Manifestation of Proliferative Kidney Disease in Chinook Salmon (Oncorhynchus tshawytscha) Following Transfer of Infected Smolts to Sea Water

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For 4 consecutive years, the prevalence and severity of proliferative kidney disease (PKD), caused by the PKX myxosporean, in chinook salmon (Oncorhynchus tshawytscha) from the Puntledge River Hatchery, Vancouver Island, British Columbia, Canada was investigated after the fish were transferred to sea water. In the spring of 1991 through 1994, smolts were transferred from the hatchery to the Pacific Biological Station (PBS), Nanaimo, British Columbia at about the same time that their cohorts were released for seaward migration. In 1991, fish were maintained in PKX-free fresh water, and in 1992 fish were maintained in a seawater neetpen. In 1993 and 1994 fish were maintained in seawater tanks maintained at 15-17°C. In all years, virtually all the fish were subclinically infected with PKX at the time of transfer from the hatchery, and a prevalence of almost 100% was eventually observed in the fish after they were transferred to PBS. This study demonstrates that PKD can occur in sea water in post smolts originating from watersheds where the PKX parasite is present. It is, therefore, possible that PKD may impact ocean survival of salmon originating from PKX enzootic watershed, particularly because the disease causes osmoregulatory imbalances and anemia.

Key words: PKX, PKD, Oncorhynchus tshawytscha, chinook salmon, Myxosporea, ocean survival

Proliferative kidney disease (PKD) is a well-recognized disease of salmonid fishes reared in freshwater hatcheries throughout Europe (Clifton-Hadley et al., 1984) and western North America (Hedrick et al., 1993). The causative agent of PKD is PKX, the extrasporogonic stage of an unidentified myxosporean (Kent and Hedrick, 1986). Recently, Kent et al. (1993) proposed that the PKX organism may be an extrasporogonic form of Sphaerospora oncorhynchi, which completes sporulation in adult salmon. The PKX organism primarily infects the kidney, where it provokes chronic renal interstitial nephritis (Ferguson and Needham 1978; Clifton-Hadley et al., 1987a; MacConnell et al., 1989). Infected fish are often anemic (Hoffmann and Lommel, 1984; Clifton-Hadley et al., 1987b), which may be the ultimate cause of death. Fish with severe PKD may also exhibit lower serum protein (Hoffmann and Lommel, 1984) and elevated serum magnesium and calcium levels (Foott and Hedrick, 1990).

The disease is primarily a problem in hatchery-reared salmonids during their first summer when water temperatures reach 12-15°C (Ferguson and Ball, 1979; Ferguson, 1981; Clifton-Hadley et al., 1986). Fish generally become infected in the spring (April or May), and the infectious stage persists in the water throughout the summer (Kent and Hedrick, 1986; Clifton-Hadley et al., 1986, 1987a; Foott and Hedrick, 1987). The parasite is first detected in fish about 3-4 wk post-exposure (p.e.), the disease is prominent 6 through 20 wk p.e., and the infection in the kidney interstitium and associated lesions is resolved in surviving fish after about 20 wk p.e., regardless of temperature (Kent and Hedrick, 1986; Clifton-Hadley et al., 1987a; MacConnell et al., 1989).

Although the disease was first considered a serious problem in rainbow trout (O. mykiss) (c.f. Clifton-Hadley et al., 1984), PKD has also been observed in other Pacific salmonids including chinook (O. tshaw-
Chinook salmon are especially susceptible to PKD in fresh water (Hedrick et al., 1984), and Hedrick and Aronstein (1987) demonstrated that the disease can persist in this species after they are transferred to sea water.

The Puntledge River Hatchery on Vancouver Island, British Columbia (B.C.) is an important facility for the Canadian Department of Fisheries and Oceans Salmon Enhancement Program, and has a rearing target of approximately $2.5 \times 10^6$ coho, 100,000 steelhead ($O. mykiss$), and $4 \times 10^6$ chinook each year. For the past several years, PKD and concurrent furunculosis have caused high mortality in the coho and steelhead at this hatchery during the summer months (Hoskins and Kieser, 1986). The chinook salmon at the hatchery are released into the Puntledge River for seaward migration in late May or early June, which is prior to the seasonal timing of PKD outbreaks. The returns of chinook to the hatchery have been extremely low in recent years (approximately 0.0012% of released fish). Because fish usually become infected with PKX in the early spring, we suspected that the chinook smolts from this hatchery might be infected at the time of their release. The following study was conducted to determine the prevalence of PKX and associated renal lesions in chinook salmon after they migrate to sea water.

**Materials and Methods**

**1991 study**

Approximately 250 chinook smolts (avg. wt. 4.9 g) from the Puntledge River Hatchery were transferred to the Pacific Biological Station (PBS), Nanaimo, B. C. on 25 May 1991. This was 1 week after fish were released from the hatchery for their seaward migration. The fish were held in a tank receiving dechlorinated city fresh water (15–18°C). The kidneys of 29 chinook held at PBS were examined by histology after 84 d to determine the prevalence of infection by PKX, and to determine if the fish had developed PKD.

**1992 study**

A more detailed study was conducted in 1992. Approximately 1000 chinook smolts (avg. wt. 7.3 g) were transferred from the Puntledge River Hatchery to PBS on 3 June 1992, one week after their cohorts had been released for seaward migration. The fish were placed in a tank receiving dechlorinated fresh water from municipal sources, maintained at 15°C. The fish were vaccinated against vibriosis using bath immersion for 3 h at 1:1000 with a commercial vaccine (Microtek, Sydney, British Columbia). Five hundred of these fish were transferred to a seawater netpen at the experimental fish farm at PBS in June 1992. Temperature and salinity data in the netpen were collected from a depth of 3 m. The water temperatures in the seawater netpens were 16 to 19°C throughout the study, with a mean of 17.1°C. Salinity in the netpen site ranged from 19 to 28 ppt throughout the study, with a mean of 25.7 ppt.

Control (uninfected) fish for the study were transferred to a seawater netpen at approximately the same time as the PKX-exposed group. Control fish were from another stock of chinook that were reared from the egg stage at PBS on PKX-free water.

Fish were examined periodically throughout the summer of 1992 (Fig. 1). Twenty one to 24 fish from the infected group, and 5 fish from the control group were collected randomly and examined by histology at each sampling.

**1993 study**

Approximately 500 chinook (avg. wt. 5.7 g) were transferred to PBS on 11 June 1993, which was the same day that their cohorts were released from the hatchery for seaward migration. The fish were divided into two groups and placed in 725 l aquaria receiving flowing sea water maintained at 15–17°C. Samples of 10 fish per group were collected bi-weekly, and were examined by histology starting at the day of transfer (Fig. 1).

**1994 study**

Approximately 100 chinook (avg. wt. 4.9 g) were transferred to PBS on 9 June 1994, at the same time that their cohorts were released from the hatchery for seaward migration. These fish were maintained in a 725 l aquarium receiving flowing sea water maintained at 15–17°C. Samples of 10 fish were collected bi-weekly and examined by histology starting at the day of transfer (Fig. 1).

**Histology**

The prevalence of infection by the PKX myxospo-
PKX in chinook in sea water

Rean and the severity of PKD were assessed by histological examination of the kidney. The kidney of each fish was preserved in Davidson's solution (Humason, 1979), processed for histology using standard techniques, and sections were stained with hematoxylin and eosin. In the 1992 study, the intensity of PKX infection was assessed by counting the organisms in the renal interstitium and associated blood vessels in $15 \times 600$ fields/kidney in histological slides. The severity of PKD was determined by measuring the degree of renal interstitial hyperplasia (RIH), which includes increase in renal hematopoiesis, diffuse interstitial nephritis and focal granulomata formation. Renal interstitial hyperplasia was assessed by determining the decrease in number of renal tubule cross sections in $10 \times 400$ fields/kidney section in histological slides as described by Kent and Hedrick (1987) and Arkush and Hedrick (1990). Determination of renal tubule concentrations was restricted to the kidney posterior to the corpuscles of Stannius. A tubule was counted when it was separated from adjacent tubules by interstitial tissue or a visible basement membrane.

Results

1991 study

Almost all (27 of 29) of the chinook transferred to PBS were infected with PKX, and all infected fish exhibited moderate to severe RIH due to diffuse chronic inflammation in the renal interstitium. Fish showed no pathologic signs suggestive of other diseases, except a few fish had tail rot. Approximately 30% of the fish died before the study was terminated in August 1991, and most of this mortality was attributed to PKD.

1992 study

All chinook examined shortly after transfer to PBS were infected with the PKX myxosporan (Fig. 1). Numerous parasites and prominent RIH were observed in all the fish from the infected group sampled during the first 30 days; after this the infection prevalence and intensity decreased (Figs. 2 and 3). Renal interstitial hyperplasia was due to chronic, diffuse inflammation, and an increase in hematopoietic activity in the renal interstitium. The renal lesions resolved after the infection was eliminated, and at 85 days, the densities of renal tubules in both
Fig. 2. Intensity of PKX infection in kidney sections in chinook salmon after transfer to sea water in 1992.

Fig. 3. Tubule densities in PKX-infected (■) and control (○) chinook salmon after transfer to sea water in 1992.

groups of infected fish were not different from those of the controls.

The PKX-infected fish in the netpen showed a cumulative mortality of 11% over the first 66 days of the experiment. Most of this mortality was attributed to PKD because fish showed pathological changes consistent with the disease (i.e. renomegaly and pale gills) and no indication of other diseases or infectious agents. Although mortality in these fish was relatively minimal, most of the surviving fish in the pen were lethargic. When fish were captured for examination, some immediately rolled over and sank to the bottom of the collecting bucket. Many of these fish had pale gills, indicative of anemia. Although the PKX infected fish were vaccinated against *Vibrio anguillarum* and *V. ordalii*, mortalities associated with vibriosis were observed in these fish starting from day 66 through day 90. Vibriosis was diagnosed by observing large, multifocal hemorrhages in the liver, which is typical for the disease in chinook smolts (Evelyn and Kent, 1992). In addition, bacteriology was conducted on four moribund fish collected at day 70, and *V. anguillarum* was isolated from the kidneys in pure culture from all fish using Tryptic Soy Agar with 1% NaCl. We attributed 16% mortality in the PKX infected fish to vibriosis. In addition, there was a 20% unaccountable loss of the fish held in the netpens (e.g., predation by seals and birds, and dead fish that slipped through the bottom of the net). Vibriosis and PKD were not observed in the control fish, and this group had less than 5% mortality during the study.

1993 study

The PKX organism was not detected in the chinook at the time of transfer, and gradually increased in prevalence to 100% 41 days later (Fig. 1). The study was terminated at 53 days post-transfer, at which time all the fish were infected. As with previous years, many fish had heavy infections associated with severe interstitial nephritis.

1994 study

The chinook exhibited a similar pattern of infection as the 1993 study. The PKX organism was not detected at the time of transfer and then increased in prevalence to 100% in subsequent samples (Fig. 1). The study was terminated at 74 days post-transfer, at which time the prevalence and severity of infection had decreased.

Discussion

Whereas considerable research has been conducted on the pathogenesis of diseases in anadromous salmonids during their freshwater phase of development, relatively little information is available on the effects of these diseases on fish after they migrate to sea water. Banner *et al.* (1983) reported that bacterial kidney disease, caused by *Renibacterium salmoninarum*, was associated with high mortality in chinook after they were transferred to sea water, and Frantsi *et al.* (1975) reported that Atlantic salmon (*Salmo salar*) with advanced bacterial kidney disease had higher mortality upon seawater entry than lightly infected or uninfected fish. The fungus *Ichthyophonus* sp. has caused impairment of seawater acclimation in salmon smolts (Uno, 1990). Boyce and Clarke (1983) showed that fish infected with the freshwater cestode *Eubothrium salvelini* had a reduced ability to adapt to sea water. Gill infections by the protozoan flagellate *Ichthyobodo necator* may also cause poor survival when hatchery-reared
PKX in chinook in sea water

coho and chinook salmon enter sea water (Wedemeyer et al., 1980).

Transfer of the chinook smolts to sea water apparently did not affect the severity or course of PKD. The fish from all years showed a similar course of infection and severity of PKD as that reported in previous studies for fish held in fresh water (Kent and Hedrick, 1986, 1987; Clifton-Hadley et al., 1987a; Arkush and Hedrick, 1990). To be comparable with the present study, it should be noted that the area of the ×400 field used to calculate the density of tubules in the studies by Kent and Hedrick (1987) and Arkush and Hedrick (1990) was determined incorrectly. The tubule concentrations for these studies should have been multiplied by a factor of 4.3.

In 1992, the chinook smolts exhibited 100% infection at the time of transfer to sea water, whereas in 1993 and 1994 the infection was not detected until the fish were in sea water for several weeks (Fig. 1). This difference was not due to the timing of transfer because the fish were transferred to PBS between 3 and 11 June for all three years. Foott and Hedrick (1987) reported that water temperatures must reach about 12°C to initiate PKX infections. Therefore, the earlier occurrence of detectable PKX parasites in 1992 was probably due to warmer water temperatures at the Puntledge River Hatchery during May 1992 compared with the two subsequent years temperatures during May. The mean water temperature in May 1992 was 12.2, versus 9.0 and 11.3 for May 1993 and 1994, respectively.

The PKX-infected fish held in the netpen in 1992 exhibited vibriosis about 2 mo after transfer to sea water, whereas other chinook of the same year class held at this site were not affected by this disease. All the chinook at this site, including the PKX-infected fish, had been vaccinated with a commercial vaccine against *Vibrio anguillarum* and *V. ordalii* before the fish were introduced to sea water. These commercial vaccines are usually very efficacious when applied properly. Angelidis et al. (1987) reported that rainbow trout with PKD had a lower immune response to a vibrio vaccine than uninfected fish. However, more research on the immunosuppressive effects of PKD should be conducted because in a contrasting study Foott and Hedrick (1990) found that rainbow trout with PKD showed better protection to vibriosis following vaccination than uninfected fish that were also vaccinated.

Examination of four year classes of chinook transferred to PBS indicates that almost all chinook smolts from the Puntledge River Hatchery are infected with PKX when they are released for their seaward migration. The prevalence of the infection in the fish held at PBS was probably not an overestimate of the prevalence of infection in their cohorts in the estuary and ocean because the PKX myxosporian is not transmitted from fish to fish in aquaria (D'Silva et al., 1984), and is only contracted in fresh water. Furthermore, the fish were not exposed to additional infectious stages of the parasite in fresh water at PBS in 1991 because the fish were maintained on dechlorinated city water.

The chinook smolts released from the hatchery may have developed PKD to a similar severity as those maintained at PBS. The Puntledge River and estuary are usually well above 12°C during the summer. Observations from similar river systems on Vancouver Island suggested that juvenile chinook may spend about 1 to 2 mo in the estuary following migration to sea water (Healey, 1980a, b; Levings et al., 1986, 1989). The Puntledge River Hatchery chinook would be categorized as ocean-type under-yearlings (Healey, 1980b), and many of these fish remain in warm, surface waters during their first summer in sea water (Healey, 1980b; Levings et al., 1986; Healey, 1991). In addition, fish with poor osmoregulatory capabilities probably remain in the brackish waters of the estuary longer than their healthy cohorts (Zaugg, 1989).

Spiny dogfish (*Squalus acanthias*) prey heavily on chinook salmon smolts (Beamish et al., 1992), and predation may be an important factor influencing survival of juvenile salmon in coastal waters (Healey, 1980b). As suggested by Hedrick and Aronstein (1987), the lethargy and apparent anemia observed in the fish with PKD in 1992 probably diminished their swimming stamina, and would have made them more susceptible to predation if they had been released to the wild. Therefore, it is possible that the 1992 infected smolts in the estuary or ocean surface waters had higher mortality than their cohorts in the seawater netpen at PBS.

In conclusion, PKD may have an impact on the ocean survival of chinook reared at the Puntledge River Hatchery because almost all of the chinook smolts are infected with the parasite at the time of release from the hatchery. They then may develop PKD shortly after entering seaward migration. This
phenomenon may also occur at other chinook hatcheries where the parasite is enzootic.

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References


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