PROLIFERATIVE KIDNEY DISEASE (PKD)

IN NORTH AMERICA

Proceedings of a Workshop on Proliferative Kidney Disease (PKD) among Salmonid Fish in North America.

April 25 and 26, 1985. Held at the University of California Davis, California U.S.A.

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Preface

Last April 25 and 26 (1985) we held a very productive workshop on proliferative kidney disease (PKD) at the University of California, Davis. This meeting was designed to present information from the laboratories actively participating in research on the disease. The first day was devoted to formal presentations by speakers from the University of Idaho, Department of Fisheries and Oceans (Canada), US Fish and Wildlife Service (USFWS) and the University of California, Davis.

The second day was devoted to an open discussion moderated by Dr. Jim Warren (USFWS). The current knowledge of the disease was summarized and the techniques used to diagnose PKD reviewed. Existing policies to control the disease in N. America were discussed and a list of recommendations for future studies developed.

What follows in this publication is a summary of the formal presentations given at this meeting. Because of the time required to prepare the material for press, the authors have in most cases updated their contributions to our current understanding of PKD in N. America. In so doing, this proceeding represents the most detailed accounts of PKD as it occurs in N. America.

I have taken the liberty of adding a section on the diagnosis of PKD that I hope will help those fish health specialists not currently familiar with the disease. In addition, a draft for a Fish Disease Leaflet on PKD has been submitted for review to USFWS.

I would like to take this opportunity to thank all those that helped organize and then carried through to make the workshop a success, especially Dr. Fred Conte from UC Davis, Aquaculture Extension. I thank each of the speakers for their time and efforts with the spoken and written texts. Sea Grant College of California is also acknowledged for their assistance in the publication of the proceedings.

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A Review of Proliferative Kidney Disease of Salmonid Fish

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Introduction

The purpose of my talk today is to present an introduction and thus provide background information on what is known about Proliferative Kidney Disease (PKD). PKD was probably first described in Germany by Plehn and called 'Amobeninfektion der Niere'. Since this time, numerous conditions suggestive of PKD, or perhaps of concurrent infections of PKD and viral or nutritional diseases have been reported and reviewed by Clifton-Hadley et al., (1984a). The disease condition we know as PKD was first named by Roberts & Shepherd (1974). While many names have been presented in the literature both prior to and since this time, this name has received widespread acceptance. The first indepth descriptive report on PKD was that by Ferguson and Needham (1978). Several reports including a recent review of PKD by Clifton-Hadley et al., (1984a) have since been published.

Occurrence and Importance

Over the past five years there has been an increasing number of cases of PKD in the United Kingdom as well as an increase in severity of the disease. Because of large economic losses in farmed salmonids PKD is considered to be

one of the most economically damaging diseases in United Kingdom aquaculture (Clifton-Hadley, et al., 1984a). To date, PKD has been reported in 13 countries. While few known cases of PKD occurred in Germany before 1980, several outbreaks were seen in 1980 and 1981 (Hoffman & Daugschat, 1981). The disease was first reported in North America in 1981 by Smith et al., (1984). Since that time it has been reported at other hatcheries in the U.S.A. within the state of California (Hedrick et al., 1985).

Causes of the Disease

It is generally agreed that the causative agent of PKD known as PKX, is a protozoan parasite that can be readily seen in infected tissues by both light and electron microscopy. It was first demonstrated in electron micrographs by Ferguson & Adair (1977) and later by Ferguson and Needham, (1978). Certain authors have suggested that the parasite is an amoeba or haplosporidan (Clifton-Hadley et al., 1984a), but recent evidence by Kent and Hedrick (1985), suggests that PKX is a myxosporidan.

Mortality due to PKD

Mortality in infected fish is generally from 5 to 15 percent, but varies and may be as great as 90% as evidence by outbreaks in both Europe and the U.S.A. The disease is often seen in association with other infectious diseases, such as infectious pancreatic necrosis (IPN), infectious hematopoietic necrosis (IHN), furunculosis, bacterial gill disease, etc. Occasionally, one may find a high morbidity rate, but low mortality.

Other losses, less easily defined, result from decreased feed efficiency, poor tolerance to handling stress, less tolerance to low oxygen concentrations, greater susceptibility to secondary infections, and increased mortality due to antibiotic treatment of concomittant diseases. Salmonids appear to be most susceptible to PKD, but infected pike and grayling have also been found.

Epidemiology

The time of peak infectivity appears to be in the spring of the year, probably May, and seems to coincide with an increase in water temperature and/or an increase in numbers of infective agents in the water or both. Approximately 4 weeks lapse before the organism can be found histologically, and another 3 to 4 weeks before the onset of gross clinical signs and mortality.

Occurrence of the disease is dependent upon water temperature. Generally, the disease only occurs at 14-15^{o}C and higher and disappears as temperature drops. It may persist later in the year in Idaho where water temperature is constant at 15^{o}C.

The disease is closely associated with water quality and usually only occurs at hatcheries using water from rivers and streams, but seldom in those using enclosed springs or wells. It occurs both in acid and alkaline waters and often in waters with high organic loads, suggesting that high concentrations of dissolved organics may predispose fish to PKD.

While PKD usually occurs in fingerlings, it is often found in larger fish as well. Evidence exists demonstrating that infected fish are probably immune to further PKD infection.

Life Cycle and Transmission of PKD

The life cycle of PKD is poorly understood. While it has been demonstrated that the peak of infectivity is probably May and that an incubation period of 6-8 weeks exists, there is little evidence as to the mode of entry of the infective agent into the fish. To date, experimental transmission only occurs when non-infected fish have been inoculated with phosphate buffered kidney homogenates (Clifton-Hadley et al., 1984B; D'Silva et al., 1984) or spleen or blood (Kent and Hedrick, 1985). Attempts to infect fish by cohabitation of non-infected fish with infected fish, or by feeding infected kidney tissue to non-infected fish have failed. Evidence presented by Kent and Hedrick (1985) suggests that PKX is a myxosporidan that sporulates in the lumens of the kidney tubules.

Clinical Signs, Gross Pathology and Histopathology

The clinical signs and the gross pathology have been well described by Ferguson & Needham (1978). They include anemia, ascites, darkened coloration, exophthalmia and extreme swelling of kidneys and often spleens (Fig. 1).

The most consistent and intense histopathological changes occur in kidneys and consist primarily of pronounced granulomatous interstitial nephritis, often with replacement of nephrons by proliferated tissue (Fig. 2). A similar granulomatous response often occurs in the spleen. In addition to seeing PKX in kidneys and spleens they may also be found in livers, musculature, pancreas, gills, intestine and spinal canal. Organisms are often seen adherant to renal and hepatic vessel walls resulting in a necrotizing vasculitis and partial to complete occlusion of vessels.

The anemia that occurs in clinically ill fish has been classified as a chronic hemolytic anemia (Hoffman & Lommel, 1984).

Diagnostic Methods

Currently, the only method used in the diagnosis of PKD is by histological examination of affected tissues. Both stained tissue sections and kidney imprints prepared by touching infected tissues to clean glass microslides, then staining with Giemsa or methylene blue are used, (Clifton-Hadley et al., 1983). Kidney imprints provide a rapid means of identifying PKX, (Fig. 3).

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Figure 1. Formalin-preserved rainbow trout showing typically swollen kidney and spleen (from Smith et al., 1984).

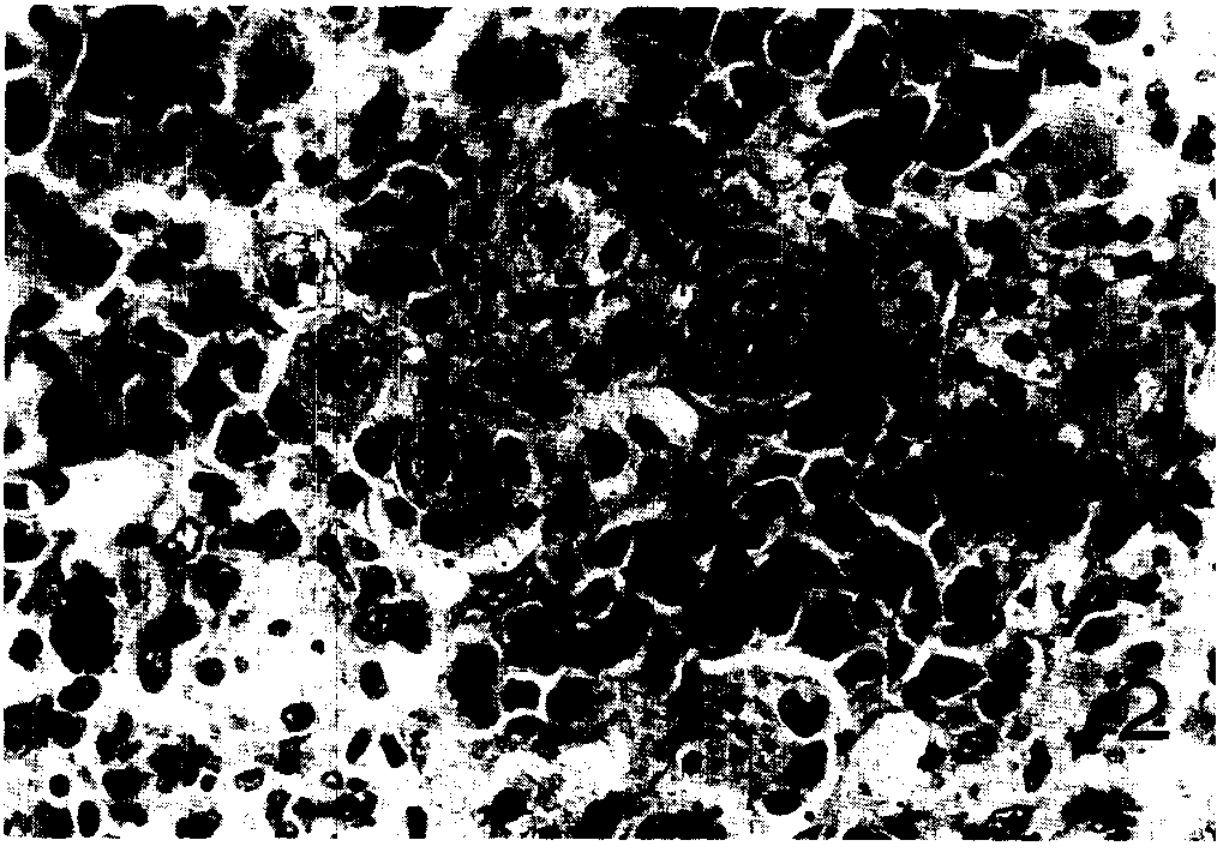

Figure 2. Parasites in kidney section of diseased fish (Arrowheads) (H-PAS, x 870) (from Smith et al., 1984).

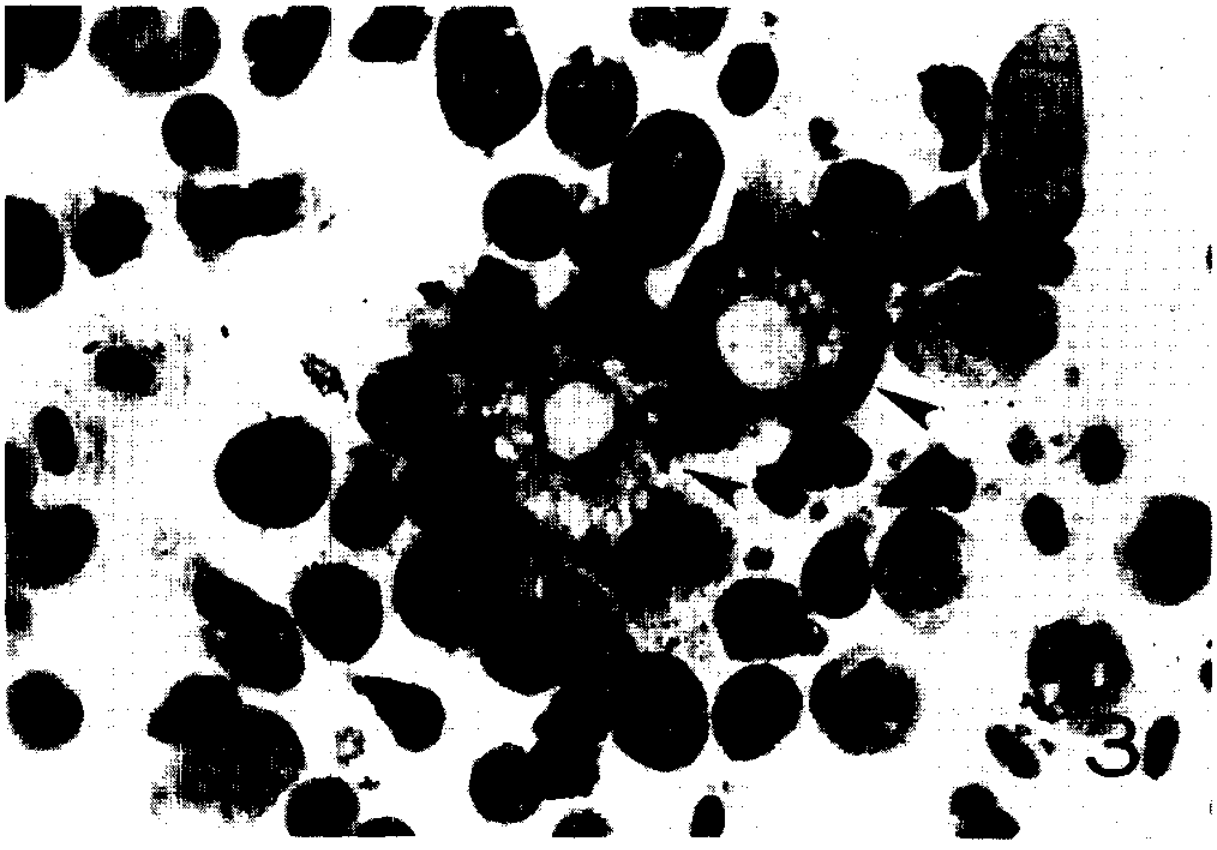

Figure 3. Kidney Imprint showing PKX cells (Arrowheads). Leishman-Giemsa x 2200.

The Occurrence of Proliferative Kidney Disease in British Columbia

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Introduction

Proliferative kidney disease (PKD) has been reported as an important cause of mortalities in cultured rainbow trout (Salmo gairdneri) as well as other salmonid species in several European countries (1). Mortalities associated with this disease have ranged from negligible to severe. In North America, this malady was detected for the first time in 1981 in Idaho (2). Since then PKD has been found in the State of California (3) and the Province of British Columbia, and more recently, in Washington State (R. Brunson, pers. comm.).

The first confirmed outbreak of PKD in British Columbia occurred in 1983 at the Puntledge River Salmon Hatchery in winter run steelhead trout (Salmo gairdneri) fry and has been found subsequently in salmonids in the Marble River, Robertson Creek, and Chehalis River hatcheries. The locations of these facilities are shown in Figure 1. The present report is intended as a short review of the disease outbreaks associated with PKD at each of these facilities.

Puntledge River Salmon Hatchery

This facility is located on the Puntledge River near Courtenay, Vancouver Island. It is actually a complex of 2 sites separated by approximately 9 km. Both sites utilize the river as the only water supply and are the only fish culture facilities within the Puntledge River drainage. As far as can be determined, no fish from other areas have ever been reared or released into the Puntledge system. Species reared include chinook (Oncorhynchus tshawytscha), coho (O. kisutch) and pink (O. gorbuscha) salmon, and cutthroat (S. clarkii) and steelhead trout.

The first diagnosed outbreak of PKD in 1983 involved approximately 45,000 steelhead fry distributed unequally among 7 covered aluminum Capilano style rearing troughs. Mortalities started in early July and continued until mid-September. Initially, losses were thought to be due to furunculosis but the causative agent (Aeromonas salmonicida) could only be isolated on Tryptic Soy Agar (TSA) medium, using routine laboratory procedures, from a few of the moribund or dead fish. Further, oral treatment of sulfamerazine started August 25 failed to significantly influence the mortality pattern.

Although PKD was suspected as the primary cause of the losses, a final diagnosis was not made until October when specimens were sent to Dr. H. Ferguson, University of Guelph, Guelph, Ontario for confirmation. At the time, the disease was confirmed in 3 of the 7 troughs. Disease signs included lethargic behavior, exophthalmos, pale gills, reduced growth, low hematocrit values, microcytosis and swollen posterior kidneys. The average hematocrit value for affected fry collected near the outlet screens was 15.7%. An average value of 12% was obtained from the most severely affected fish whereas those that were classified as unaffected because of their position near the inlet screen gave an average value of 35%.

Over the period in which the outbreak occurred losses in the most severely affected trough were approximately 9%. Mean water temperature, total weekly mortalities, and mean fish weight for this trough are given in Table 1. A summary of this initial encounter with the disease in British Columbia in relation to temperature profiles in the culture troughs is given in Table 2.

Histological hematoxylin-eosin stained sections of kidney tissue from affected fish showed marked cellular proliferation and numerous parasitic cells similar to those called "PKX" by Seagrave, Bucke and Alderman (4).

The disease recurred at Puntledge during peak summer water temperatures in 1984 and 1985. Species in which PKD has been diagnosed include coho salmon and cutthroat trout, in addition to the steelhead trout. Because of concurrent outbreaks of furunculosis at this facility, it has not been possible to determine accurately the losses caused by PKD. However, mortalities attributed solely to PKD are generally estimated at 10%. Perhaps more important is the acute infection by A. salmonicida which appears to be associated with debilitation of the fish by PKD.

To determine whether the steelhead from the affected stock at the Puntledge Hatchery would suffer PKD losses during a second summer of rearing, 5,000 fish from the 45,000 fry in which the disease was initially encountered in 1983 were held and monitored throughout the summer of 1984. Although a low level of furunculosis infection continued to occur in the stock, no PKD or PKX cells were found after October 1983 and the fish were successfully released in the fall of 1984 as apparently PKD-free smolts.

It is now clear to us that PKD occurred at the Puntledge River Hatchery prior to 1983. During a severe outbreak of furunculosis and saprolegniosis in coho fingerlings in the summer of 1982 the fish failed to respond to remedial measures and mortalities subsided only after the water temperatures decreased in September. The fish were being reared in large gravel raceways. A number of dead and dying sticklebacks were also noted in the raceway but no samples of this species were collected. Reexamination in

1983 of histological samples of coho collected during this outbreak has revealed the presence of PKX cells. This and other evidence suggests that the disease occurred at Puntledge in 1982 and 1981 but remained undiagnosed or masked by other fish health problems.

Robertson Creek Salmon Hatchery

PKD was diagnosed at this facility near Port Alberni, Vancouver Island in coho salmon and steelhead trout during the summer of 1984 and recurred in both species in 1985. M. Kent, University of California, Davis, California assisted with the diagnoses in 1984. Similarly as at Puntledge, outbreaks of furunculosis occurred at Robertson Creek prior to 1984 that failed to respond fully to chemotherapy. In at least some of these cases, failure of the therapy may have been due to a concurrent PKD infection.

The 1984 outbreak at Robertson Creek involved approximately 1.64 million coho fry in large serial, gravel raceways and 0.25 million steelhead fry in 6 parallel concrete raceways. PKX cells were first found in early June during a routine health check of the coho. Losses were negligible and no other infectious agents, including A. salmonicida, were detected. By July 4 the mortalities had increased to 417/day in the coho and the mean water temperature had reached 17°C. All fish in a sample of 30 moribund animals were heavily infected by the parasite. Disease signs encountered were primarily exophthalmos, pale gills, lethargic behavior, and swollen posterior kidneys. A low prevalence of furunculosis was also found in this sample. A sample of moribund fish collected July 11 gave the results summarized in Table 3.

Because of rapidly increasing losses and the large number of lethargic fish, corrective measures were started July 5. Oxytetracycline combined with sulfamerazine was orally administered and a coldwater system turned on.

Water for the Robertson Creek Hatchery is normally drawn from Robertson Creek which originates in Boot Lagoon, a small bay in nearby Great Central Lake. Since surface temperature in the Lagoon often exceeds 20°C by July 1, a system of pumping water from below the thermocline of the lake was installed several years ago. By using this system it was possible to maintain a mean water temperature below 15°C after July 10. Table 4 summarizes the course of the disease in the coho from June 27 to December 18.

Because of the susceptibility of steelhead trout at the Puntledge River Hatchery to PKD and furunculosis, the water supply to this species was also cooled. Unfortunately, we were unable to sample the steelhead until July 18 when 16.9% of 30 moribund fish were positive for PKX.

All findings given in Table 4 are based on the examination of imprints of posterior kidney tissue stained with the commercially available Diff-Quik[1] stain. Samples were declared positive if one or more typical PKX cells were found in a search of 50 microscope fields using 450X magnification. Figure 2 illustrates the morphology of cells considered to be typical PKX.

As shown in Table 4, a sample of 30 moribund and 30 randomly collected coho on July 4 had PKD infection rates of 100% and 93.3%,

[1]Diff-Quik stain is manufactured by Dade Diagnostics, Puerto Rico and distributed by American Scientific Products, U.S.A.

respectively. Following 50 days of exposure to the cold water, the organism could not be detected in coho samples collected on August 23 and 24. This suggests that PKD cannot sustain itself in Robertson Creek coho at water temperatures less than 15°C. Unfortunately, no control fish were held at ambient temperatures to follow the course of the disease at higher temperatures. However, these results can be compared to the course of the disease at Puntledge in 1983 where infected fish continued to be found throughout August and into September until the mean ambient temperature fell below 15°C.

On August 23, 1984, after the PKX organism could no longer be detected in the sample of moribund fish, a small lot of 8,000 fish, collected randomly from the main stock of 1.64 million, were moved to an aluminum Capilano style trough. The water supply to the trough was normal Robertson Creek water at 20°C. Sampling of the fish on August 27, September 13 and October 10, when the respective ambient mean water temperatures were 20, 18.5 and 15°C, failed to detect either A. salmonicida or PKX. Except for a low prevalence of saprolegniosis the fish remained normal, suffered no losses, and were returned to the main stock shortly after October 10.

The disease recurred at Robertson Creek in 1985 in both steelhead trout and coho salmon. The water temperature was again maintained below 15°C and the losses remained negligible. A summary of the losses for Robertson Creek Hatchery during June, July, August, September, and October, for both 1984 and 1985 are given in Table 5. Part of the apparent success of the use of the cold water in both 1984 and 1985 could be due to possible absence of the infective stage for PKD below the thermocline of Great Central Lake. Thus, infective stages would not be available to reinfect

fish which have recovered. The massive infection rate in salmonids may depend more on the seasonality of an unidentified host which sheds infective stages of the PKX organism in high numbers rather than on the water temperature.

Chehalis River Salmon Hatchery

This occurrence of PKD is important because it establishes the presence of the causative organism in the lower Fraser Valley. The Chehalis River is a tributary to the Harrison River approximately 6 km upstream of the Harrison River's confluence with the Fraser River. No movements of fish between the Chehalis facility and other PKD-positive sites are known to have taken place.

The Chehalis Hatchery began operation in 1981. Species reared are coho, chinook, and chum (Oncorhynchus keta) salmon as well as steelhead and cutthroat trout. Water for both egg incubation and rearing is obtained from deep wells and the river.

On September 3, 1984 we were contacted by the Hatchery's manager who reported low chronic losses, poor growth, and loss of appetite in 0.1 million coho fingerlings in a large concrete raceway.

In a random sample of 20 fish, 4 were found to be moribund with pale gills and swollen posterior kidneys. Kidney tissue imprints prepared from these 4 moribund fish, and stained with Diff-Quik, contained moderate numbers of PKX cells. No bacterial pathogens were isolated on TSA streaked with kidney tissue from all 20 fish and no signs of other infectious agents were noted. This has been the only occurrence of PKD in British Columbia that has not been associated with furunculosis.

Because the mean winter temperature was 15.5°C and decreasing, no remedial measures were carried out and no further samples collected. By September 15 the feeding response of the fish had returned to normal. To date, no further outbreaks of PKD have occurred at this facility.

The low prevalence of the disease at Chehalis could be due to lower water temperatures, or possibly to a combination of low temperatures and the lack of certain biological elements in the water supplies. The normal water temperatures at Chehalis are much cooler than at the Robertson Creek or Puntledge River facilities. In 1984, the mean temperature at Chehalis did not reach 15°C until July 22, it peaked at 18.6° on August 9, and again dropped below 15°C by August 23. Between August 23 and September 4 it fluctuated between 14.8° and 15.6°C.

Marble River Salmon Hatchery

During July 1985 the Marble River operation, located on the Marble River, which drains into Holberg Inlet, north Vancouver Island, was rearing 0.11 million 6 gram chinook salmon divided among 3 floating netpens in the river. On July 8, losses sharply increased and peaked at 631/day on July 13. A diagnosis of acute furunculosis was made and the fish were started on a 10 day treatment of orally administered Terramycin/sulfamerazine. On July 19, mortalities had declined to 59/day and the fish were released except for 2,000 retained for a further health check of the stock. On the following day, 12 fish in a random sample of 45 collected from the 2,000 were positive for PKX and one for furunculosis. The only remarkable disease signs found in this random sample were 3 fish with moderately swollen kidneys. Between July 1 and July 18 total mortalities were approximately 2,740 fish or 2.5%.

Table 6 gives the mortality data and maximum surface water temperature during this period for the most severely affected pen. On July 20, the remaining chinook were released.

A single coho fingerling collected from a cultured stock at Marble River showed the typical disease signs of PKD, and a Diff-Quik stained imprint of kidney tissue contained numerous PKX. No other samples were examined from this stock of coho.

Discussion

Since June 1983 outbreaks of PKD have occurred in four salmonid culture facilities in British Columbia. Although the causative organism may be found in coho fry in June, significant mortalities in affected coho have not occurred until July when water temperatures exceed a mean value of 15°C. Conversely, by mid-September, when water temperatures have declined to less than 15°C, the epizootics subside. In every case, except one, these outbreaks of PKD have been associated with furunculosis.

Although the mortalities at the Puntledge River Hatchery approached 10%, it is difficult to estimate the losses due only to PKD because of concurrent furunculosis. Smith et al. (2) encountered a similar problem in estimating the percent mortality in rainbow trout at the State Fish Hatchery, Hagerman, Idaho. Heavily infected Pacific salmon fry are severely debilitated and probably very sensitive to environmental stress as well as being highly susceptible to other infectious agents. Our observations indicate that PKD-debilitated salmonid stocks should not be handled or otherwise stressed; food must be carefully fed to avoid feeding

frenzies, or withdrawn completely; water temperatures should be maintained at less than 15°C, or as low as possible, and prophylactic measures to prevent other infectious agents from gaining a foothold in affected stocks should be considered. By careful management and avoidance of secondary infectious diseases, it is possible to prevent serious losses among PKD-infected salmonids.

Because of stocking practices at the facilities in British Columbia in which the disease has been encountered, we do not believe that the causative parasite has been recently introduced but rather it is naturally-occurring and has hitherto gone unrecognized, or has just occurred because of unidentified intrinsic factors. For example, PKD occurred at the Puntledge River Hatchery in 1982 but was masked by concurrent outbreaks of furunculosis and saprolegniosis. It is possible that similar, but undiagnosed, outbreaks of the disease have occurred at the Robertson Creek Hatchery prior to 1984.

Bodies resembling myxosporean trophozoites and pansporoblasts have been observed in the lumens of the kidney tubules of affected fish. Evidence has been published that suggests that these are maturing spores or stages of PKX cells and that they occur in recovering fish (5). Because of the massive tissue proliferation in the kidneys of infected salmonids and the apparent failure of the causative parasite to develop into a mature spore, it is probable that Pacific salmonids are blind or aberrant hosts. Possibly, a natural host in which infective stages are produced in great numbers exists in the water supplies of the affected hatcheries in British Columbia.

In general, our observations confirm the findings of other workers that indicate that recovered fish are not susceptible to reinfection. The

failure of recovered Puntledge River Hatchery steelhead trout to contract PKD in the second summer of culturing tends to support this indication of resistance to reinfection. Reinfection failed to occur in this case even though coho and steelhead fry in the same water supply contracted the disease. Further, moving recovered Robertson Creek Hatchery coho salmon into water at 20°C failed to precipitate a recurrence of the disease.

Infected wild fish were not detected in brief disease surveys of wild salmonids in the water supplies to the Chehalis River, Robertson Creek, and Puntledge River hatcheries. However, of 2.9 million coho released into the Puntledge River system in July 1984, 10% were found to be infected based on a sample of 300 collected 2 months later. In this case, it is highly likely that these fish were infected prior to their release.

Table 1. PKD associated losses in trough 7 containing approximately 18,250 steelhead fry at the Puntledge Hatchery in 1983.

Date	Weekly losses	Mean water temperature (°C)	Mean fish weight (grams)
July 25-31	66	17°	1.8
Aug. 1-7	327	17.7	2.2
Aug. 8-14	396	18.7	2.5
Aug. 15-21	273	18.3	2.3
Aug. 22-28	271	18.8	2.6
Aug. 29-Sept. 4	211	17.9	3.3
Sept. 5-11	117	17.1	3.8
Sept. 12-18	101	16.3	3.9
Sept. 19-25	40	15.6	4.3

Total loss - 1,802

Table 2. Temperature profile and course of disease during the PKD outbreak in steelhead trout at the Puntledge Hatchery in 1983.

Date	Water Temperature °C			Comments
	High	Low	Mean	
July 17	16	14	15	Mean water temperature reached 15° for the first time.
July 31	18	16.3	17.1	Start of high losses.
Aug. 3	-	-	-	First signs of PKD detected in moribund fry.
Aug. 12	20	18.2	19.1	Maximum temperature, peak losses
Aug. 25	-	-	-	Furunculosis diagnosed; oral chemotherapy started.
Sept. 17	16	15.5	15.75	Losses returned to normal level.
Sept. 28	14.5	12.5	13.5	Last PKD-infected fish found. Samples sent to H. Ferguson for confirmation of PKD.
Oct. 3				
Nov. 1	10.5	10.5	10.5	No PKD-infected fish found. No signs of PKD.

Table 3. Number of furunculosis and PKD positive fish in a sample of 30 moribund coho fry collected July 11, 1984 at the Robertson Creek Hatchery.

Disease	No. infected
PKD	16
Furunculosis	7
PKD + furunculosis	7
Non-infected	0

Table 4. Summary of the PKD outbreak in 1.64 million coho fry at the Robertson Creek Hatchery in 1984.

Date	Mean Temp. °C	Approx. losses/day	No. of fish sampled		% PKD positive		Remarks
			Moribund	Random	Moribund	Random	
June 27	16.5	169	12	0	100	-	No furunculosis.
July 4	17.7	417	30	30	100	93.3	Coldwater pumps on.
July 11	14.0	400	30	0	76.7	-	Furunculosis diagnosed, see Table 3.
July 18	14.0	655	22	0	68.2	-	
July 24	14.5	150	8	10	37.5	70	
July 27	14.5	186	12	0	50	-	Increased coldwater volume.
Aug. 2	12.6	269	30	0	46.7	-	Furunculosis present.
Aug. 9	13.8	208	18	0	11.1	-	
Aug. 16	12.4	61	17	0	11.8	-	
Aug. 23	13.5	218	30	0	0	-	
Aug. 24	13.6	107	29	40	0	0	
Aug. 27	13.3	298	10	60	0	0	Furunculosis present.

Table 4 (cont'd).

Date	Mean Temp. °C	Approx. losses/day	No. of fish sampled		% PKD positive		Remarks
			Moribund	Random	Moribund	Random	
Sept. 12	12.5	278	16	60	0	0	
Sept. 11	13.2	115	15	0	0	-	
Sept. 12	13.0	210	5	0	0	-	
Sept. 18	E	133	20	60	0	2	No furunculosis.
Nov. 15	6.7	42	7	60	E	E	
Dec. 18	3.5	44	11	60	0	0	

E - data missing or samples not processed.

Table 5. Stock size and monthly mortalities associated with PKD and furunculosis at the Robertson Creek Salmon Hatchery during the summer of 1984 and 1985.

Species	Year	Stock Size (millions)	Monthly Mortalities					Approx. Total % loss
			June	July	Aug.	Sept.	Oct.	
Coho	1984	1.64	7,830	7,638	5,118	6,263	5,050	2
	1985	1.5	5,274	8,561	7,729	13,921[1]	3,177	2.5
Steelhead	1984	0.22	7,993	709	1,336	4,684	3,982	8.3
	1985	0.18	14,788[2]	693	1,056	178	207	9.4

[1]Large numbers of ducks in raceways for a short period.

[2]Losses associated with egg and alevin incubation problems.

Table 6. Maximum water temperatures and mortalities associated with PKD in 39,000 chinook salmon in floating netpens in the Marble River.

	Mortalities	Water Temperature °C
July 1	14	14.5
2	10	15.0
3	13	16.0
4	26	16.5
5	9	16.5
6	11	17.0
7	10	16.5
8	42	15.0
9	86	20.0
10	180	19.0
11	202	16.6
12	345	18.0
13[1]	443	18.0
14	251	18.0
15	148	18.0
16	94	18.0
17	31	18.0
18	30	18.0

[1]Ten day regimen of orally administered Terramycin/sulfamerazine started.

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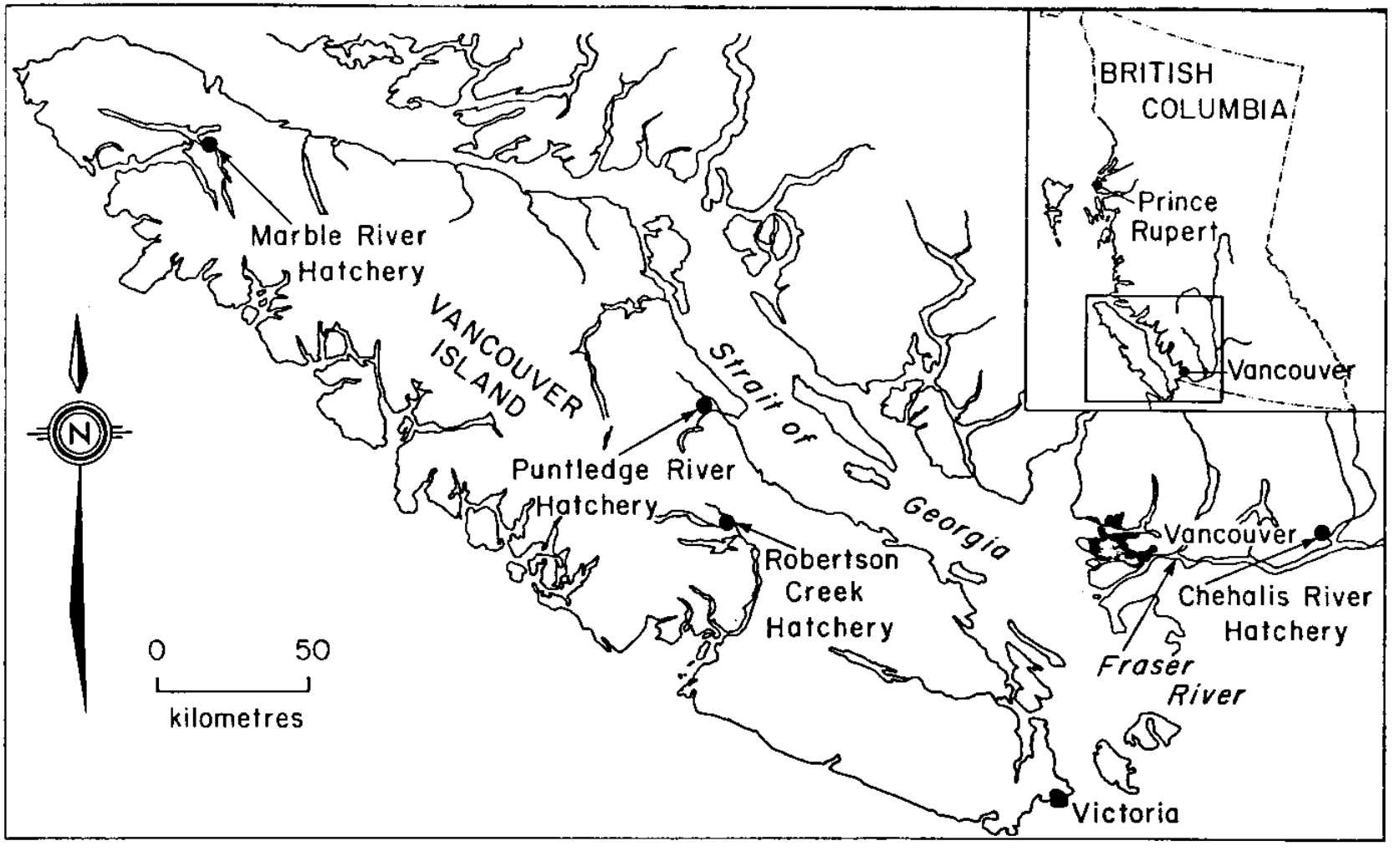

Figure 1. Map of British Columbia showing the general location of the hatcheries in which PKD has been encountered.

Figure 2. A Diff-Quik stained kidney tissue imprint containing a single PKX cell.

Proliferative Kidney Disease (PKD) among Salmonid Fish in California

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Introduction

Proliferative kidney disease (PKD) has been recognized as a major problem among farm-reared trout in Europe for years but only recently has it been detected in N. America (Smith et al, 1984). Of major concern is the appearance of the disease among Pacific salmon in California (Hedrick et al, 1984), British Columbia (Hoskins and Kieser, this issue) and most recently in the state of Washington. Under certain conditions, mortality due to PKD can be substantial (Clifton-Hadley et al, 1984). The associated anemia (Hoffman and Lommel, 1984) predisposes the fish to environmental stress and secondary infectious diseases. For anadromous species, the effects of PKD may be even more pronounced because of the physiological demands on the kidney needed to adapt to life in seawater.

In California, our increased awareness of PKD has led to identification of infected fish at nine locations, involving three species of salmonids. The purpose of this paper is to provide information on the occurrences of PKD among salmonid fish in California, stressing the epizootiological aspects of the disease.

Historical Perspective

The first report of PKD in California (and the Pacific coast) came in June 1983 upon examination of an unexplained mortality among king salmon (Oncorhynchus tshawytscha) at the Mad River Hatchery (Hedrick et al, 1984). Although this was believed to be the first occurrence of the disease in California, a retrospective analysis of a syndrome known since 1958 as 'lupus' showed that identity was PKD (Hedrick et al, 1985). ' Lupus' has been reported at certain hatcheries since their construction and its seasonality and pathology are identical to that of PKD. Furthermore, preserved kidney tissues from rainbow trout (Salmo gairdneri) at American River Hatchery in 1966 with 'lupus' show the typical PKX protozoans, the causative agents of PKD. Therefore PKD has been present in California for many years, perhaps prior to construction of salmon and trout hatcheries at certain of these sites.

Geographical and Host Ranges

The locations were PKD has been detected in salmonid fish in California are shown in Figure 1. The first reported outbreak of PKD was in June 1983 at the Mad River Hatchery (Table 1). This hatchery utilizes recirculated water derived from wells near the river. King salmon were first affected and mortalities began in June, followed shortly by of steelhead trout (S. gairdneri) and coho salmon (O. kisutch).

King salmon showed the greatest mortality (cumulative 95%) while the coho and steelhead were less affected (Hedrick et al, 1984). No mortality or evidence of infection was observed among coho salmon reared in ultraviolet-

treated (UV) water. The hatchery was depopulated and disinfected in the fall of 1983 and the disease has not recurred to date.

Annual losses of king salmon at the Nimbus Hatchery and rainbow trout at the American River Hatchery have been attributed in part to PKD. The increases in mortalities correspond with higher water temperatures in the summer and early fall. Because of this problem, king salmon are now released in the delta prior to onset of disease signs. Both hatcheries utilize water from a reservoir (Lake Netoma) and our studies of this water source have shown the seasonal nature of the infective stage of PKD.

Two additional sites with PKD affected salmonids were discovered in 1984. These were both river fed hatcheries near Merced (Table 1). In the state hatchery, king salmon were involved and at the private farm rainbow trout. The disease followed a pattern similar to that at the American and Nimbus Hatcheries with peak infections in the late summer and early fall. The infection at both Merced sites was complicated by bacterial gill problems. Proliferative Kidney disease was initially found in 1984 and recurred in 1985 at these sites. The Feather River Hatchery was also found to have infected king salmon in 1984 and 1985 but at lower levels than at other sites. Steelhead at this location in 1984 were found to be infected with both Ceratomyxa shasta and PKX. Infected king salmon were also detected in the Tehama-Colusa rearing facility in the Sacramento River Drainage in October 1985.

A recent location where PKD has occurred offers a unique opportunity to study the source of the infective stage. This site is the Hot Creek Hatchery in the Sierra-Nevada range (Figure 1). The hatchery water supplies are springs located 100-200 m from the production ponds where PKD was detected in

rainbow trout in March of 1985. The water runs year round at 15C and contains a few escaped salmonids (rainbow and brown trout, Salmo trutta) and some native non-salmonids. An abundant cyprinid, the tui chub (Gila bicolor), was found to contain a parasite in the lumens of the kidney tubules that resembles the later stages of PKX.

Studies on the relationship of this parasite to PKX are in progress in the laboratory and the similarities of this myxosporean (Sphaerospora) are discussed in the contribution to this volume by Kent and Hedrick. In addition, other possible sources of the infective stage are under examination.

Seasonality of the Disease

The seasonal nature of the disease has been well established from epizootiological studies in areas enzootic for PKD (Clifton-Hadley et al, 1984). Unknown however, is whether this is dependent on a true seasonality of the infective stage or solely water temperatures or, stocking schedules of fish.

To distinguish between these alternatives, we began introducing groups of 20-30 rainbow trout to the water supply at the American River Hatchery each month of the year. After a 4 wk exposure the fish were transferred to the UC Davis Fish Pathology Laboratory for an additional 4 wk rearing in 15C pathogen-free water. The fish were then sacrificed and examined for pathology and the PKX parasite. The results of an entire years study are shown in Table 2. To further examine the effect of temperature at the time of exposure during the winter months (when the ambient temperature dropped from 15-17C to 7-9C) an additional group of fish was exposed to water heated to 17C. This study clearly showed that infective stage was prevalent between May and

October. During the remainder of the year it disappeared and fish did not become infected regardless of water temperature at time of exposure. Water temperatures may however influence the emergence of the infective stage from an as yet unknown host (perhaps a nonsalmonid) or cause spores in the environment to become infectious (see Kent and Hedrick, this issue).

Immunity to PKD

Resistance among survivors of previous epizootics to second infections of PKD has been noted by several investigators (Ferguson and Ball, 1979; Hoskins and Kieser, this issue). None of these studies however, have utilized controls that demonstrate the effects that size and age might have on susceptibility to the disease. We examined the resistance of survivors of the 1984 outbreak at American River Hatchery by re-exposing these fish and a parallel group of rainbow trout from the same stock, size and age reared at a hatchery with no history of PKD. The yearlings not previously infected with PKD readily contracted the disease while survivors showed a complete resistance to infection (Table 3).

It was also determined from examinations of fish in production ponds at American River Hatchery that exposure alone is not sufficient to develop immunity but that recovery from active infection is necessary. This was demonstrated by fish introduced to the hatchery in October. These fish probably received a light exposure to the parasite, since our group exposure experiments showed that the infective stage was still present although diminishing (Table 2). However, these fish did not show PKD infection until the following June (Table 4) even though they were examined monthly throughout the year. These data support the hypothesis that PKX stimulates an immune

response in infected fish that may aid in convalescence and in providing a strong resistance to reinfection. The nature of this resistance is yet to be determined but probably involves both a humoral (Olesen and Jorgensen, 1985; Klontz et al. 1985) and cellular response.

Control of PKD

Infections with PKX have as yet to be controlled by any tested chemotherapeutics (Bucke et al 1981) and in this respect represent the same problem as other myxosporidans and viral pathogens. Avoidance of contact between the pathogen has been the most successful method to ameliorate the effects of PKD on hatchery-reared salmonids. Ferguson and Ball (1979) first proposed a stocking stategy that moved fish into waters enzootic for PKD during the fall when neither water temperature or perhaps abundance of the infective stage were conducive to manifestation of the disease. The fish then show a 'manageable' involvement the following year although some infection and mortality occurs.

The use of colder water during expected times of peak involvement have also been employed when possible (Hoskins and Kieser, this issue). Although this may not prevent infection, it does seem to minimize the effects of the disease by improving complications due to both environmental and secondary pathogens.

Our group experiments have shown that fish exposed at ambient water temperatures of 9C in the spring develop PKD when transferred to 15C in

laboratory. In addition, reports from Europe by Rafferty et al (1985) have shown that infection and disease can proceed steadily although slowly at temperatures down to 7C.

In contrast, Clifton-Hadley (1985) and Ferguson and Ball (1979) found the disease to be poorly manifested or absent in fish at 12 and 9C respectively. Clearly, more definitive studies on the role of temperature on initial infection and development of full clinical disease are needed.

Disinfection of the water supply to remove the infective stage of the parasite has shown some promise. Observations made at Mad River in 1983 indicated the potential efficacy of ultraviolet (UV) treatment of the water to prevent PKD. Coho salmon receiving UV treated water were apparently completely protected while other groups of coho, steelhead and chinook salmon without treated water contracted the disease (Hedrick et al, 1984). We are conducting additional studies to determine the potential of this form of water treatment. This has been shown to be effective in controlling other myxosporean pathogens such as Myxobolus cerebralis (Hoffman, 1985) and C. shasta (Sanders et al 1982).

Another strategy that might be used is the development of resistant strains of trout for rearing in waters enzootic for PKD. The marked difference between severity of PKD in steelhead trout at the Nimbus Hatchery and groups of rainbow trout introduced at American River Hatchery is noteworthy. Both hatcheries share the same water supply from the Netoma reservoir but PKD is a much more severe problem among the rainbow trout brought in from outside broodstocks than the hatchery stocks of steelhead reared year after year at Nimbus. It is possible that the co-existance of the pathogen and steelhead stocks has resulted in development of resistance in

these populations. This might be exploited in a genetic program aimed at developing PKD resistance.

Further studies on more recently developed anti-protozoan drugs and a better understanding of the life cycle of the parasite are being pursued in our laboratory as short and long term programs aimed at controlling the disease. In the meantime, management procedures aimed at minimizing the potential spread of the disease through movement of actively infected fish should be employed. The salmonid may be an aberant host in which PKX fails to develop and therefore, unable to transmit the parasite. However, if the appropriate host is infected by contact with the parasite which may be transported with present in the water used to transport by a cycle of transmission of PKX might become established.

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Table 1 - Recorded outbreaks of proliferative kidney disease (PKD) in California, USA

Date	Location	Species	Water Temperature
June 1983	Mad River	Oncorhynchus tshawytscha O. kisutch Salmo gairdneri *	13C
June 1984	American River	S. gairdneri	16C
June 1984	Nimbus	O. tshawytscha S. gairdneri *	16C
June 1984	Merced	O. tshawytscha	15C
July 1984	Private Farm at Snelling	S. gairdneri	15C
August 1984	Feather River	O. tshawytscha S. gairdneri *	15C
March 1985	Hot Creek	S. gairdneri	15C
October 1985	Tehama Colusa Canal	O. tshawytscha	17C

* Steelhead trout

Table 2 - Prevalence of proliferative kidney disease (PKD) among rainbow trout (Salmo gairdneri) introduced monthly into water enzootic for the disease.

Group No.	Date Exposure Starts*	% PKX	% L	% Pathology	Water Temp(^{O}C)
1	8-06-84	65	59	65	19
2	9-06-84	16	33	25	19
3	10-17-84	30	30	18	17
4	11-17-84	0(0)	0(0)	0(0)	13
5	12-17-84	0(0)	0(0)	0(0)	11
6	1-17-85	0(0)	0(0)	0(0)	7
7	2-17-85	0(0)	0(0)	0(0)	7
8	3-15-85	0(0)	0(0)	0(0)	10
9	4-17-85	37(33)	25(0)	23(33)	12
10	5-15-85	81	33	68	13
11	6-17-85	97	38	88	15
12	7-16-85	73	27	57	17

* Groups of 20-30 PKD-free rainbow trout introduced each month to American River Hatchery, exposed for 4 weeks, transferred to FPL, incubated 4 weeks (at 16^{O}C), then examined for PKD, PKX and intraluminal stanges (L), by histological methods.

+ Parallel group of fish exposed at the hatchery but in water heated to 17^{O}C.

Table 3 - Incidence of PKD and PKX following exposure of previously infected and uninfected rainbow trout[+] to water containing the infective stage.

	Prevously Infected*	Previously Uninfected
PKD	0/30[A]	32/46 (69.6%)
PKX	0/30	44/46 (95.7%)

[+]Both groups of fish were 17 months of age and the average weights were 180 and 201g for previously infected and uninfected respectively.

[*]At the peak of infection in the previous year, the level of infection with PKX was 83%.

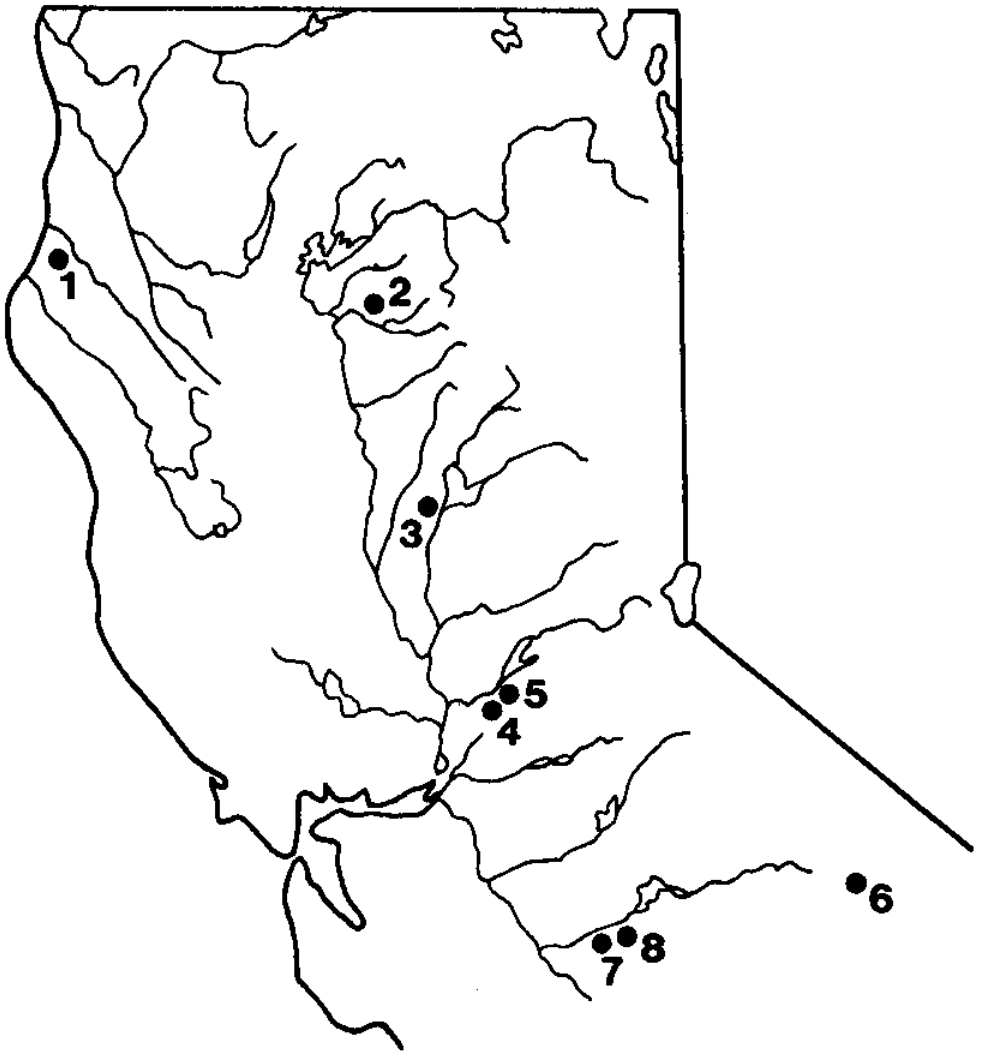
[A]Fish were exposed for 2 months at American River Hatchery prior to sampling.

Table 4

First detection and subsequent disappearance of PKX in selected groups of fish introduced to American River Hatchery

Species	Date Introduced	Appearance (^{O}C)	Disappearance (^{O}C)
RbT	12-83	6-84 (14)	9-84 (20)
RbT	4-84 (hatched)	6-84 (14)	10-84 (19)
RbT	8-84	9-84 (20)	12-84 (11)
RbT	10-84	6-85 (14)	12-85 (11)
RbT	12-84	6-85 (14)	12-85 (11)

SITES IN CALIFORNIA WHERE PKD HAS BEEN DETECTED IN SALMONID FISH



No.	LOCATION
1	Mad River Hatchery
2	Tehama Colusa Canal
3	Feather River Hatchery
4	American River Hatchery
5	Nimbus Hatchery
6	Hot Creek Hatchery
7	Private Farm
8	Merced Hatchery

Figure 1. Sites in California where proliferative kidney disease (PKD) has been detected in salmonid fish.

Affinites of PKX with the phylum Myxozoa

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Introduction

The taxonomic status of PKX has been controversial. Plehn (1924) described a disease in salmonids that she believed was caused by an amoeba in the kidney which was most likely PKD, and because Ghittino et al. (1977) and Ferguson and Needham (1978) observed PKX to form pseudopodia, they also considered it an amoeba. Although no spores were observed, Seagrave et al. (1980) proposed that the organism might be a haplosporidan (phylum Aceto-spora). Division by internal clevage, the prescence of multivesicular bodies and "haplosporosomes" were cited as common features with PKX. Myxosporean trophozoites and developing spores have been observed in the kidney tubules of fish recovering from PKD (Hedrick et al. 1984; Kent and Hedrick 1985a,b) and presented here is evidence that they are later stages of PKX. Therefore, PKX belongs most appropriately in the phylum Myxozoa.

Sequential Development

Paraffin Sections. A clear sequential development from typical PKX in the renal interstitium to developing spores in the kidney tubules was observed in PKX infected rainbow trout Salmo gairdneri by Kent (1985) and Kent and Hedrick (1985a). As the disease progressed, PKX parasites migrated to the epithelium of the tubule and some released their internal daughter cells into the lumen (Figure 1). These continued to develop and formed as many as six

internal daughter cells, representing the sporoblast, within an enveloping cell (Figure 2). This is typical of myxosporean sporogenesis, with two cells forming the valves, two cells representing the capsulogenic cells and two forming the sporoplasm (Mitchell 1977). However, no large multinucleated "plasmodia", typical of most myxosporeans, were observed and the enveloping cell is analagous to the "pseudoplasmodium" described for Sphaerospora (Lom et al. 1982). Two spherical polar capsules approximately 2 um in diameter were frequently observed in developing spores (Figure 3), and they are quite prominent when stained with Giemsa (Figure 4). The presence of polar capsules is an important criterion for placement in the phylum Myxozoa (Lom and Noble 1984).

Although polar capsules were frequently seen in the PKX spores, clearly defined valves has not been observed. Additionally, the spores remained within the pseudoplasmodium and quickly disintegrated when removed from the fish. These features indicate that the spore does not complete its development in the salmonid species that we have studied.

Electron Microscopy. A similar developmental sequence was observed by electron microscopy. Figure 5 shows a PKX parasite with two daughter cells in the epithelium of the tubule. The daughter cells are released into the lumen, and represent the simplest intraluminal sporongonic stage observed (Figure 6). The daughter cells in PKX (Figure 5) are remarkably similar to the early intraluminal forms (Figure 6). Haplosporosomes are only observed in the primary cell of typical PKX (Ferguson and Needham 1978; Seagrave et al. 1980; Smith et al. 1984; Kent and Hedrick 1985a), which would account for their absence in the intraluminal stages.

As observed by light microscopy, the intraluminal forms developed into immature spores. Five sporoblastic cells were observed in one parasite (Figure 7) and the most mature spores seen contained polar capsules with coiled filaments within capsulogenic cells (Figure 8). However, valvogenic cell differentiation was not detected.

Wet Mounts. Intraluminal stages of PKX are readily visible in wet mount (squash) preparations. They are characterized by prominent refractile granules in the enveloping cell of the pseudoplasmodium, and often contain two developing polar capsules within the monosporous sporoblast (Figures 9,10).

Temporal Prevalence. The prevalence of typical PKX, the associated interstitial nephritis, and the intraluminal myxosporean forms were followed in a group of rainbow trout introduced at the American River Hatchery on 16 August 1984. One PKX parasite was detected in one of 30 fish 3 wk after introduction, and numerous organisms were observed in several fish the following week (Figure 11). The intraluminal forms were detected at 7 wk. Although they persisted for several months after typical PKX and the associated interstitial nephritis subsided, the spores remained in an immature state. This study demonstrates that fish remain infected with the PKX organism, in its intraluminal rather than its interstitial form, much longer than previously reported.

Epizootiology. Epizootiological data support the hypothesis that the intraluminal myxosporeans are later stages of PKX. The myxosporean spores

described here have been detected in fish from all the PKD enzootic areas that we have studied in California. We have also observed them in PKD infected fish from Vancouver Island, B.C., Canada and in exposed trout from Idaho and Washington. Similar forms have also been observed in brown trout Salmo trutta, Atlantic salmon S. salar and rarely, rainbow trout in Europe (R.S. Clifton-Hadley, Ministry of Aquaculture, Fisheries and Food, Weymouth, England, pers. comm.). In the "group exposure study" (Hedrick et al. this issue), the intraluminal myxosporeans were found in all the groups that became infected with typical PKX. They were not detected in fish exposed during the winter months when the infectious stage of PKX was not present at the American River Hatchery.

Transmission. To further elucidate the affinities of typical PKX with the intraluminal myxosporeans, uninfected fish were injected with blood and spleen of PKX-infected fish (Kent and Hedrick 1985b). The myxosporean forms have been found only in the kidney tubules and never in the blood or spleen where typical PKX is often observed. Uninfected fish which were injected with either spleen or blood became infected with the intraluminal myxosporeans as well as typical PKX. This provides strong evidence that both forms belong to the same organism.

Similarities of PKX to Known Myxosporeans. Many similarities exist between PKX and a protozoan of carp blood, which was described as a possible early myxosporean stage by Bucsek and Csaba (1981). Lom et al. (1983) reported that this parasite, designated UBO or Csaba's blood protozoan, may be an early stage of Sphaerospora renicola, and a similar protozoan of the carp's

swimmbladder was shown to be an early stage of S. angulata (Molnar 1984). Both parasites form endogenous daughter cells which are released and subsequently sporulate in lumens of the kidney tubules. A similar pattern of development occurs with PKX, and Csaba et al. (1984) reported that the carp swimmbladder parasite resembles PKX.

The development of the intraluminal forms of the PKX myxosporean is similar to the sporogonic stages of Sphaerospora spp. The outer enveloping cell and the internal sporoblast of the intraluminal PKX, which most likely arises from the secondary cell of typical PKX, is equivalent to the pseudoplasmodium of Sphaerospora described by Lom et al. (1982). The pseudoplasmodium of Sphaerospora is uninucleate, but this stage of PKX often contained up to three nuclei. The PKX myxosporean is monosporous as was reported for S. molnari (Lom et al. 1983). Sporogensis of the PKX myxosporean was frequently observed. However, trophozoites were more numerous than spores even late in the infection, as Bond (1938) reported for S. renicola in the killifish Fundulus heteroclitus.

A comparison of immature spores of Sphaerospora to PKX shows similar features such as an elongated shape, small polar capsules and indistinct valves. In contrast, the mature spores of Sphaerospora have a spherical shape, prominent valves and larger polar capsules. Ferguson (1984) reported Sphaerospora in the kidney of brown trout from PKD enzootic waters, and Lom et al. (1985) observed pseudoplasmodia without spores in brown trout and grayling Thymallus thymallus in Czechoslovakia. These fishes are known hosts for PKX (Seagrave et al. 1981) and the pseudoplasmodia were possibly the intraluminal stages of PKX.

Although the PKX myxosporean shows similarities to Sphaerospora, its spores also resemble those of Parvicapsula spp. This myxosporean is also elongated, has two small polar casules and one species sporulates in the epithelium of the kidney tubules of coho salmon Oncorhynchus kisutch (Hoffman 1981; Jonhstone 1984). Parvicasula, in contrast to PKX, develops in the usual myxosporean manner with large plasmodia and pansporoblasts, but it may also form pseudoplasmodia (J. Lom pers. comm; Johnstone 1984).

Spore morphology has been the most important criterion for species descriptions in the Myxozoa (Mitchell 1977), but the origin of sporoblasts (either within true plasmodia or pseudoplasmodia) should also be included in these descriptions (Lom et al. 1982, Lom and Noble 1984). Identification of the primary host and completely formed spores are needed to determine the precise taxonomic status of the PKX myxosporean.

Host-Parasite Relationships. Seagrave et al. (1980) proposed that salmonids may be abnormal hosts for PKX, which would account for the severe inflammatory response and lack of complete spore development. It is well documented that immunosuppressed higher vertebrates can be abnormal hosts for parasites of many taxa, and under this condition infections by their own parasites will intensify. Since inhibition of inflammation may allow parasites to complete their development in abnormal hosts and salmonids may be abnormal hosts for PKX, fish were treated with cortisol implants to test the effects of immunosuppression on the development of the parasite and inflammation that is commonly associated with PKD.

Treated fish were immunosuppressed as indicated by high plasma cortisol levels, chronic mortality due to opportunistic pathogens and depressed

leucocrites when compared to PKX infected fish which had not received cortisol implants (Kent 1985). The fish treated with cortisol had twenty times the density of PKX, but showed less interstitial hypercellularity. At 4 wk post injection (8 wk post-exposure to PKX), all of the fish treated with cortisol that were exposed to PKX exhibited the interstitial and intraluminal forms, while no intraluminal forms were detected in the non-treated fish. Although suppression of the inflammatory response allowed more parasites to reach the sporogonic stage, the spores developed no further than those seen in natural epizootics. This adds additional support to the hypothesis that salmonids are abnormal hosts for PKX.

Because salmonids may not be primary hosts for PKX and complete sporulation may occur in other fishes, we have initiated surveys of feral non-salmonids from PKX enzootic waters. Spores that are remarkably similar to the PKX myxosporean were observed in the lumens of the kidney tubules of tui chub Gila bicolor from the Hot Creek Hatchery water supply, and developed Sphaerospora spores were later detected in these fish (Hedrick et al. 1986). Further studies are now underway to determine the association of PKX with the myxosporeans from tui chubs.

Summary

Experimental and observational data provided strong evidence that typical PKX is an early stage of a myxosporean that sporulates in the lumens kidney tubules. However, salmonids may not by primary hosts, and the reservoir host and complete spores must be identified to determine the source of infection and precise taxonomic status of PKX.

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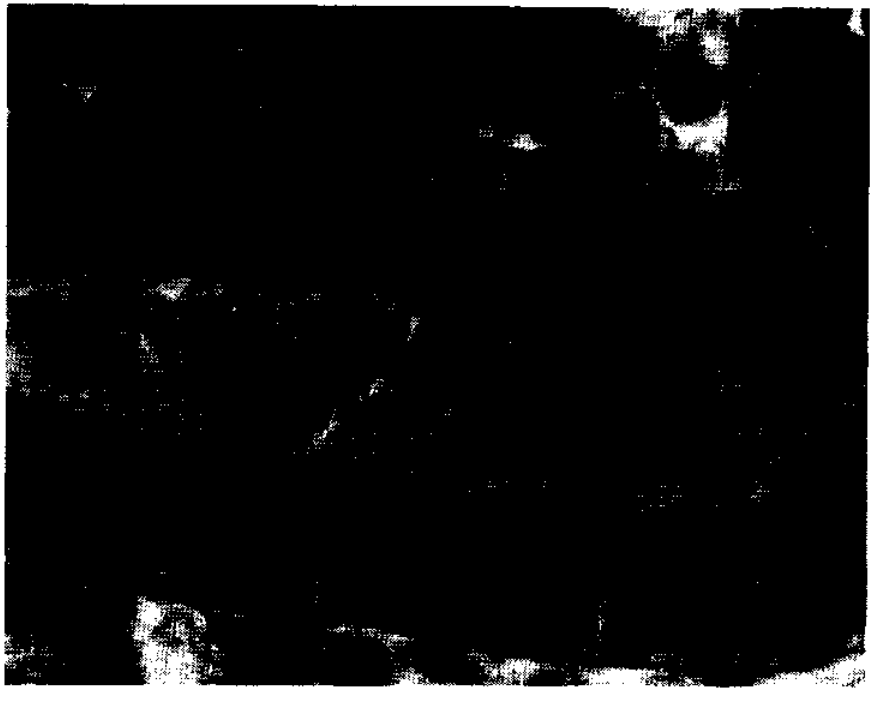
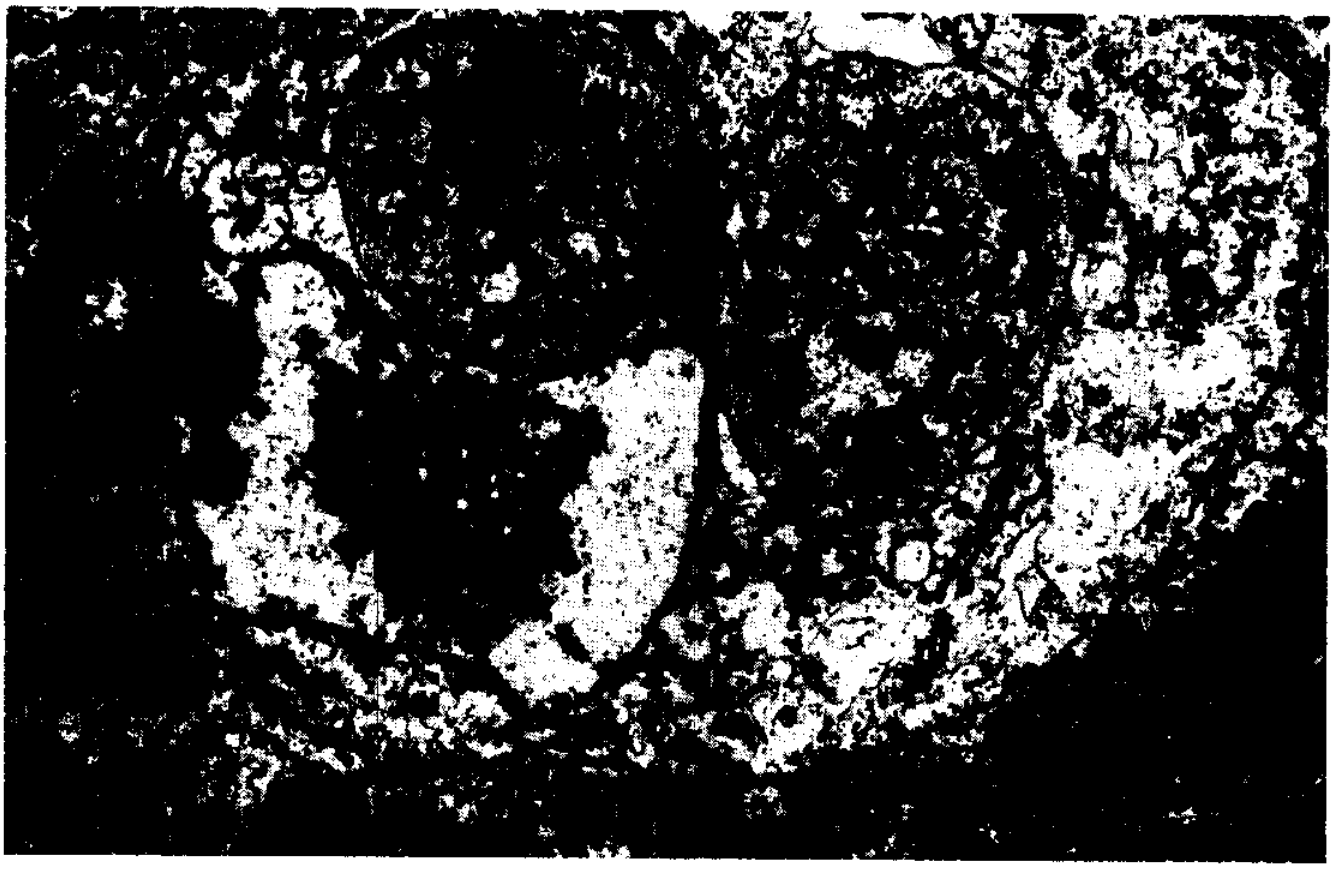
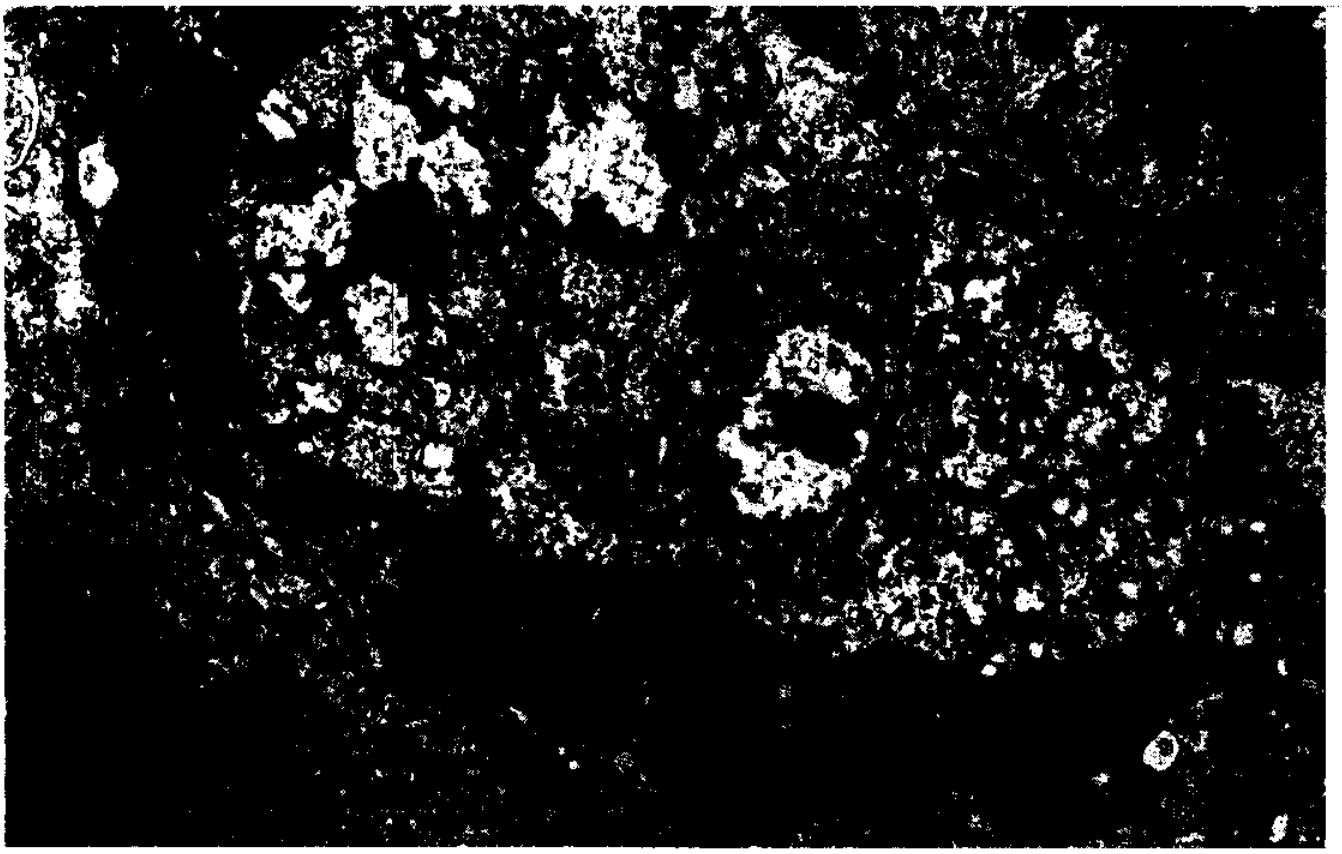
Figures 1-8. Paraffin sections (1-4, bar=10 um) and electron micrographs (5-8, bar=2 um) of PKX in the kidney of rainbow trout Salmo gairdneri. 1. PKX in the epithelium of a tubule releasing a daughter cell (S), hematoxylin and eosin (HE). 2. Intraluminal sporoblasts, HE. 3. Intraluminal spore with two polar capsules (arrow), HE. 4. Intraluminal spore with polar capsules (arrow), Giemsa. 5. PKX with daughter cells (S) in the epithelium of a tubule. 6. Released daughter cells, equivalent to the earliest intraluminal stage. 7. Intraluminal pseudoplasmodium with five sporogonic cells surrounded by an enveloping cell (E). 8. Developing intraluminal spore with two polar capsules (P).





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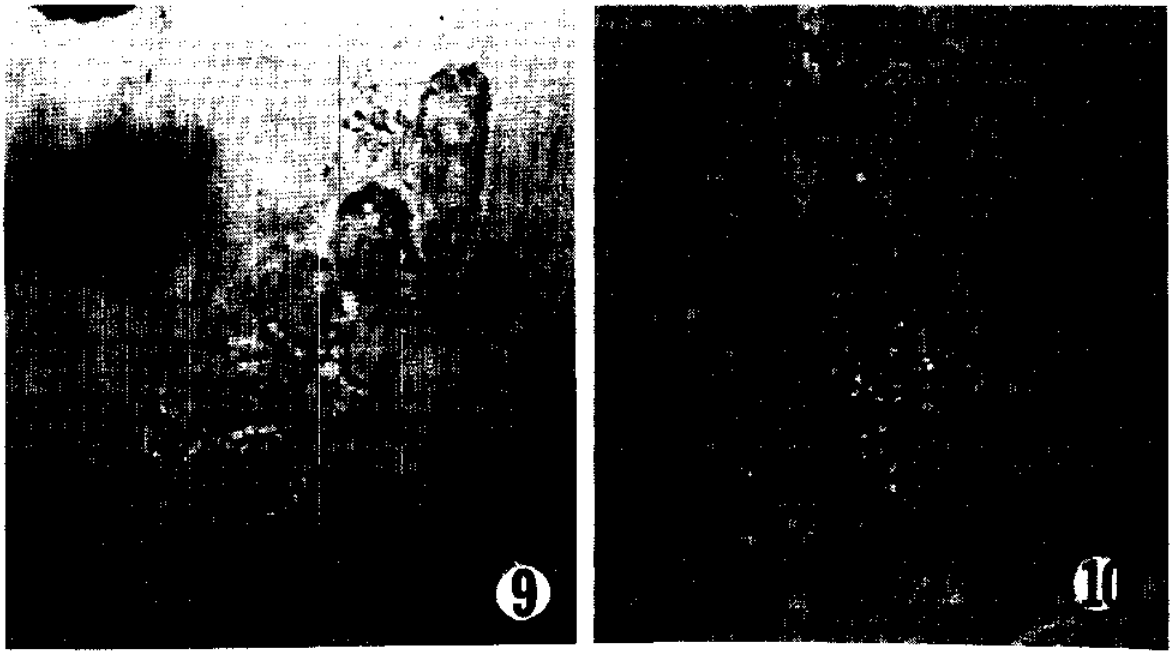

Figures 9,10. Wet mounts of intraluminal PKX pseudoplasmodia with refractile granules (R) and developing polar capsules (pc) in the lumen of kidney tubules in a rainbow trout Salmo gairdneri. Bar=10 um.

Diagnosis of Proliferative Kidney Disease (PKD), Caused by the Protozoan PKX

1. History and Environmental Conditions

In any diagnosis of a pathogen, a history of the fish stock and the geographical location should be considered. Nearly all locations where salmonids have contracted PKD have had annually recurring episodes, often with a marked seasonality. As with myxosporeans such as Ceratomyxa shasta and Myxobolus cerebralis, presence of the infective stage of PKD in a watershed can be established by exposures of sentinel fish.

The seasonal nature of the disease may also be useful in the diagnosis of PKD. The disease occurs most frequently at temperatures of 60F (15.6C) or above and this usually is from mid-summer through early fall (June to October).

The disease is most often seen at hatcheries that utilize impounded river water and seldom at spring-fed sites. It occurs primarily in under yearling (0+) trout and salmon. Information on the stocks, sites and environmental conditions should therefore be considered by the fish health specialist when making a diagnostic of PKD.

2. External signs of PKD

The signs shown by affected fish include, darkening in body color, distended abdomen due to ascites, pale gills indicating anemia, pronounced lateral body swelling and bilateral exophthalmia. These signs are unfortunately similar to those caused by other chronic diseases that impair

kidney function and should not be relied upon even in a presumptive diagnosis of PKD. Under certain conditions (e.g. early infections) no external signs may be observable.

3. Internal signs of PKD

Enlargement of the kidney and spleen are the most noteable internal signs. The kidney may be greyish throughout or mottled and is markedly swollen (sometimes more pronounced posteriorly). In severe cases the capsule may have a folded or corragated appearance. Ascites, when present, is usually clear.

These signs can be confused with those of Renibacterum salmoniarum that causes bacterial kidney disease (BKD) and Ichthyophonus hoferi and other hypertrophies of the kidney. Microscopic studies including histology, however, can readily distinguish PKD from the other conditons.

4. Microscopic Examination

a. Light microscopy

Wet mounts made from affected kidney tissues and examined by brightfield or phase microscopy can be utilized for the diagnosis of PKD. However, adequate numbers of PKX and experience is required to distinguish trophozoites of PKX from host macrophages, particularly when the latter are laden with cellular debris.

Imprints of affected kidney tissues stained with Giemsa or Leishman-Giemsa, provide a useful tool for detection of PKX. However, as with wet mounts, macrophages can be confused with PKX, particularly when the

staining between cytoplasm and nuclear material is not differential. With both imprints and wet mounts, the attachment of macrophages to PKX aids in distinguishing the parasite from other cells present in the intersitium of the kidney. It should be stressed that fresh kidney tissue is needed because autolytic changes (such as extensive vacuolation) in the host cells can interfere or confuse the diagnosis of PKX.

b. Histology

Observation of the PKX parasite in histological section is currently the method of definitive diagnosis of PKD. Sections from affected kidney tissues provide an opportunity to observe the parasite and the accompanying cellular response. The lesions are characterized by a diffuse granulomatous reaction, often surrounding one or more PKX parasites. Early in infection there may be hyperplasia of the hematopoietic cells but as the disease progresses these are replaced by macrophages and lymphocytes. Degeneration and subsequent loss of the tubules are evident. The lesions may persist for several weeks after typical PKX parasites are no longer detectable. These lesions in combination with the observation of later stages of the PKX in the lumens of the tubules can also be used to demonstrate late infection or recent convalescence form PKD.

c. Later Developmental Stages of PKX

As described by Kent and Hedrick (this issue), later developmental stages of the PKX myxosporean have been identified. These stages are found in the lumens of the kidney tubules and persist for several months following recovery from clinical PKD. Therefore, they can be useful indicators of previous infection. Although the early sporogonic stages can be confused with other

myxosporeans, certain characteristics are unique for PKX. Spores develop in pseudoplasmodia within the lumen of kidney tubules. They are monosporous and contain two anteriorly located spherical polar capsules 2 u in diameter. No clear valve formation has been observed. In contrast, other myxosporeans (Parvicapsula, Myxidium etc.) that may be found in the lumens of the kidney tubules of salmonid fish form true plasmodia and are polysporous with many spores developing within a pansporoblast. Furthermore, valvogenesis is usually evident. With Parvicapsula sporogenesis is evident in the epithelium of the tubules as well. An observation of these unique spores of PKX, therefore, considerably extends the period in which diagnoses of can be made.