Novotny***

The intensive culture of fish in natural marine waters is historically documented as having great potential (because of the amount of water available), and is rapidly moving into expanding production. Production in Japan is measured in thousands of metric tons and in the U.S. and Norway, production is now being measured in hundreds of metric tons.

As production moves from pilot scale to full commercial production, the problems of fish diseases rear their ugly heads and dominate the scene. Although commercial farmers are always interested in the latest developments in the therapeutic treatment of fish diseases, the long-range interest is in disease prevention. Undoubtedly, the future will show that properly balanced diets for use in the marine culture of fish will be of importance in preventing or limiting the ravages of epizootics. But there will be an equal or greater emphasis on the use of specific vaccines.

Use of Vaccine

The vaccine of greatest interest to marine fish farms (at present) is the one used to prevent vibriosis. The disease and the causative organism have been well defined by regional scientists such as Ordal and Pacha. The preparation and successful use of an oral vaccine has been demonstrated in Oregon by Fryer in the laboratory and Garrison in the field. At Manchester, vibriosis has been successfully prevented in coho salmon and cutthroat trout for periods of 4 to 6 months by using direct injections of the vaccine. The use of adjuvants may increase protection through the marketing period, but this requires additional testing.

**Summary of moderator's discussion*

***National Marine Fisheries Service, Manchester, Washington*

There will be an interest in both oral and injected vaccines by the growers. Mr. Barry Freidman of Union Carbide indicated to me today (at this workshop) that the present estimates of the cost of the vibrio vaccine will be $0.14 to $0.22 per gram of cells. On the basis of present advice on vaccination procedures, the following cost comparisons can be made:

Oral Vaccination

The minimum level is 5 g of vaccine/kg of food for 15 days, followed by a maximum of 15 days without vaccine before exposure. This could mean 15 days before entry into seawater, or if *Vibrio anguillarum* is not present, it could mean using the oral vaccine up to within a few days of entry into seawater. The degree of exposure to the live organism that is necessary to induce natural antibody production (if there is any) is not known. The Oregon research group is suggesting that a re-vaccination be made at 90 days after the first vaccination for a period of 7 days, although this needs to be tested in the field, and may present some complications.

Assuming a single vaccination period of 15 days, followed by a 15-day "rest" period prior to seawater entry, we have the following for 1 million coho smolts (accelerated zero-age fish):

(1) Entry into seawater: 18 g fish (25/lb)--18,000 Kg (40,000 lbs)

End of vaccination: 13.5 g fish (37.5/lb)--13,500 Kg (30,000 lbs)

Start of vaccination: 9 g fish (50/lb)--9,000 Kg (20,000 lbs)

This is a net gain of 4,500 Kg (10,000 lb) during vaccination. Assuming a net food conversion of 1.5:1, the food used during vaccination is 6,750 Kg (15,000 lb).

(2) The cost, then will range from $4725 to $7425 per million fish.

Injection

Recent field trials using automatic syringes show a minimum injection rate of 700 fish/hour (10,000 fish). Test data reported today (using women trained as fish markers) by Brian Allee on 20,000 fish show a rate of 900 to 1100 fish/hour/person. At 900/hour, this is 7200 fish/worker/day. At a wage rate of $20.00/day, the labor cost of injection is $0.0027/fish, or $2700/million fish.

On the basis of our present tests, there are no indications that an intraperitoneal injection in excess of 2.5 mg of wet-packed cells per fish will afford appreciable increases in protection. At this rate, the cost of vaccine is $0.00035 to $0.00055/fish, or $350 to $550 per million fish. The total cost, then, will be $3050 to $3250 per million fish. The cost of capital equipment is about $50/worker.

Thus, the oral and injected vaccines are competitive at the projected level of present vaccine prices. And, the injected vaccine affords better protection.

Legal Aspects of the Use of Vaccines in Fish

All animal vaccines fall under the regulations of the 1913 Federal Virus-Serum-Toxin Act. This Act of Congress places the entire control under the U.S. Department of Agriculture (USDA). The Food and Drug Administration (FDA) does not enter into the picture unless the vaccine is used in combination with a drug (including antibiotics). This means that an oral vaccine, an injected vaccine, or an injected vaccine with nondrug adjuvants need only be cleared by the USDA.

The basic problem of vaccine use is not with the growers--it is with the manufacturer of the vaccine. Once a manufacturer has obtained a license to sell the vaccine in interstate commerce, the grower can use the vaccine at his discretion. However, a license for fish vaccine production has never been requested before, and it may take up to 2 years for a manufacturer to satisfy the USDA requirements, which are stringent and too numerous to mention here.

The USDA is not a villain in this matter. To the contrary, they are charged with encouraging the development of vaccines. Until a licensed product is available, a waiver to the license may be obtained for experimental use. And, the experiments can involve large numbers of fish. The USDA is interested in field data to support the data on hand showing the need for and efficacy of the vaccine. Growers interested in using experimental vaccines should contact Dr. David Long (301-436-8675), USDA, Animal-Plant-Health Inspection Service, Federal Center Building, Room 833, 6505 Bellcrest Road, Hyattsville, Maryland 20782.

Need for a Grower's Cooperative

There is a bill in the state legislature that calls for funding an animal diagnostic laboratory at Washington State University to assist livestock growers with their animal disease problems. The bill sets up an assessment program on slaughtered livestock of $0.04/hog; $0.01/sheep; $0.04 to$0.065/cow; and $0.10/horse. Perhaps the time has come for the salmon growers to consider a similar measure by self-assessment, and seek assistance from the federal or state government as a collective for diagnostic services. The disease problems will not disappear, and as the number of growers increases, the available "free" services will be diluted.

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